Module Title: Parasitology
Module Code: BS42012

This is the main reading list. In the reviews section you will find primarily reviews that support the lectures and **i**n the research papers section you will find primary research papers that also support the lectures. Some of these papers will form the basis of coursework.

Abstracts are provided below and you should use both reading lists to supplement the lecture content of the module.

Note that some lecturers may also provide additional material.

You must download this list, which also serves to obtain active links or DOI.

Reviews

Trends Parasitol. 2016 Nov;32(11):899-911. doi: 10.1016/j.pt.2016.08.009. Epub 2016 Sep 6.

Putting Infection Dynamics at the Heart of Chagas Disease.

Lewis MD[1], Kelly JM[2].
Author information
Abstract
In chronic Trypanosoma cruzi infections, parasite burden is controlled by effective, but nonsterilising immune responses. Infected cells are difficult to detect because they are scarce and focally distributed in multiple sites. However, advances in detection technologies have established a link between parasite persistence and the pathogenesis of Chagas heart disease. Long-term persistence likely involves episodic reinvasion as well as continuous infection, to an extent that varies between tissues. The primary reservoir sites in humans are not definitively known, but analysis of murine models has identified the gastrointestinal tract. Here, we highlight that quantitative, spatial, and temporal aspects of T. cruzi infection are central to a fuller understanding of the association between persistence, pathogenesis, and immunity, and for optimising treatment.

Annu Rev Microbiol. 2008;62:445-70. doi: 10.1146/annurev.micro.61.080706.093134.

Antigenic variation in Plasmodium falciparum.

Scherf A[1], Lopez-Rubio JJ, Riviere L.
Author information
Abstract
The persistence of the human malaria parasite Plasmodium falciparum during blood stage proliferation in its host depends on the successive expression of variant molecules at the surface of infected erythrocytes. This variation is mediated by the differential control of a family of surface molecules termed PfEMP1 encoded by approximately 60 var genes. Each individual parasite expresses a single var gene at a time, maintaining all other members of the family in a transcriptionally silent state. PfEMP1/var enables parasitized erythrocytes to adhere within the microvasculature, resulting in severe disease. This review highlights key regulatory mechanisms thought to be critical for monoallelic expression of var genes. Antigenic variation is orchestrated by epigenetic factors including monoallelic var transcription at separate spatial domains at the nuclear periphery, differential histone marks on otherwise identical var genes, and var silencing mediated by telomeric heterochromatin. In addition, controversies surrounding var genetic elements in antigenic variation are discussed.

Curr Opin Microbiol. 2016 Dec;34:97-103. doi: 10.1016/j.mib.2016.08.005. Epub 2016 Sep 9.

Exploiting the Achilles' heel of membrane trafficking in trypanosomes.

Zoltner M[1], Horn D[1], de Koning HP[2], Field MC[3].
Author information
Abstract
Pathogenic protozoa are evolutionarily highly divergent from their metazoan hosts, reflected in many aspects of their biology. One particularly important parasite taxon is the trypanosomatids. Multiple transmission modes, distinct life cycles and exploitation of many host species attests to great prowess as parasites, and adaptability for efficient, chronic infection. Genome sequencing has begun uncovering how trypanosomatids are well suited to parasitism, and recent genetic screening and cell biology are revealing new aspects of how to control these organisms and prevent disease. Importantly, several lines of evidence suggest that membrane transport processes are central for the sensitivity towards several frontline drugs.

Nat Rev Microbiol. 2017 Feb 27;15(4):217-231. doi: 10.1038/nrmicro.2016.193. [Epub ahead of print]

Anti-trypanosomatid drug discovery: an ongoing challenge and a continuing need.

Field MC[1], Horn D[1], Fairlamb AH[1], Ferguson MA[1], Gray DW[1], Read KD[1], De Rycker M[1], Torrie LS[1], Wyatt PG[1], Wyllie S[1], Gilbert IH[1].
Author information

Abstract
The WHO recognizes human African trypanosomiasis, Chagas disease and the leishmaniases as neglected tropical diseases. These diseases are caused by parasitic trypanosomatids and range in severity from mild and self-curing to near invariably fatal. Public health advances have substantially decreased the effect of these diseases in recent decades but alone will not eliminate them. In this Review, we discuss why new drugs against trypanosomatids are required, approaches that are under investigation to develop new drugs and why the drug discovery pipeline remains essentially unfilled. In addition, we consider the important challenges to drug discovery strategies and the new technologies that can address them. The combination of new drugs, new technologies and public health initiatives is essential for the management, and hopefully eventual elimination, of trypanosomatid diseases from the human population.

Trends Parasitol. 2014 Jun;30(6):289-98. doi: 10.1016/j.pt.2014.04.003. Epub 2014 Apr 26.

Nitro drugs for the treatment of trypanosomatid diseases: past, present, and future prospects.

Patterson S1, Wyllie S2.

Abstract
There is an urgent need for new, safer, and effective treatments for the diseases caused by the protozoan parasites Trypanosoma brucei, Trypanosoma cruzi, and Leishmania spp. In the search for more effective drugs to treat these 'neglected diseases' researchers have chosen to reassess the therapeutic value of nitroaromatic compounds. Previously avoided in drug discovery programs owing to potential toxicity issues, a nitro drug is now being used successfully as part of a combination therapy for human African trypanosomiasis. We describe here the rehabilitation of nitro drugs for the treatment of trypanosomatid diseases and discuss the future prospects for this compound class.

J Clin Invest. 2008 Apr;118(4):1301-10. doi: 10.1172/JCI33945.

Kinetoplastids: related protozoan pathogens, different diseases.

Stuart K1, Brun R, Croft S, Fairlamb A, Gürtler RE, McKerrow J, Reed S, Tarleton R.

Abstract
Kinetoplastids are a group of flagellated protozoans that include the species Trypanosoma and Leishmania, which are human pathogens with devastating health and economic effects. The sequencing of the genomes of some of these species has highlighted their genetic relatedness and underlined differences in the diseases that they cause. As we discuss in this Review, steady progress using a combination of molecular, genetic, immunologic, and clinical approaches has substantially increased understanding of these pathogens and important aspects of the diseases that they cause. Consequently, the paths for developing additional measures to control these "neglected diseases" are becoming increasingly clear, and we believe that the opportunities for developing the drugs, diagnostics, vaccines, and other tools necessary to expand the armamentarium to combat these diseases have never been better.

J Med Chem. 2013 Oct 24;56(20):7719-26. doi: 10.1021/jm400362b. Epub 2013 Sep 9.

Drug discovery for neglected diseases: molecular target-based and phenotypic approaches.

Gilbert IH1.

Abstract
Drug discovery for neglected tropical diseases is carried out using both target-

based and phenotypic approaches. In this paper, target-based approaches are discussed, with a particular focus on human African trypanosomiasis. Target-based drug discovery can be successful, but careful selection of targets is required. There are still very few fully validated drug targets in neglected diseases, and there is a high attrition rate in target-based drug discovery for these diseases. Phenotypic screening is a powerful method in both neglected and non-neglected diseases and has been very successfully used. Identification of molecular targets from phenotypic approaches can be a way to identify potential new drug targets.

Cell Microbiol. 2013 Dec;15(12):1976-83. doi: 10.1111/cmi.12183. Epub 2013 Sep 4.

Malaria's deadly grip: cytoadhesion of Plasmodium falciparum-infected erythrocytes.

Smith JD[1], Rowe JA, Higgins MK, Lavstsen T.

Author information

Abstract

Cytoadhesion of Plasmodium falciparum-infected erythrocytes to host microvasculature is a key virulence determinant. Parasite binding is mediated by a large family of clonally variant adhesion proteins, termed P. falciparum erythrocyte membrane protein 1 (PfEMP1), encoded by var genes and expressed at the infected erythrocyte surface. Although PfEMP1 proteins have extensively diverged under opposing selection pressure to maintain ligand binding while avoiding antibody-mediated detection, recent work has revealed they can be classified into different groups based on chromosome location and domain composition. This grouping reflects functional specialization of PfEMP1 proteins for different human host and microvascular binding niches and appears to be maintained by gene recombination hierarchies. Inone extreme, a specific PfEMP1 variant is associated with placental binding and malaria during pregnancy, while other PfEMP1 subtypes appear to be specialized for infection of malaria naïve hosts. Here, we discuss recent findings on the origins and evolution of the var gene family, the structure-function of PfEMP1 proteins, and a distinct subset of PfEMP1 variants that have been associated with severe childhood malaria.

Trends Parasitol. 2013 Mar;29(3):110-8. doi: 10.1016/j.pt.2012.12.005. Epub 2013 Jan 30.

Drug resistance in African trypanosomiasis: the melarsoprol and pentamidine story.

Baker N[1], de Koning HP, Mäser P, Horn D.

Author information

Abstract

Melarsoprol and pentamidine represent the two main classes of drugs, the arsenicals and diamidines, historically used to treat the diseases caused by African trypanosomes: sleeping sickness in humans and Nagana in livestock. Cross-resistance to these drugs was first observed over 60 years ago and remains the only example of cross-resistance among sleeping sickness therapies. A Trypanosoma brucei adenosine transporter is well known for its role in the uptake of both drugs. More recently, aquaglyceroporin 2 (AQP2) loss of function was linked to melarsoprol-pentamidine cross-resistance. AQP2, a channel that appears to facilitate drug accumulation, may also be linked to clinical cases of resistance. Here, we review these findings and consider some new questions as well as future prospects for tackling the devastating diseases caused by these parasites.

PLoS Pathog. 2013 Oct;9(10):e1003629. doi: 10.1371/journal.ppat.1003629. Epub 2013 Oct 24.

Protein trafficking through the endosomal system prepares intracellular parasites for a home invasion.

Tomavo S[1], Slomianny C, Meissner M, Carruthers VB.

Author information

Abstract

Toxoplasma (toxoplasmosis) and Plasmodium (malaria) use unique secretory organelles for migration, cell invasion, manipulation of host cell functions, and cell egress. In particular, the apical secretory micronemes and rhoptries of apicomplexan parasites are essential for successful host infection. New findings reveal that the contents of these organelles, which are transported through the endoplasmic reticulum (ER) and Golgi, also require the parasite endosome-like system to access their respective organelles. In this review, we discuss recent findings that demonstrate that these parasites reduced their endosomal system and modified classical regulators of this pathway for the biogenesis of apical organelles.

Nat Rev Microbiol. 2012 Apr 30;10(6):431-8. doi: 10.1038/nrmicro2779.

Trypanosomal immune evasion, chronicity and transmission: an elegant balancing act.

MacGregor P[1], Szöőr B, Savill NJ, Matthews KR.

Author information

Abstract

During their life cycle, trypanosomes must overcome conflicting demands to ensure their survival and transmission. First, they must evade immunity without overwhelming the host. Second, they must generate and maintain transmission stages at sufficient levels to allow passage into their tsetse vector. Finally, they must rapidly commit to onward development when they enter the tsetse fly. On the basis of recent quantification and modelling of

Trypanosoma brucei infection dynamics, we propose that the interplay between immune evasion and development achieves both infection chronicity and transmissibility. Moreover, we suggest that a novel form of bistable regulation ensures developmental commitment on entry into the tsetse fly midgut.

Mol Biochem Parasitol. 2014 Jul;195(2):123-9. doi: 10.1016/j.molbiopara.2014.05.001. Epub 2014 May 22.

Antigenic variation in African trypanosomes.

Horn D[1].

Author information

Abstract

Studies on Variant Surface Glycoproteins (VSGs) and antigenic variation in the African trypanosome, Trypanosoma brucei, have yielded a remarkable range of novel and important insights. The features first identified in T. brucei extend from unique to conserved-among-trypanosomatids to conserved-among-eukaryotes. Consequently, much of what we now know about trypanosomatid biology and much of the technology available has its origin in studies related to VSGs. T. brucei is now probably the most advanced early branched eukaryote in terms of experimental tractability and can be approached as a pathogen, as a model for studies on fundamental processes, as a model for studies on eukaryotic evolution or often all of the above. In terms of antigenic variation itself, substantial progress has been made in understanding the expression and switching of the VSG coat, while outstanding questions continue to stimulate innovative new approaches. There are large numbers of VSG genes in the genome but only one is expressed at a time, always immediately adjacent to a telomere. DNA repair processes allow a new VSG to be copied into the single transcribed locus. A coordinated transcriptional switch can also allow a new VSG gene to be activated without any detectable change in the DNA sequence, thereby maintaining singular expression, also known as allelic exclusion. I review the story behind VSGs; the genes, their expression and switching, their central role in T. brucei virulence, the discoveries that emerged along the way and the persistent questions relating to allelic exclusion in particular.

Mol Biochem Parasitol. 2014 Jul;195(2):96-106. doi: 10.1016/j.molbiopara.2014.06.005. Epub 2014 Jul 1.

Networks of gene expression regulation in Trypanosoma brucei.

Clayton CE[1].

Author information

Abstract

Regulation of gene expression in Kinetoplastids relies mainly on post-transcriptional mechanisms. Recent high-throughput analyses, combined with mathematical modelling, have demonstrated possibilities for transcript-specific regulation at every stage: trans splicing, polyadenylation, translation, and degradation of both the precursor and the mature mRNA. Different mRNA degradation pathways result in different types of degradation kinetics. The original idea that the fate of an mRNA - or even just its degradation kinetics - can be defined by a single "regulatory element" is an over-simplification. It is now clear that every mRNA can bind many different proteins, some of which may compete with each other. Superimposed upon this complexity are the interactions of those proteins with effectors of gene expression. The amount of protein that is made from a gene is therefore determined by a comple

Mol Biochem Parasitol. 2014 Jul;195(2):77-81. doi: 10.1016/j.molbiopara.2014.07.008. Epub 2014 Aug 8.

The revolution of whole genome sequencing to study parasites.

Forrester SJ[1], Hall N[2].

Author information

Abstract

Genome sequencing has revolutionized the way in which we approach biological research from fundamental molecular biology to ecology and epidemiology. In the last 10 years the field of genomics has changed enormously as technology has improved and the tools for genomic sequencing have moved out of a few dedicated centers and now can be performed on bench-top instruments. In this review we will cover some of the key discoveries that were catalyzed by some of the first genome projects and discuss how this field is developing, what the new challenges are and how this may impact on research in the near future.

Trends Genet. 2006 Nov;22(11):614-20. Epub 2006 Aug 14.

Switching trypanosome coats: what's in the wardrobe?

Taylor JE[1], Rudenko G.

Author information

Abstract

The African trypanosome Trypanosoma brucei is best known for its extraordinarily sophisticated antigenic variation of a protective variant surface glycoprotein (VSG) coat. T. brucei has >1000 VSG genes and pseudogenes, of which one is transcribed at a time from one of multiple telomeric VSG expression sites. Switching the active VSG gene can involve DNA rearrangements replacing the old VSG with a new one, or alternatively transcriptional control. The astonishing revelation from the T. brucei genome sequence is that <7% of the sequenced VSGs seem to have fully functional

coding regions. This preponderance of pseudogenes in the VSG gene repertoire will necessitate a rethink of how antigenic variation in African

Parasitology. 2014 Jan;141(1):77-82. doi: 10.1017/S0031182013000243. Epub 2013 Apr 8.

High-throughput decoding of drug targets and drug resistance mechanisms in African trypanosomes.

Horn D1.

Abstract
The availability of genome sequence data has facilitated the development of high-throughput genetic screening approaches in microbial pathogens. In the African trypanosome, Trypanosoma brucei, genome-scale RNA interference screens have proven particularly effective in this regard. These genetic screens allow for identification of the genes that contribute to a particular pathway or mechanisms of interest. The approach has been used to assess loss-of-fitness, revealing the genes and proteins required for parasite viability and growth. The outputs from these screens predict essential and dispensable genes and facilitate drug target prioritization efforts. The approach has also been used to assess resistance to anti-trypanosomal drugs, revealing the genes and proteins that facilitate drug uptake and action. These outputs also highlight likely mechanisms underlying clinically relevant drug resistance. I first review these findings in the context of what we know about current drugs. I then describe potential contributions that these high-throughput approaches could make to the development and implementation of new drugs.

J Trop Med. 2012;2012:340538. doi: 10.1155/2012/340538. Epub 2012 Mar 27.

Towards Point-of-Care Diagnostic and Staging Tools for Human African Trypanosomiaisis.

Matovu E1, Kazibwe AJ, Mugasa CM, Ndungu JM, Njiru ZK.

Abstract
Human African trypanosomiasis is a debilitating disease prevalent in rural sub-Saharan Africa. Control of this disease almost exclusively relies on chemotherapy that should be driven by accurate diagnosis, given the unacceptable toxicity of the few available drugs. Unfortunately, the available diagnostics are characterised by low sensitivities due to the inherent low parasitaemia in natural infections. Demonstration of the trypanosomes in body

fluids, which is a prerequisite before treatment, often follows complex algorithms. In this paper, we review the available diagnostics and explore recent advances towards development of novel point-of-care diagnostic tests.

Nature. 2010 Apr 1;464(7289):728-32. doi: 10.1038/nature08893.

N-myristoyltransferase inhibitors as new leads to treat sleeping sickness.

Frearson JA1, Brand S, McElroy SP, Cleghorn LA, Smid O, Stojanovski L, Price HP, Guther ML, Torrie LS, Robinson DA, Hallyburton I, Mpamhanga CP, Brannigan JA, Wilkinson AJ, Hodgkinson M, Hui R, Qiu W, Raimi OG, van Aalten DM, Brenk R, Gilbert IH, Read KD, Fairlamb AH, Ferguson MA, Smith DF, Wyatt PG.
Author information

Abstract

African sleeping sickness or human African trypanosomiasis, caused by Trypanosoma brucei spp., is responsible for approximately 30,000 deaths each year. Available treatments for this disease are poor, with unacceptable efficacy and safety profiles, particularly in the late stage of the disease when the parasite has infected the central nervous system. Here we report the validation of a molecular target and the discovery of associated lead compounds with the potential to address this lack of suitable treatments. Inhibition of this target-T. brucei N-myristoyltransferase-leads to rapid killing of trypanosomes both in vitro and in vivo and cures trypanosomiasis in mice. These high-affinity inhibitors bind into the peptide substrate pocket of the enzyme and inhibit protein N-myristoylation in trypanosomes. The compounds identified have promising pharmaceutical properties and represent an opportunity to develop oral drugs to treat this devastating disease. Our studies validate T. brucei N-myristoyltransferase as a promising therapeutic target for human African trypanosomiasis.

Cell Mol Life Sci. 2014 Apr;71(7):1245-63. doi: 10.1007/s00018-013-1491-1. Epub 2013 Nov 13.

Mechanisms of cellular invasion by intracellular parasites.

Walker DM1, Oghumu S, Gupta G, McGwire BS, Drew ME, Satoskar AR.
Author information

Abstract

Numerous disease-causing parasites must invade host cells in order to prosper. Collectively, such pathogens are responsible for a staggering amount of human sickness and death throughout the world. Leishmaniasis, Chagas disease, toxoplasmosis, and malaria are neglected diseases and therefore are linked to socio-economical and geographical factors, affecting well-over

half the world's population. Such obligate intracellular parasites have co-evolved with humans to establish a complexity of specific molecular parasite-host cell interactions, forming the basis of the parasite's cellular tropism. They make use of such interactions to invade host cells as a means to migrate through various tissues, to evade the host immune system, and to undergo intracellular replication. These cellular migration and invasion events are absolutely essential for the completion of the lifecycles of these parasites and lead to their for disease pathogenesis. This review is an overview of the molecular mechanisms of protozoan parasite invasion of host cells and discussion of therapeutic strategies, which could be developed by targeting these invasion pathways. Specifically, we focus on four species of protozoan parasites Leishmania, Trypanosoma cruzi, Plasmodium, and Toxoplasma, which are responsible for significant morbidity and mortality.

Trends Parasitol. 2012 Dec;28(12):539-45. doi: 10.1016/j.pt.2012.09.002. Epub 2012 Oct 8.

Trypanosome resistance to human innate immunity: targeting Achilles' heel.

Stephens NA1, Kieft R, Macleod A, Hajduk SL.

Author information

Abstract

Trypanosome lytic factors (TLFs) are powerful, naturally occurring toxins in humans that provide sterile protection against infection by several African trypanosomes. These trypanocidal complexes predominantly enter the parasite by binding to the trypanosome haptoglobin/hemoglobin receptor (HpHbR), trafficking to the lysosome, causing membrane damage and, ultimately, cell lysis. Despite TLF-mediated immunity, the parasites that cause human African Trypanosomiasis (HAT), Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense, have developed independent mechanisms of resistance to TLF killing. In this review we describe the parasite defenses that allow trypanosome infections of humans and discuss how targeting these apparent strengths of the parasite may reveal their Achilles' heel, leading to new approaches in the treatment of HAT.

Trends Parasitol. 2014 May;30(5):251-8. doi: 10.1016/j.pt.2014.03.004. Epub 2014 Apr 12.

Life and times: synthesis, trafficking, and evolution of VSG.

Manna PT1, Boehm C1, Leung KF2, Natesan SK2, Field MC3.

Author information

Abstract

Evasion of the acquired immune response in African trypanosomes is principally mediated by antigenic variation, the sequential expression of distinct variant surface glycoproteins (VSGs) at extremely high density on the cell surface. Sequence diversity between VSGs facilitates escape of a subpopulation of trypanosomes from antibody-mediated killing. Significant

advances have increased understanding of the mechanisms underpinning synthesis and maintenance of the VSG coat. In this review, we discuss the biosynthesis, trafficking, and turnover of VSG, emphasising those unusual mechanisms that act to maintain coat integrity and to protect against immunological attack. We also highlight new findings that suggest the presence of unique or highly divergent proteins that may offer therapeutic opportunities, as well as considering aspects of VSG biology that remain to be fully explored.

Research papers

Proc Natl Acad Sci U S A. 2018 Sep 18;115(38):9616-9621. doi: 10.1073/pnas.1807915115. Epub 2018 Sep 5.

Clinical and veterinary trypanocidal benzoxaboroles target CPSF3.

Wall RJ1, Rico E1, Lukac I1, Zuccotto F1, Elg S1, Gilbert IH1, Freund Y2, Alley MRK2, Field MC1, Wyllie S1, Horn D3.

Author information

Abstract

African trypanosomes cause lethal and neglected tropical diseases, known as sleeping sickness in humans and nagana in animals. Current therapies are limited, but fortunately, promising therapies are in advanced clinical and veterinary development, including acoziborole (AN5568 or SCYX-7158) and AN11736, respectively. These benzoxaboroles will likely be key to the World Health Organization's target of disease control by 2030. Their mode of action was previously unknown. We have developed a high-coverage overexpression library and use it here to explore drug mode of action in *Trypanosoma brucei* Initially, an inhibitor with a known target was used to select for drug resistance and to test massive parallel library screening and genome-wide mapping; this effectively identified the known target and validated the approach. Subsequently, the overexpression screening approach was used to identify the target of the benzoxaboroles, Cleavage and Polyadenylation Specificity Factor 3 (CPSF3, Tb927.4.1340). We validated the CPSF3 endonuclease as the target, using independent overexpression strains. Knockdown provided genetic validation of CPSF3 as essential, and GFP tagging confirmed the expected nuclear localization. Molecular docking and CRISPR-Cas9-based editing demonstrated how acoziborole can specifically block the active site and mRNA processing by parasite, but not host CPSF3. Thus, our findings provide both genetic and chemical validation for CPSF3 as an important drug target in trypanosomes and reveal inhibition of mRNA maturation as the mode of action of the trypanocidal benzoxaboroles. Understanding the mechanism of action of benzoxaborole-based therapies can assist development of improved therapies, as well as the prediction and monitoring of resistance, if or when it arises.

PLoS Pathog. 2018 Feb 9;14(2):e1006850. doi: 10.1371/journal.ppat.1006850. eCollection 2018 Feb.

Host-parasite co-metabolic activation of antitrypanosomal aminomethyl-benzoxaboroles.

Zhang N1, Zoltner M1, Leung KF2, Scullion P1, Hutchinson S1, Del Pino RC1, Vincent IM3, Zhang YK4, Freund YR4, Alley MRK4, Jacobs RT4, Read KD1, Barrett MP3, Horn D1, Field MC1.

Author information

Abstract

Recent development of benzoxaborole-based chemistry gave rise to a collection of compounds with great potential in targeting diverse infectious diseases, including human African Trypanosomiasis (HAT), a devastating neglected tropical disease. However, further medicinal development is largely restricted by a lack of insight into mechanism of action (MoA) in pathogenic kinetoplastids. We adopted a multidisciplinary approach, combining a high-throughput forward genetic screen with functional group focused chemical biological, structural biology and biochemical analyses, to tackle the complex MoAs of benzoxaboroles in Trypanosoma brucei. We describe an oxidative enzymatic pathway composed of host semicarbazide-sensitive amine oxidase and a trypanosomal aldehyde dehydrogenase TbALDH3. Two sequential reactions through this pathway serve as the key underlying mechanism for activating a series of 4-aminomethylphenoxy-benzoxaboroles as potent trypanocides; the methylamine parental compounds as pro-drugs are transformed first into intermediate aldehyde metabolites, and further into the carboxylate metabolites as effective forms. Moreover, comparative biochemical and crystallographic analyses elucidated the catalytic specificity of TbALDH3 towards the benzaldehyde benzoxaborole metabolites as xenogeneic substrates. Overall, this work proposes a novel drug activation mechanism dependent on both host and parasite metabolism of primary amine containing molecules, which contributes a new perspective to our understanding of the benzoxaborole MoA, and could be further exploited to improve the therapeutic index of antimicrobial compounds.

Nat Microbiol. 2017 Nov;2(11):1523-1532. doi: 10.1038/s41564-017-0013-6. Epub 2017 Sep 11.

Structural basis for the shielding function of the dynamic trypanosome variant surface glycoprotein coat.

Bartossek T[1], Jones NG[2], Schäfer C[3], Cvitković M[4,5], Glogger M[1], Mott HR[6], Kuper J[3], Brennich M[7,8], Carrington M[6], Smith AS[4,5], Fenz S[1], Kisker C[3], Engstler M[9].

Author information

Abstract

The most prominent defence of the unicellular parasite Trypanosoma brucei against the host immune system is a dense coat that comprises a variant surface glycoprotein (VSG). Despite the importance of the VSG family, no complete structure of a VSG has been reported. Making use of high-resolution structures of individual VSG domains, we employed small-angle X-ray scattering to elucidate the first two complete VSG structures. The resulting models imply that the linker regions confer great flexibility between domains, which suggests that VSGs can adopt two main conformations to respond to obstacles and changes of protein density, while maintaining a protective barrier at all times. Single-molecule diffusion measurements of VSG in supported lipid bilayers substantiate this possibility, as two freely diffusing populations could be detected. This translates into a highly flexible overall

topology of the surface VSG coat, which displays both lateral movement in the plane of the membrane and variation in the overall thickness of the coat.

The structure of serum resistance-associated protein and its implications for human African trypanosomiasis.

Zoll S[1], Lane-Serff H[1], Mehmood S[2], Schneider J[1], Robinson CV[2], Carrington M[3], Higgins MK[4].
Author information
Abstract
Only two trypanosome subspecies are able to cause human African trypanosomiasis. To establish an infection in human blood, they must overcome the innate immune system by resisting the toxic effects of trypanolytic factor 1 and trypanolytic factor 2 (refs. [1,2]). These lipoprotein complexes contain an active, pore-forming component, apolipoprotein L1 (ApoL1), that causes trypanosome cell death [3] . One of the two human-infective subspecies, Trypanosoma brucei rhodesiense, differs from non-infective trypanosomes solely by the presence of the serum resistance-associated protein, which binds directly to ApoL1 and blocks its pore-forming capacity[3-5]. Since this interaction is the single critical event that renders T. b. rhodesiense human- infective, detailed structural information that allows identification of binding determinants is crucial to understand immune escape by the parasite. Here, we present the structure of serum resistance-associated protein and reveal the adaptations that occurred as it diverged from other trypanosome surface molecules to neutralize ApoL1. We also present our mapping of residues important for ApoL1 binding, giving molecular insight into this interaction at the heart of human sleeping sickness.

Genome Biol Evol. 2017 Aug 1;9(8):2093-2109. doi: 10.1093/gbe/evx152.

An Alternative Strategy for Trypanosome Survival in the Mammalian Bloodstream Revealed through Genome and Transcriptome Analysis of the Ubiquitous Bovine Parasite Trypanosoma (Megatrypanum) theileri.

Kelly S[1], Ivens A[2], Mott GA[2], O'Neill E[1], Emms D[1], Macleod O[3], Voorheis P[4], Tyler K[5], Clark M[6], Matthews J[7], Matthews K[2], Carrington M[3].
Author information
Abstract
There are hundreds of Trypanosoma species that live in the blood and tissue spaces of their vertebrate hosts. The vast majority of these do not have the ornate system of antigenic variation that has evolved in the small number of African trypanosome species, but can still maintain long-term infections in the face of the vertebrate adaptive immune system. Trypanosoma theileri is a typical example, has a restricted host range of cattle and other Bovinae, and is only occasionally reported to cause patent disease although no systematic survey of the effect of infection on agricultural productivity has been performed. Here, a detailed genome sequence and a transcriptome analysis of gene expression in bloodstream form T. theileri have been performed. Analysis of the genome sequence and expression showed that T. theileri has a typical kinetoplastid genome structure and allowed a prediction that it is

capable of meiotic exchange, gene silencing via RNA interference and, potentially, density-dependent growth control. In particular, the transcriptome analysis has allowed a comparison of two distinct trypanosome cell surfaces, T. brucei and T. theileri, that have each evolved to enable the maintenance of a long-term extracellular infection in cattle. The T. theileri cell surface can be modeled to contain a mixture of proteins encoded by four novel large and divergent gene families and by members of a major surface protease gene family. This surface composition is distinct from the uniform variant surface glycoprotein coat on African trypanosomes providing an insight into a second mechanism used by trypanosome species that proliferate in an extracellular milieu in vertebrate hosts to avoid the adaptive immune response.

Cell Microbiol. 2016 Oct;18(10):1429-43. doi: 10.1111/cmi.12584. Epub 2016 May 25.

Host and parasite genetics shape a link between Trypanosoma cruzi infection dynamics and chronic cardiomyopathy.

Lewis MD[1,2], Francisco AF[3], Taylor MC[3], Jayawardhana S[3], Kelly JM[3].
Author information

Abstract

Host and parasite diversity are suspected to be key factors in Chagas disease pathogenesis. Experimental investigation of underlying mechanisms is hampered by a lack of tools to detect scarce, pleiotropic infection foci. We developed sensitive imaging models to track Trypanosoma cruzi infection dynamics and quantify tissue-specific parasite loads, with minimal sampling bias. We used this technology to investigate cardiomyopathy caused by highly divergent parasite strains in BALB/c, C3H/HeN and C57BL/6 mice. The gastrointestinal tract was unexpectedly found to be the primary site of chronic infection in all models. Immunosuppression induced expansion of parasite loads in the gut and was followed by widespread dissemination. These data indicate that differential immune control of T. cruzioccurs between tissues and shows that the large intestine and stomach provide permissive niches for active infection. The end-point frequency of heart-specific infections ranged from 0% in TcVI-CLBR-infected C57BL/6 to 88% in TcI-JR-infected C3H/HeN mice. Nevertheless, infection led to fibrotic cardiac pathology in all models. Heart disease severity was associated with the model-dependent frequency of dissemination outside the gut and inferred cumulative heart-specific parasite loads. We propose a model of cardiac pathogenesis driven by periodic trafficking of parasites into the heart, occurring at a frequency determined by host and parasite genetics.

Cell. 2016 Jan 14;164(1-2):246-257. doi: 10.1016/j.cell.2015.11.051.

Extracellular Vesicles from Trypanosoma brucei Mediate Virulence Factor Transferand Cause Host Anemia.

Szempruch AJ[1], Sykes SE[1], Kieft R[1], Dennison L[1], Becker AC[1], Gartrell A[1], Martin WJ[2], Nakayasu ES[3], Almeida IC[4], Hajduk SL[5], Harrington JM[6].

Author information

Abstract

Intercellular communication between parasites and with host cells provides mechanisms for parasite development, immune evasion, and disease pathology. Bloodstream African trypanosomes produce membranous nanotubes that originate from the flagellar membrane and disassociate into free extracellular vesicles (EVs). Trypanosome EVs contain several flagellar proteins that contribute to virulence, and Trypanosoma brucei rhodesiense EVs contain the serum resistance-associated protein (SRA) necessary for human infectivity. T. b. rhodesiense EVs transfer SRA to non-human infectious trypanosomes, allowing evasion of human innate immunity. Trypanosome EVs can also fuse with mammalian erythrocytes, resulting in rapid erythrocyte clearance and anemia. These data indicate that trypanosome EVs are organelles mediating non-hereditary virulence factor transfer and causing host erythrocyte remodeling, inducing anemia.

Mol Cell Proteomics. 2015 Jul;14(7):1911-26. doi: 10.1074/mcp.M114.047647. Epub 2015 Apr 30.

Architecture of a Host-Parasite Interface: Complex Targeting Mechanisms RevealedThrough Proteomics.

Gadelha C[1], Zhang W[2], Chamberlain JW[3], Chait BT[2], Wickstead B[3], Field MC[4].

Author information

Abstract

Surface membrane organization and composition is key to cellular function, and membrane proteins serve many essential roles in endocytosis, secretion, and cell recognition. The surface of parasitic organisms, however, is a double-edged sword; this is the primary interface between parasites and their hosts, and those crucial cellular processes must be carried out while avoiding elimination by the host immune defenses. For extracellular African trypanosomes, the surface is partitioned such that all endo- and exocytosis is directed through a specific membrane region, the flagellar pocket, in which it is thought the majority of invariant surface proteins reside. However, very few of these proteins have been identified, severely limiting functional studies, and hampering the development of potential treatments. Here we used an integrated biochemical, proteomic and bioinformatic strategy to identify surface components of the human parasite Trypanosoma brucei. This surface proteome contains previously known flagellar pocket proteins as well as multiple novel components, and is significantly enriched in proteins that are essential for parasite survival. Molecules with receptor-like properties are almost exclusively parasite-specific, whereas transporter-like proteins are conserved in model organisms. Validation shows that the majority of surface proteome constituents are bona fide surface-associated proteins and, as expected, most present at the flagellar pocket. Moreover, the largest systematic analysis of trypanosome surface molecules to date provides evidence that the cell surface is compartmentalized into three distinct domains

with free diffusion of molecules in each, but selective, asymmetric traffic between. This work provides a paradigm for the compartmentalization of a cell surface and a resource for its analysis.

Curr Biol. 2016 Jan 25;26(2):161-72. doi: 10.1016/j.cub.2015.11.055. Epub 2015 Dec 24.

Kinetoplastid Phylogenomics Reveals the Evolutionary Innovations Ass ociated with the Origins of Parasitism.

Jackson AP[1], Otto TD[2], Aslett M[2], Armstrong SD[3], Bringaud F[4], Schlacht A[5], Hartley C[3], Sanders M[2], Wastling JM[6], Dacks JB[5], Acosta-Serrano A[7], Field MC[8], Ginger ML[9], Berriman M[2].

Author information

Abstract

The evolution of parasitism is a recurrent event in the history of life and a core problem in evolutionary biology. Trypanosomatids are important parasites and include the human pathogens Trypanosoma brucei, Trypanosoma cruzi, and Leishmania spp., which in humans cause African trypanosomiasis, Chagas disease, and leishmaniasis, respectively. Genome comparison between trypanosomatids reveals that these parasites have evolved specialized cell-surface protein families, overlaid on a well-conserved cell template. Understanding how these features evolved and which ones are specifically associated with parasitism requires comparison with related non-parasites. We have produced genome sequences for Bodo saltans, the closest known non-parasitic relative of trypanosomatids, and a second bodonid, Trypanoplasma borreli. Here we show how genomic reduction and innovation contributed to the character of trypanosomatid genomes. We show that gene loss has "streamlined" trypanosomatid genomes, particularly with respect to macromolecular degradation and ion transport, but consistent with a widespread loss of functional redundancy, while adaptive radiations of gene families involved in membrane function provide the principal innovations in trypanosomatid evolution. Gene gain and loss continued during trypanosomatid diversification, resulting in the asymmetric assortment of ancestral characters such as peptidases between Trypanosoma and Leishmania, genomic differences that were subsequently amplified by lineage-specific innovations after divergence. Finally, we show how species-specific, cell-surface gene families (DGF-1 and PSA) with no apparent structural similarity are independent derivations of a common ancestral form, which we call "bodonin." This new evidence defines the parasitic innovations of trypanosomatid genomes, revealing how a free-living phagotroph became adapted to exploiting hostile host environments.

Nature. 2012 Jan 25;482(7384):232-6. doi: 10.1038/nature10771.

High-throughput decoding of antitrypanosomal drug efficacy and resistance.

Alsford S1, Eckert S, Baker N, Glover L, Sanchez-Flores A, Leung KF, Turner DJ, Field MC, Berriman M, Horn D.

Abstract

The concept of disease-specific chemotherapy was developed a century ago. Dyes and arsenical compounds that displayed selectivity against trypanosomes were central to this work, and the drugs that emerged remain in use for treating human African trypanosomiasis (HAT). The importance of understanding the mechanisms underlying selective drug action and resistance for the development of improved HAT therapies has been recognized, but these mechanisms have remained largely unknown. Here we use all five current HAT drugs for genome-scale RNA interference target sequencing (RIT-seq) screens in Trypanosoma brucei, revealing the transporters, organelles, enzymes and metabolic pathways that function to facilitate antitrypanosomal drug action. RIT-seq profiling identifies both known drug importers and the only known pro-drug activator, and links more than fifty additional genes to drug action. A bloodstream stage-specific invariant surface glycoprotein (ISG75) family mediates suramin uptake, and the AP1 adaptin complex, lysosomal proteases and major lysosomal transmembrane protein, as well as spermidine and N-acetylglucosamine biosynthesis, all contribute to suramin action. Further screens link ubiquinone availability to nitro-drug action, plasma membrane P-type H(+)-ATPases to pentamidine action, and trypanothione and several putative kinases to melarsoprol action. We also demonstrate a major role for aquaglyceroporins in pentamidine and melarsoprol cross-resistance. These advances in our understanding of mechanisms of antitrypanosomal drug efficacy and resistance will aid the rational design of new therapies and help to combat drug resistance, and provide unprecedented molecular insight into the mode of action of antitrypanosomal drugs.

Cell Host Microbe. 2013 Jan 16;13(1):108-17. doi: 10.1016/j.chom. 2012.11.011. Epub 2013 Jan 16.

Host metabolism regulates intracellular growth of Trypanosoma cruzi.

Caradonna KL1, Engel JC, Jacobi D, Lee CH, Burleigh BA.

Author information

Abstract

Metabolic coupling of intracellular pathogens with host cells is essential for successful colonization of the host. Establishment of intracellular infection by the protozoan Trypanosoma cruzi leads to the development of human Chagas' disease, yet the functional contributions of the host cell toward the infection process remain poorly characterized. Here, a genome-scale functional screen identified interconnected metabolic networks centered around host energy production, nucleotide metabolism, pteridine biosynthesis,

and fatty acid oxidation as key processes that fuel intracellular T. cruzi growth. Additionally, the host kinase Akt, which plays essential roles in various cellular processes, was critical for parasite replication. Targeted perturbations in these host metabolic pathways or Akt-dependent signaling pathways modulated the parasite's replicative capacity, highlighting the adaptability of this intracellular pathogen to changing conditions in the host. These findings identify key cellular process regulating intracellular T. cruzi growth and illuminate the potential to leverage host pathways to limit T. cruzi infection.

Nature. 2013 Dec 12;504(7479):248-53. doi: 10.1038/nature12782. Epub 2013 Nov 27.

Targeting Plasmodium PI(4)K to eliminate malaria.

McNamara CW1, Lee MC2, Lim CS3, Lim SH3, Roland J4, Nagle A4, Simon O3, Yeung BK3, Chatterjee AK4, McCormack SL4, Manary MJ5, Zeeman AM6, Dechering KJ7, Kumar TR8, Henrich PP8, Gagaring K4, Ibanez M4, Kato N4, Kuhen KL4, Fischli C9, Rottmann M10, Plouffe DM4, Bursulaya B4, Meister S5, Rameh L11, Trappe J12, Haasen D12, Timmerman M7, Sauerwein RW13, Suwanarusk R14, Russell B15, Renia L14, Nosten F16, Tully DC4, Kocken CH6, Glynne RJ4, Bodenreider C3, Fidock DA17, Diagana TT3, Winzeler EA18.

Author information

Abstract

Achieving the goal of malaria elimination will depend on targeting Plasmodium pathways essential across all life stages. Here we identify a lipid kinase, phosphatidylinositol-4-OH kinase (PI(4)K), as the target of imidazopyrazines, a new antimalarial compound class that inhibits the intracellular development of multiple Plasmodium species at each stage of infection in the vertebrate host. Imidazopyrazines demonstrate potent preventive, therapeutic, and transmission-blocking activity in rodent malaria models, are active against blood-stage field isolates of the major human pathogens P. falciparum and P. vivax, and inhibit liver-stage hypnozoites in the simian parasite P. cynomolgi. We show that imidazopyrazines exert their effect through inhibitory interaction with the ATP-binding pocket of PI(4)K, altering the intracellular distribution of phosphatidylinositol-4-phosphate. Collectively, our data define PI(4)K as a key Plasmodium vulnerability, opening up new avenues of target-based discovery to identify drugs with an ideal activity profile for the prevention, treatment and

Science. 2010 Sep 3;329(5996):1175-80. doi: 10.1126/science.1193225.

Spiroindolones, a potent compound class for the treatment of malaria.

Rottmann M1, McNamara C, Yeung BK, Lee MC, Zou B, Russell B, Seitz P, Plouffe DM, Dharia NV, Tan J, Cohen SB, Spencer KR, González-Páez GE, Lakshminarayana SB, Goh A, Suwanarusk R, Jegla T, Schmitt EK, Beck HP,

Brun R, Nosten F, Renia L, Dartois V, Keller TH, Fidock DA, Winzeler EA, Diagana TT.

Author information

Abstract

Recent reports of increased tolerance to artemisinin derivatives--the most recently adopted class of antimalarials--have prompted a need for new treatments. The spirotetrahydro-beta-carbolines, or spiroindolones, are potent drugs that kill the blood stages of Plasmodium falciparum and Plasmodium vivax clinical isolates at low nanomolar concentration. Spiroindolones rapidly inhibit protein synthesis in P. falciparum, an effect that is ablated in parasites bearing nonsynonymous mutations in the gene encoding the P-type cation-transporter ATPase4 (PfATP4). The optimized spiroindolone NITD609 shows pharmacokinetic properties compatible with once-daily oral dosing and has

Drug resistance. Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance.

Mok S1, Ashley EA2, Ferreira PE1, Zhu L1, Lin Z1, Yeo T1, Chotivanich K3, Imwong M4, Pukrittayakamee S3, Dhorda M5, Nguon C6, Lim P7, Amaratunga C8, Suon S6, Hien TT9, Htut Y10, Faiz MA11, Onyamboko MA12, Mayxay M13, Newton PN14, Tripura R15, Woodrow CJ2, Miotto O16, Kwiatkowski DP17, Nosten F18, Day NP2, Preiser PR1, White NJ2, Dondorp AM2, Fairhurst RM8, Bozdech Z19.

Author information

Abstract

Artemisinin resistance in Plasmodium falciparum threatens global efforts to control and eliminate malaria. Polymorphisms in the kelch domain-carrying protein K13 are associated with artemisinin resistance, but the underlying molecular mechanisms are unknown. We analyzed the in vivo transcriptomes of 1043 P. falciparum isolates from patients with acute malaria and found that artemisinin resistance is associated with increased expression of unfolded protein response (UPR) pathways involving the major PROSC and TRiC chaperone complexes. Artemisinin-resistant parasites also exhibit decelerated progression through the first part of the asexual intraerythrocytic development cycle. These findings suggest that artemisinin-resistant parasites remain in a state of decelerated development at the young ring stage, whereas their up-regulated UPR pathways mitigate protein damage caused by artemisinin. The expression profiles of UPR-related genes also associate with the geographical origin of parasite isolates, further suggesting their role in emerging artemisinin resistance in the Greater Mekong Subregion.

Proc Natl Acad Sci U S A. 2012 Jul 3;109(27):10996-1001. doi: 10.1073/pnas.1202885109. Epub 2012 Jun 18.

Aquaglyceroporin 2 controls susceptibility to melarsoprol and pentamidine in African trypanosomes.

Baker N1, Glover L, Munday JC, Aguinaga Andrés D, Barrett MP, de Koning

HP, Horn D.
Author information

Abstract
African trypanosomes cause sleeping sickness in humans, a disease that is typically fatal without chemotherapy. Unfortunately, drug resistance is common and melarsoprol-resistant trypanosomes often display cross-resistance to pentamidine. Although melarsoprol/pentamidine cross-resistance (MPXR) has been an area of intense interest for several decades, our understanding of the underlying mechanisms remains incomplete. Recently, a locus encoding two closely related aquaglyceroporins, AQP2 and AQP3, was linked to MPXR in a high-throughput loss-of-function screen. Here, we show that AQP2 has an unconventional "selectivity filter." AQP2-specific gene knockout generated MPXR trypanosomes but did not affect resistance to a lipophilic arsenical, whereas recombinant AQP2 reversed MPXR in cells lacking native AQP2 and AQP3. AQP2 was also shown to be disrupted in a laboratory-selected MPXR strain. Both AQP2 and AQP3 gained access to the surface plasma membrane in insect life-cycle-stage trypanosomes but, remarkably, AQP2 was specifically restricted to the flagellar pocket in the bloodstream stage. We conclude that the unconventional aquaglyceroporin, AQP2, renders cells sensitive to both melarsoprol and pentamidine and that loss of AQP2 function could explain cases of innate and acquired MPXR.

Nat Biotechnol. 2014 Aug;32(8):819-21. doi: 10.1038/nbt.2925. Epub 2014 Jun 1.

Genome editing in the human malaria parasite Plasmodium falciparum using the CRISPR-Cas9 system.

Ghorbal M1, Gorman M2, Macpherson CR2, Martins RM2, Scherf A2, Lopez-Rubio JJ1.
Author information
Abstract
Genome manipulation in the malaria parasite Plasmodium falciparum remains largely intractable and improved genomic tools are needed to further understand pathogenesis and drug resistance. We demonstrated the CRISPR-Cas9 system for use in P. falciparum by disrupting chromosomal loci and generating marker-free, single-nucleotide substitutions with high efficiency. Additionally, an artemisinin-resistant strain was generated by introducing a previously implicated polymorphism, thus illustrating the value of efficient genome editing in malaria research.

PMID: 24880488 [PubMed - in process]

Nature. 2013 Jul 11;499(7457):223-7. doi: 10.1038/nature12361. Epub 2013 Jul 3.

PfSETvs methylation of histone H3K36 represses virulence genes in Plasmodium falciparum.

Jiang L1, Mu J, Zhang Q, Ni T, Srinivasan P, Rayavara K, Yang W, Turner L, Lavstsen T, Theander TG, Peng W, Wei G, Jing Q, Wakabayashi Y, Bansal A, Luo Y, Ribeiro JM, Scherf A, Aravind L, Zhu J, Zhao K, Miller LH.

Author information

Abstract

The variant antigen Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1), which is expressed on the surface of P. falciparum-infected red blood cells, is a critical virulence factor for malaria. Each parasite has 60 antigenically distinct var genes that each code for a different PfEMP1 protein. During infection the clonal parasite population expresses only one gene at a time before switching to the expression of a new variant antigen as an immune-evasion mechanism to avoid the host antibody response. The mechanism by which 59 of the 60 var genes are silenced remains largely unknown. Here we show that knocking out the P. falciparum variant-silencing SET gene (here termed PfSETvs), which encodes an orthologue of Drosophila melanogaster ASH1 and controls histone H3 lysine 36 trimethylation (H3K36me3) on var genes, results in the transcription of virtually all var genes in the single parasite nuclei and their expression as proteins on the surface of individual infected red blood cells. PfSETvs-dependent H3K36me3 is present along the entire gene body, including the transcription start site, to silence var genes. With low occupancy of PfSETvs at both the transcription start site of var genes and the intronic promoter, expression of var genes coincides with transcription of their corresponding antisense long noncoding RNA. These results uncover a previously unknown role of PfSETvs-dependent H3K36me3 in silencing var genes in P. falciparum that might provide a general mechanism by which orthologues of PfSETvs repress gene expression in other eukaryotes. PfSETvs knockout parasites expressing all PfEMP1 proteins may also be applied to the development of a malaria vaccine.

Nature. 2010 May 20;465(7296):359-62. doi: 10.1038/nature09022.

Calcium-dependent protein kinase 1 is an essential regulator of exocytosis in Toxoplasma.

Lourido S1, Shuman J, Zhang C, Shokat KM, Hui R, Sibley LD.

Author information

Abstract

Calcium-regulated exocytosis is a ubiquitous process in eukaryotes, whereby secretory vesicles fuse with the plasma membrane and release their contents in response to an intracellular calcium surge. This process regulates various cellular functions such as plasma membrane repair in plants and animals, the discharge of defensive spikes in Paramecium, and the secretion of insulin from pancreatic cells, immune modulators from lymphocytes, and chemical

transmitters from neurons. In animal cells, serine/threonine kinases including cAMP-dependent protein kinase, protein kinase C and calmodulin kinases have been implicated in calcium-signal transduction leading to regulated secretion. Although plants and protozoa also regulate secretion by means of intracellular calcium, the method by which these signals are relayed has not been explained. Here we show that the Toxoplasma gondii calcium-dependent protein kinase 1 (TgCDPK1) is an essential regulator of calcium-dependent exocytosis in this opportunistic human pathogen. Conditional suppression of TgCDPK1 revealed that it controls calcium-dependent secretion of specialized organelles called micronemes, resulting in a block of essential phenotypes including parasite motility, host-cell invasion, and egress. These phenotypes were recapitulated by using a chemical biology approach in which pyrazolopyrimidine-derived compounds specifically inhibited TgCDPK1 and disrupted the parasite's life cycle at stages dependent on microneme secretion. Inhibition was specific to TgCDPK1, because expression of a resistant mutant kinase reversed sensitivity to the inhibitor. TgCDPK1 is conserved among apicomplexans and belongs to a family of kinases shared with plants and ciliates, suggesting that related CDPKs may have a function in calcium-regulated secretion in other organisms. Because this kinase family is absent from mammalian hosts, it represents a validated target that may be exploitable for chemotherapy against T. gondii and related apicomplexans.

Nature. 2013 Sep 19;501(7467):430-4. doi: 10.1038/nature12516. Epub 2013 Aug 21.

Mechanism of Trypanosoma brucei gambiense resistance to human serum.

Uzureau P1, Uzureau S, Lecordier L, Fontaine F, Tebabi P, Homblé F, Grélard A, Zhendre V, Nolan DP, Lins L, Crowet JM, Pays A, Felu C, Poelvoorde P, Vanhollebeke B, Moestrup SK, Lyngsø J, Pedersen JS, Mottram JC, Dufourc EJ, Pérez-Morga D, Pays E.
Author information

Abstract

The African parasite Trypanosoma brucei gambiense accounts for 97% of human sleeping sickness cases. T. b. gambiense resists the specific human innate immunity acting against several other tsetse-fly-transmitted trypanosome species such as T. b. brucei, the causative agent of nagana disease in cattle. Human immunity to some African trypanosomes is due to two serum complexes designated trypanolytic factors (TLF-1 and -2), which both contain haptoglobin-related protein (HPR) and apolipoprotein LI (APOL1). Whereas HPR association with haemoglobin (Hb) allows TLF-1 binding and uptake via the trypanosome receptor TbHpHbR (ref. 5), TLF-2 enters trypanosomes independently of TbHpHbR (refs 4, 5). APOL1 kills trypanosomes after insertion into endosomal/lysosomal membranes. Here we report that T. b. gambiense resists TLFs via a hydrophobic β-sheet of the T. b. gambiense-specific glycoprotein (TgsGP), which prevents APOL1 toxicity and induces stiffening of membranes upon interaction with lipids. Two additional features contribute to resistance to TLFs: reduction of sensitivity to APOL1 requiring cysteine protease activity, and TbHpHbR inactivation due to a L210S substitution. According to such a multifactorial defence mechanism, transgenic expression of T. b. brucei TbHpHbR in T. b. gambiense did not cause parasite lysis in normal human serum. However, these transgenic parasites were killed in hypohaptoglobinaemic serum, after high TLF-1 uptake in the absence of haptoglobin (Hp) that competes for Hb and receptor binding. TbHpHbR inactivation preventing high APOL1 loading in hypohaptoglobinaemic serum may have evolved because of the overlapping endemic area of T. b. gambiense infection and malaria, the main cause of haemolysis-induced hypohaptoglobinaemia in western and central Africa.

Nature. 2011 Nov 9;480(7378):534-7. doi: 10.1038/nature10606.

Basigin is a receptor essential for erythrocyte invasion by Plasmodium falciparum.

Crosnier C1, Bustamante LY, Bartholdson SJ, Bei AK, Theron M, Uchikawa M, Mboup S, Ndir O, Kwiatkowski DP, Duraisingh MT, Rayner JC, Wright GJ.
Author information

Abstract

Erythrocyte invasion by Plasmodium falciparum is central to the pathogenesis of malaria. Invasion requires a series of extracellular recognition events

between erythrocyte receptors and ligands on the merozoite, the invasive form of the parasite. None of the few known receptor-ligand interactions involved are required in all parasite strains, indicating that the parasite is able to access multiple redundant invasion pathways. Here, we show that we have identified a receptor-ligand pair that is essential for erythrocyte invasion in all tested P. falciparum strains. By systematically screening a library of erythrocyte proteins, we have found that the Ok blood group antigen, basigin, is a receptor for PfRh5, a parasite ligand that is essential for blood stage growth. Erythrocyte invasion was potently inhibited by soluble basigin or by basigin knockdown, and invasion could be completely blocked using low concentrations of anti-basigin antibodies; importantly, these effects were observed across all laboratory-adapted and field strains tested. Furthermore, Ok(a-) erythrocytes, which express a basigin variant that has a weaker binding affinity for PfRh5, had reduced invasion efficiencies. Our discovery of a cross-strain dependency on a single extracellular receptor-ligand pair for erythrocyte invasion by P. falciparum provides a focus for new anti-malarial therapies.

Science. 2010 May 14;328(5980):910-2. doi: 10.1126/science.1188191.

A plant-like kinase in Plasmodium falciparum regulates parasite egress from erythrocytes.

Dvorin JD1, Martyn DC, Patel SD, Grimley JS, Collins CR, Hopp CS, Bright AT, Westenberger S, Winzeler E, Blackman MJ, Baker DA, Wandless TJ, Duraisingh MT.

Author information

Abstract

Clinical malaria is associated with the proliferation of Plasmodium parasites in human erythrocytes. The coordinated processes of parasite egress from and invasion into erythrocytes are rapid and tightly regulated. We have found that the plant-like calcium-dependent protein kinase PfCDPK5, which is expressed in invasive merozoite forms of Plasmodium falciparum, was critical for egress. Parasites deficient in PfCDPK5 arrested as mature schizonts with intact membranes, despite normal maturation of egress proteases and invasion ligands. Merozoites physically released from stalled schizonts were capable of invading new erythrocytes, separating the pathways of egress and invasion. The arrest was downstream of cyclic guanosine monophosphate-dependent protein kinase (PfPKG) function and independent of protease processing. Thus, PfCDPK5 plays an essential role during the blood stage of malaria replication.

Nature. 2014 Mar 13;507(7491):253-7. doi: 10.1038/nature12970. Epub 2014 Feb 23.

A cascade of DNA-binding proteins for sexual commitment and development in Plasmodium.

Sinha A1, Hughes KR1, Modrzynska KK2, Otto TD3, Pfander C3, Dickens NJ4, Religa AA4, Bushell E3, Graham AL4, Cameron R4, Kafsack BF5, Williams AE6, Llinás M7, Berriman M3, Billker O3, Waters AP4.

Author information

Abstract

Commitment to and completion of sexual development are essential for malaria parasites (protists of the genus Plasmodium) to be transmitted through mosquitoes. The molecular mechanism(s) responsible for commitment have been hitherto unknown. Here we show that PbAP2-G, a conserved member of the apicomplexan AP2 (ApiAP2) family of DNA-binding proteins, is essential for the commitment of asexually replicating forms to sexual development in Plasmodium berghei, a malaria parasite of rodents. PbAP2-G was identified from mutations in its encoding gene, PBANKA_143750, which account for the loss of sexual development frequently observed in parasites transmitted artificially by blood passage. Systematic gene deletion of conserved ApiAP2 genes in Plasmodium confirmed the role of PbAP2-G and revealed a second ApiAP2 member (PBANKA_103430, here termed PbAP2-G2) that significantly modulates but does not abolish gametocytogenesis, indicating that a cascade of ApiAP2 proteins are involved in commitment to the production and maturation of gametocytes. The data suggest a mechanism of commitment to gametocytogenesis in Plasmodium consistent with a positive feedback loop involving PbAP2-G that could be exploited to prevent the transmission of this pernicious parasite.

Nature. 2008 Dec 11;456(7223):750-4. doi: 10.1038/nature07585.

Antigenic variation in Giardia lamblia is regulated by RNA interference.

Prucca CG1, Slavin I, Quiroga R, Elías EV, Rivero FD, Saura A, Carranza PG, Luján HD.

Author information

Abstract

Giardia lamblia (also called Giardia intestinalis) is one of the most common intestinal parasites of humans. To evade the host's immune response, Giardia undergoes antigenic variation-a process that allows the parasite to develop chronic and recurrent infections. From a repertoire of approximately 190 variant-specific surface protein (VSP)-coding genes, Giardia expresses only one VSP on the surface of each parasite at a particular time, but spontaneously switches to a different VSP by unknown mechanisms. Here we show that regulation of VSP expression involves a system comprising RNA-dependent RNA polymerase, Dicer and Argonaute, known components of the RNA interference machinery. Clones expressing a single surface antigen efficiently transcribe several VSP genes but only accumulate transcripts encoding the VSP to be expressed. Detection of antisense RNAs corresponding to the silenced VSP genes and small RNAs from the silenced but not for the expressed vsp implicate the RNA interference pathway in

antigenic variation. Remarkably, silencing of Dicer and RNA-dependent RNA polymerase leads to a change from single to multiple VSP expression in individual parasites.

Science. 2009 Jan 23;323(5913):530-3. doi: 10.1126/science.1165740. Epub 2008 Dec 18.

Rapid membrane disruption by a perforin-like protein facilitates parasite exit from host cells.

Kafsack BF1, Pena JD, Coppens I, Ravindran S, Boothroyd JC, Carruthers VB.

Author information

Abstract

Perforin-like proteins are expressed by many bacterial and protozoan pathogens, yet little is known about their function or mode of action. Here, we describe Toxoplasma perforin-like protein 1 (TgPLP1), a secreted perforin-like protein of the intracellular protozoan pathogen Toxoplasma gondii that displays structural features necessary for pore formation. After intracellular growth, TgPLP1-deficient parasites failed to exit normally, resulting in entrapment within host cells. We show that this defect is due to an inability to rapidly permeabilize the parasitophorous vacuole membrane and host plasma membrane during exit. TgPLP1 ablation had little effect on growth in culture but resulted in a reduction greater than five orders of magnitude of acute virulence in mice. Perforin-like proteins from other intracellular pathogens may play a similar role in microbial egress and virulence.

Science. 2011 Feb 11;331(6018):775-8. doi: 10.1126/science.1199326.

Leishmania RNA virus controls the severity of mucocutaneous leishmaniasis.

Ives A1, Ronet C, Prevel F, Ruzzante G, Fuertes-Marraco S, Schutz F, Zangger H, Revaz-Breton M, Lye LF, Hickerson SM, Beverley SM, Acha-Orbea H, Launois P, Fasel N, Masina S.

Author information

Abstract

Mucocutaneous leishmaniasis is caused by infections with intracellular parasites of the Leishmania Viannia subgenus, including Leishmania guyanensis. The pathology develops after parasite dissemination to nasopharyngeal tissues, where destructive metastatic lesions form with chronic inflammation. Currently, the mechanisms involved in lesion development are poorly understood. Here we show that metastasizing parasites have a high Leishmania RNA virus-1 (LRV1) burden that is recognized by the host Toll-like receptor 3 (TLR3) to induce proinflammatory cytokines and chemokines. Paradoxically, these TLR3-mediated immune

responses rendered mice more susceptible to infection, and the animals developed an increased footpad swelling and parasitemia. Thus, LRV1 in the metastasizing parasites subverted the host immune response to Leishmania and promoted parasite persistence.

PMID: 21311023 [PubMed - indexed for MEDLINE] PMCID: PMC3253482

Cell Microbiol. 2014 Sep;16(9):1285-300. doi: 10.1111/cmi.12297. Epub 2014 May 1.

Bioluminescence imaging of chronic Trypanosoma cruzi infections reveals tissue-specific parasite dynamics and heart disease in the absence of locally persistent infection.

Lewis MD1, Fortes Francisco A, Taylor MC, Burrell-Saward H, McLatchie AP, Miles MA, Kelly JM.
Author information

Abstract
Chronic Trypanosoma cruzi infections lead to cardiomyopathy in 20-30% of cases. A causal link between cardiac infection and pathology has been difficult to establish because of a lack of robust methods to detect scarce, focally distributed parasites within tissues. We developed a highly sensitive bioluminescence imaging system based on T. cruzi expressing a novel luciferase that emits tissue-penetrating orange-red light. This enabled long-term serial evaluation of parasite burdens in individual mice with an in vivo limit of detection of significantly less than 1000 parasites. Parasite distributions during chronic infections were highly focal and spatiotemporally dynamic, but did not localize to the heart. End-point ex vivo bioluminescence imaging allowed tissue-specific quantification of parasite loads with minimal sampling bias. During chronic infections, the gastro-intestinal tract, specifically the colon and stomach, was the only site where T. cruzi infection was consistently observed. Quantitative PCR-inferred parasite loads correlated with ex vivo bioluminescence and confirmed the gut as the parasite reservoir. Chronically infected mice developed myocarditis and cardiac fibrosis, despite the absence of locally persistent parasites. These data identify the gut as a permissive niche for long-term T. cruzi infection and show that canonical features of Chagas disease can occur without continual myocardium-specific infection.

PMID: 24712539 [PubMed - in process] PMCID: PMC4190689

Cell. 2014 Mar 13;156(6):1247-58. doi: 10.1016/j.cell.2014.01.049. Epub 2014 Feb 27.

Discovery of unconventional kinetochores in kinetoplastids.

Akiyoshi B1, Gull K2.
Author information

Abstract
The kinetochore is the macromolecular protein complex that directs chromosome segregation in eukaryotes. It has been widely assumed that the core kinetochore consists of proteins that are common to all eukaryotes. However, no conventional kinetochore components have been identified in any kinetoplastid genome, thus challenging this assumption of universality. Here, we report the identification of 19 kinetochore proteins (KKT1-19) in Trypanosoma brucei. The majority is conserved among kinetoplastids, but none of them has detectable homology to conventional kinetochore proteins. These proteins instead have a variety of features not found in conventional kinetochore proteins. We propose that kinetoplastids build kinetochores using a distinct set of proteins. These findings provide important insights into the longstanding problem of the position of the root of the eukaryotic tree of life.

Comment in
Cell division: the prehistorichore? [Curr Biol. 2014]
PMID: 24582333 [PubMed - indexed for MEDLINE] PMCID: PMC3978658

Science. 2010 Aug 13;329(5993):841-5. doi: 10.1126/science.1193032. Epub 2010 Jul 15.

Association of trypanolytic ApoL1 variants with kidney disease in African Americans.

Genovese G1, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, Bowden DW, Langefeld CD, Oleksyk TK, Uscinski Knob AL, Bernhardy AJ, Hicks PJ, Nelson GW, Vanhollebeke B, Winkler CA, Kopp JB, Pays E, Pollak MR.
Author information

Abstract
African Americans have higher rates of kidney disease than European Americans. Here, we show that, in African Americans, focal segmental glomerulosclerosis (FSGS) and hypertension-attributed end-stage kidney disease (H-ESKD) are associated with two independent sequence variants in the APOL1 gene on chromosome 22 {FSGS odds ratio = 10.5 [95% confidence interval (CI) 6.0 to 18.4]; H-ESKD odds ratio = 7.3 (95% CI 5.6 to 9.5)}. The two APOL1 variants are common in African chromosomes but absent from European chromosomes, and both reside within haplotypes that harbor signatures of positive selection. ApoL1 (apolipoprotein L-1) is a serum factor that lyses trypanosomes. In vitro assays revealed that only the kidney disease-associated ApoL1 variants lysed Trypanosoma brucei rhodesiense. We speculate that evolution of a critical survival factor in Africa may have contributed to the high rates of renal disease in African Americans.

Comment in

Variation in APOL1 gene may contribute to high rates of kidney disease in African Americans. [Circ Cardiovasc Genet. 2011]

Genetics: Kidney disease susceptibility may be drawback of parasite resistance in African Americans. [Nat Rev Nephrol. 2010]

PMID: 20647424 [PubMed - indexed for MEDLINE] PMCID: PMC2980843

Proc Natl Acad Sci U S A. 2009 Nov 17;106(46):19509-14. doi: 10.1073/pnas.0905669106. Epub 2009 Oct 26.

Hydrodynamic gene delivery of baboon trypanosome lytic factor eliminates both animal and human-infective African trypanosomes.

Thomson R1, Molina-Portela P, Mott H, Carrington M, Raper J.
Author information

Abstract
Several species of African trypanosomes cause fatal disease in livestock, but most cannot infect humans due to innate trypanosome lytic factors (TLFs). Human TLFs are pore forming high-density lipoprotein (HDL) particles that contain apolipoprotein L-I (apoL-I) the trypanolytic component, and haptoglobin-related protein (Hpr), which binds free hemoglobin (Hb) in blood and facilitates the uptake of TLF via a trypanosome haptoglobin-hemoglobin receptor. The human-infective Trypanosoma brucei rhodesiense escapes lysis by TLF by expression of serum resistance-associated (SRA) protein, which binds and neutralizes apoL-I. Unlike humans, baboons are not susceptible to infection by T. b. rhodesiense due to previously unidentified serum factors. Here, we show that baboons have a TLF complex that contains orthologs of Hpr and apoL-I and that full-length baboon apoL-I confers trypanolytic activity to mice and when expressed together with baboon Hpr and human apoA-I, provides protection against both animal infective and the human-infective T. brucei rhodesiense in vivo. We further define two critical lysines near the C terminus of baboon apoL-1 that are necessary and sufficient to prevent binding to SRA and thereby confer resistance to human-infective trypanosomes. These findings form the basis for the creation of TLF transgenic livestock that would be resistant to animal and human-infective trypanosomes, which would result in the reduction of disease and the zoonotic transmission of human infective trypanosomes.

PMID: 19858474 [PubMed - indexed for MEDLINE] PMCID: PMC2780755

Nature. 2014 Jan 30;505(7485):681-5. doi: 10.1038/nature12864. Epub 2013 Dec 15.

Genome-wide dissection of the quorum sensing signalling pathway in Trypanosoma brucei.

Mony BM1, MacGregor P1, Ivens A2, Rojas F2, Cowton A2, Young J2, Horn D3, Matthews K2.
Author information

Abstract
The protozoan parasites Trypanosoma brucei spp. cause important human and livestock diseases in sub-Saharan Africa. In mammalian blood, two developmental forms of the parasite exist: proliferative 'slender' forms and arrested 'stumpy' forms that are responsible for transmission to tsetse flies. The slender to stumpy differentiation is a density-dependent response that resembles quorum sensing in microbial systems and is crucial for the parasite life cycle, ensuring both infection chronicity and disease transmission. This response is triggered by an elusive 'stumpy induction factor' (SIF) whose intracellular signalling pathway is also uncharacterized. Laboratory-adapted (monomorphic) trypanosome strains respond inefficiently to SIF but can generate forms with stumpy characteristics when exposed to cell-permeable cAMP and AMP analogues. Exploiting this, we have used a genome-wide RNA interference library screen to identify the signalling components driving stumpy formation. In separate screens, monomorphic parasites were exposed to 8-(4-chlorophenylthio)-cAMP (pCPT-cAMP) or 8-pCPT-2'-O-methyl-5'-AMP to select cells that were unresponsive to these signals and hence remained proliferative. Genome-wide Ion Torrent based RNAi target sequencing identified cohorts of genes implicated in each step of the signalling pathway, from purine metabolism, through signal transducers (kinases, phosphatases) to gene expression regulators. Genes at each step were independently validated in cells naturally capable of stumpy formation, confirming their role in density sensing in vivo. The putative RNA-binding protein, RBP7, was required for normal quorum sensing and promoted cell-cycle arrest and transmission competence when overexpressed. This study reveals that quorum sensing signalling in trypanosomes shares similarities to fundamental quiescence pathways in eukaryotic cells, its components providing targets for quorum-sensing interference-based therapeutics.

PMID: 24336212 [PubMed - indexed for MEDLINE] PMCID: PMC3908871

Genome Res. 2011 Jun;21(6):915-24. doi: 10.1101/gr.115089.110. Epub 2011 Mar 1.

High-throughput phenotyping using parallel sequencing of RNA interference targets in the African trypanosome.

Alsford S1, Turner DJ, Obado SO, Sanchez-Flores A, Glover L, Berriman M, Hertz-Fowler C, Horn D.
Author information

Abstract

African trypanosomes are major pathogens of humans and livestock and represent a model for studies of unusual protozoal biology. We describe a high-throughput phenotyping approach termed RNA interference (RNAi) target sequencing, or RIT-seq that, using Illumina sequencing, maps fitness-costs associated with RNAi. We scored the abundance of >90,000 integrated RNAi targets recovered from trypanosome libraries before and after induction of RNAi. Data are presented for 7435 protein coding sequences, >99% of a non-redundant set in the Trypanosoma brucei genome. Analysis of bloodstream and insect life-cycle stages and differentiated libraries revealed genome-scale knockdown profiles of growth and development, linking thousands of previously uncharacterized and "hypothetical" genes to essential functions. Genes underlying prominent features of trypanosome biology are highlighted, including the constitutive emphasis on post-transcriptional gene expression control, the importance of flagellar motility and glycolysis in the bloodstream, and of carboxylic acid metabolism and phosphorylation during differentiation from the bloodstream to the insect stage. The current data set also provides much needed genetic validation to identify new drug targets. RIT-seq represents a versatile new tool for genome-scale functional analyses and for the exploitation of genome sequence data.

Comment in

Understanding sleeping sickness. [Nat Methods. 2011]
PMID: 21363968 [PubMed - indexed for MEDLINE] PMCID: PMC3106324

Nature. 2001 Dec 13;414(6865):759-63.

A pol I transcriptional body associated with VSG mono-allelic expression in Trypanosoma brucei.

Navarro M1, Gull K.
Author information

Abstract
In the mammalian host, African trypanosomes generate consecutive waves of parasitaemia by changing their antigenic coat. Because this coat consists of a single type of variant surface glycoprotein (VSG), the question arises of how a trypanosome accomplishes the transcription of only one of a multi-allelic family of VSG expression site loci to display a single VSG type on the surface at any one time. No major differences have been detected between the single active expression site and the cohort of inactive expression sites. Here we identify an extranucleolar body containing RNA polymerase I (pol I) that is transcriptionally active and present only in the bloodstream form of the parasite. Visualization of the active expression site locus by tagging with green fluorescent protein shows that it is specifically located at this unique pol I transcriptional factory. The presence of this transcriptional body in postmitotic nuclei and its stability in the nucleus after DNA digestion provide evidence for a coherent structure. We propose that the recruitment of a single expression site and the concomitant exclusion of inactive loci from a discrete transcriptional body define the mechanism responsible for VSG mono-allelic expression.

PMID: 11742402 [PubMed - indexed for MEDLINE]

Proc Natl Acad Sci U S A. 2013 Dec 3;110(49):19932-7. doi: 10.1073/pnas.1311535110. Epub 2013 Oct 28.

Chronic exposure to arsenic in drinking water can lead to resistance to antimonial drugs in a mouse model of visceral leishmaniasis.

Perry MR1, Wyllie S, Raab A, Feldmann J, Fairlamb AH.
Author information

Abstract
The Indian subcontinent is the only region where arsenic contamination of drinking water coexists with widespread resistance to antimonial drugs that are used to treat the parasitic disease visceral leishmaniasis. We have previously proposed that selection for parasite resistance within visceral leishmaniasis patients who have been exposed to trivalent arsenic results in cross-resistance to the related metalloid antimony, present in the pentavalent state as a complex in drugs such as sodium stibogluconate (Pentostam) and meglumine antimonate (Glucantime). To test this hypothesis, Leishmania donovani was serially passaged in mice exposed to arsenic in drinking water at environmentally relevant levels (10 or 100 ppm). Arsenic accumulation in organs and other tissues was proportional to the level of exposure and similar to that previously reported in human liver biopsies. After five monthly passages in mice exposed to arsenic, isolated parasites were found to be completely refractory to 500 μg · mL(-1) Pentostam compared with the control passage group (38.5 μg · mL(-1)) cultured in vitro in mouse peritoneal macrophages. Reassessment of resistant parasites following further passage for 4 mo in mice without arsenic exposure showed that resistance was stable. Treatment of infected mice with Pentostam confirmed that resistance observed in vitro also occurred in vivo. We conclude that arsenic contamination may have played a significant role in the development of Leishmania antimonial resistance in Bihar because inadequate treatment with antimonial drugs is not exclusive to India, whereas widespread antimonial resistance is.

KEYWORDS:
drug resistance; environmental pollution; sodium antimony gluconate; treatment failure

Comment in

Chronic arsenic exposure and microbial drug resistance. [Proc Natl Acad Sci U S A. 2013]

PMID: 24167266 [PubMed - indexed for MEDLINE] PMCID: PMC3856816

Sci Transl Med. 2012 Feb 1;4(119):119re1. doi: 10.1126/scitranslmed.3003326.

The anti-trypanosome drug fexinidazole shows potential for treating visceral leishmaniasis.

Wyllie S1, Patterson S, Stojanovski L, Simeons FR, Norval S, Kime R, Read KD, Fairlamb AH.
Author information

Abstract
Safer and more effective oral drugs are required to treat visceral leishmaniasis, a parasitic disease that kills 50,000 to 60,000 people each year in parts of Asia, Africa, and Latin America. Here, we report that fexinidazole, a drug currently in phase 1 clinical trials for treating African trypanosomiasis, shows promise for treating visceral leishmaniasis. This 2-substituted 5-nitroimidazole drug is rapidly oxidized in vivo in mice, dogs, and humans to sulfoxide and sulfone metabolites. Both metabolites of fexinidazole were active against Leishmania donovani amastigotes grown in macrophages, whereas the parent compound was inactive. Pharmacokinetic studies with fexinidazole (200 mg/kg) showed that fexinidazole sulfone achieves blood concentrations in mice above the EC(99) (effective concentration inhibiting growth by 99%) value for at least 24 hours after a single oral dose. A once-daily regimen for 5 days at this dose resulted in a 98.4% suppression of infection in a mouse model of visceral leishmaniasis, equivalent to that seen with the drugs miltefosine and Pentostam, which are currently used clinically to treat this tropical disease. In African trypanosomes, the mode of action of nitro drugs involves reductive activation via a NADH (reduced form of nicotinamide adenine dinucleotide)-dependent bacterial-like nitroreductase. Overexpression of the leishmanial homolog of this nitroreductase in L. donovani increased sensitivity to fexinidazole by 19-fold, indicating that a similar mechanism is involved in both parasites. These findings illustrate the potential of fexinidazole as an oral drug therapy for treating visceral leishmaniasis.

PMID: 22301556 [PubMed - indexed for MEDLINE] PMCID: PMC3457684

PLoS Negl Trop Dis. 2014 Jul 17;8(7):e2976. doi: 10.1371/journal.pntd.0002976. eCollection 2014.

Identification of sVSG117 as an immunodiagnostic antigen and evaluation of a dual-antigen lateral flow test for the diagnosis of human African trypanosomiasis.

Sullivan L1, Fleming J1, Sastry L1, Mehlert A1, Wall SJ2, Ferguson MA1.
Author information

Abstract
BACKGROUND:
The diagnosis of human African trypanosomiasis (HAT) caused by Trypanosoma brucei gambiense relies mainly on the Card Agglutination Test for Trypanosomiasis (CATT). There is no immunodiagnostic for HAT caused by T. b. rhodesiense. Our principle aim was to develop a prototype lateral flow test that might be an improvement on CATT.

METHODOLOGY/PRINCIPLE FINDINGS:
Pools of infection and control sera were screened against four different soluble form variant surface glycoproteins (sVSGs) by ELISA and one, sVSG117, showed particularly strong immunoreactivity to pooled infection sera. Using individual sera, sVSG117 was shown to be able to discriminate between T. b. gambiense infection and control sera by both ELISA and lateral flow test. The sVSG117 antigen was subsequently used with a previously described recombinant diagnostic antigen, rISG65, to create a dual-antigen lateral flow test prototype. The latter was used blind in a virtual field trial of 431 randomized infection and control sera from the WHO HAT Specimen Biobank.

CONCLUSION/SIGNIFICANCE:
In the virtual field trial, using two positive antigen bands as the criterion for infection, the sVSG117 and rISG65 dual-antigen lateral flow test prototype showed a sensitivity of 97.3% (95% CI: 93.3 to 99.2) and a specificity of 83.3% (95% CI: 76.4 to 88.9) for the detection of T. b. gambiense infections. The device was not as good for detecting T. b. rhodesiense infections using two positive antigen bands as the criterion for infection, with a sensitivity of 58.9% (95% CI: 44.9 to 71.9) and specificity of 97.3% (95% CI: 90.7 to 99.7). However, using one or both positive antigen band(s) as the criterion for T. b. rhodesiense infection improved the sensitivity to 83.9% (95% CI: 71.7 to 92.4) with a specificity of 85.3% (95% CI: 75.3 to 92.4). These results encourage further development of the dual-antigen device for clinical use.

PMID: 25033401 [PubMed - in process] PMCID: PMC4102454

Nat Commun. 2013;4:2552. doi: 10.1038/ncomms3552.

Apical membrane antigen 1 mediates apicomplexan parasite attachment but is dispensable for host cell invasion.

Bargieri DY1, Andenmatten N, Lagal V, Thiberge S, Whitelaw JA, Tardieux I, Meissner M, Ménard R.
Author information

Abstract
Apicomplexan parasites invade host cells by forming a ring-like junction with the cell surface and actively sliding through the junction inside an intracellular vacuole. Apical membrane antigen 1 is conserved in apicomplexans and a long-standing malaria vaccine candidate. It is considered to have multiple important roles during host cell penetration, primarily in structuring the junction by interacting with the rhoptry neck 2 protein and transducing the force generated by the parasite motor during internalization. Here, we generate Plasmodium sporozoites and merozoites and Toxoplasma tachyzoites lacking apical membrane antigen 1, and find that the latter two are impaired in host cell attachment but the three display normal host cell penetration through the junction. Therefore, apical membrane antigen 1, rather than an essential invasin, is a dispensable adhesin of apicomplexan zoites. These genetic data have implications on the use of apical membrane antigen 1 or the apical membrane antigen 1-rhoptry neck 2 interaction as targets of intervention strategies against malaria or other diseases caused by apicomplexans.

PMID: 24108241 [PubMed - indexed for MEDLINE] PMCID: PMC3826631

PLoS Negl Trop Dis. 2015 Jan 8;9(1):e3404. doi: 10.1371/journal.pntd.0003404. eCollection 2015.

Genome and Phylogenetic Analyses of Trypanosoma evansi Reveal Extensive Similarity to T. brucei and Multiple Independent Origins for Dyskinetoplasty.

Carnes J1, Anupama A1, Balmer O2, Jackson A3, Lewis M4, Brown R1, Cestari I1, Desquesnes M5, Gendrin C1, Hertz-Fowler C6, Imamura H7, Ivens A8, Kořený L9, Lai DH10, MacLeod A11, McDermott SM1, Merritt C1, Monnerat S1, Moon W1, Myler P1, Phan I1, Ramasamy G1, Sivam D1, Lun ZR12, Lukeš J13, Stuart K14, Schnaufer A15.
Author information

Abstract
Two key biological features distinguish Trypanosoma evansi from the T. brucei group: independence from the tsetse fly as obligatory vector, and independence from the need for functional mitochondrial DNA (kinetoplast or

kDNA). In an effort to better understand the molecular causes and consequences of these differences, we sequenced the genome of an akinetoplastic T. evansi strain from China and compared it to the T. b. brucei reference strain. The annotated T. evansi genome shows extensive similarity to the reference, with 94.9% of the predicted T. b. brucei coding sequences (CDS) having an ortholog in T. evansi, and 94.6% of the non-repetitive orthologs having a nucleotide identity of 95% or greater. Interestingly, several procyclin-associated genes (PAGs) were disrupted or not found in this T. evansi strain, suggesting a selective loss of function in the absence of the insect life-cycle stage. Surprisingly, orthologous sequences were found in T. evansi for all 978 nuclear CDS predicted to represent the mitochondrial proteome in T. brucei, although a small number of these may have lost functionality. Consistent with previous results, the F1FO-ATP synthase γ subunit was found to have an A281 deletion, which is involved in generation of a mitochondrial membrane potential in the absence of kDNA. Candidates for CDS that are absent from the reference genome were identified in supplementary de novo assemblies of T. evansi reads. Phylogenetic analyses show that the sequenced strain belongs to a dominant group of clonal T. evansi strains with worldwide distribution that also includes isolates classified as T. equiperdum. At least three other types of T. evansi or T. equiperdum have emerged independently. Overall, the elucidation of the T. evansi genome sequence reveals extensive similarity of T. brucei and supports the contention that T. evansi should be classified as a subspecies of T. brucei.

Mol Microbiol. 2014 Dec 30. doi: 10.1111/mmi.12920.

Lineage-specific activities of a multipotent mitochondrion of trypanosomatid flagellates.

Škodová-Sveráková I1, Verner Z, Skalický T, Votýpka J, Horváth A, Lukeš J.
Author information

Abstract

Trypanosomatids are a very diverse group composed of monoxenous and dixenous parasites belonging to the excavate class Kinetoplastea. Here we studied the respiration of five monoxenous species (Blechomonas ayalai, Herpetomonas muscarum, H. samuelpessoai, Leptomonas pyrrhocoris and Sergeia podlipaevi) introduced into culture, each representing a novel yet globally distributed and/or species-rich clade, and compare them with well-studied flagellates Trypanosoma brucei, Phytomonas serpens, Crithidia fasciculata and Leishmania tarentolae. Differences in structure and activities of respiratory chain complexes, respiration and other biochemical parameters recorded under laboratory conditions reveal their substantial diversity, likely a reflection of different host environments. Phylogenetic relationships of the analysed trypanosomatids do not correlate with their biochemical parameters, with the differences within clades by far exceeding those among clades. As

the S. podlipaevi canonical respiratory chain complexes have very low activities, we believe that its mitochondrion is utilised for purposes other than oxidative phosphorylation. Hence, the single reticulated mitochondrion of diverse trypanosomatids seems to retain multipotency, with the capacity to activate its individual components based on the host environment.

MBio. 2014 Dec 30;6(1). pii: e02097-14. doi: 10.1128/mBio.02097-14.

CRISPR-Cas9-Mediated Single-Gene and Gene Family Disruption in Trypanosoma cruzi.

Peng D1, Kurup SP1, Yao PY1, Minning TA1, Tarleton RL2.
Author information

Abstract
Trypanosoma cruzi is a protozoan parasite of humans and animals, affecting 10 to 20 million people and innumerable animals, primarily in the Americas. Despite being the largest cause of infection-induced heart disease worldwide, even among the neglected tropical diseases (NTDs) T. cruzi is considered one of the least well understood and understudied. The genetic complexity of T. cruzi as well as the limited set of efficient techniques for genome engineering contribute significantly to the relative lack of progress in and understanding of this pathogen. Here, we adapted the CRISPR-Cas9 system for the genetic engineering of T. cruzi, demonstrating rapid and efficient knockout of multiple endogenous genes, including essential genes. We observed that in the absence of a template, repair of the Cas9-induced double-stranded breaks (DSBs) in T. cruzi occurs exclusively by microhomology-mediated end joining (MMEJ) with various-sized deletions. When a template for DNA repair is provided, DSB repair by homologous recombination is achieved at an efficiency several orders of magnitude higher than that in the absence of CRISPR-Cas9-induced DSBs. We also demonstrate the high multiplexing capacity of CRISPR-Cas9 in T. cruzi by knocking down expression of an enzyme gene family consisting of 65 members, resulting in a significant reduction of enzymatic product with no apparent off-target mutations. Lastly, we show that Cas9 can mediate disruption of its own coding sequence, rescuing a growth defect in stable Cas9-expressing parasites. These results establish a powerful new tool for the analysis of gene functions in T. cruzi, enabling the study of essential genes and their functions and analysis of the many large families of related genes that occupy a substantial portion of the T. cruzi genome.
IMPORTANCE:
Trypanosoma cruzi, the causative agent of human Chagas disease, is the leading worldwide cause of infectious myocarditis. Diagnostics for the infection are relatively poor, treatment options are limited and of variable effectiveness, and suitable vaccines are nonexistent. The T. cruzi genome is replete with genes of unknown function and greatly expanded gene families

with hundreds of members. The absence of facile genetic engineering tools, including RNA interference, for T. cruzi has prevented elucidation of gene and gene family function and the development of better infection prevention and control measures. In this study, we demonstrate that the CRISPR-Cas9 system is a versatile and powerful tool for genome manipulations in T. cruzi, bringing new opportunities for unraveling the functions of previously uncharacterized genes and how this human pathogen engages its large families of genes encoding surface proteins to interact with human and animal hosts.

PLoS Negl Trop Dis. 2014 Dec 18;8(12):e3373. doi: 10.1371/journal.pntd.0003373. eCollection 2014.

Evaluation of the diagnostic accuracy of prototype rapid tests for human african trypanosomiasis.

Sternberg JM1, Gierliński M2, Biéler S3, Ferguson MA2, Ndung'u JM3.
Author information

Abstract
BACKGROUND:
Diagnosis of human African trypanosomiasis (HAT) remains a challenge both for active screening, which is critical in control of the disease, and in the point-of-care scenario where early and accurate diagnosis is essential. Recently, the first field deployment of a lateral flow rapid diagnostic test (RDT) for HAT, "SD BIOLINE HAT" has taken place. In this study, we evaluated the performance of "SD BIOLINE HAT" and two new prototype RDTs.
METHODOLOGY/PRINCIPAL FINDINGS:
The performance of "SD BIOLINE HAT" and 2 prototype RDTs was tested using archived plasma from 250 Trypanosoma brucei gambiense patients, and 250 endemic controls. As well as comparison of the sensitivity and specificity of each device, the performance of individual antigens was assessed and the hypothetical performance of novel antigen combinations extrapolated. Neither of the prototype devices were inferior in sensitivity or specificity to "SD BIOLINE HAT" (sensitivity 0.82±0.01, specificity 0.97±0.01, 95% CI) at the 5% margins, while one of the devices (BBI) had significantly superior sensitivity (0.88±0.03). Analysis of the performance of individual antigens was used to model new antigen combinations to be explored in development of the next generation of HAT RDTs. The modelling showed that an RDT using two recombinant antigens (rLiTat1.5 and rISG65) would give a performance similar to the best devices in this study, and would also offer the most robust performance under deteriorating field conditions.
CONCLUSIONS/SIGNIFICANCE:
Both "SD BIOLINE HAT" and the prototype devices performed comparably well to one another and also to the published performance range of the card agglutination test for trypanosomiasis in sensitivity and specificity. The

performance of individual antigens enabled us to predict that an all-recombinant antigen RDT can be developed with an accuracy equivalent to " SD BIOLINE HAT." Such an RDT would have advantages in simplified manufacture, lower unit cost and assured reproducibility.

PLoS Pathog. 2014 Dec 11;10(12):e1004555. doi: 10.1371/journal.ppat.1004555. eCollection 2014.

Proteomic analysis of the acidocalcisome, an organelle conserved from bacteria to human cells.

Huang G1, Ulrich PN2, Storey M1, Johnson D3, Tischer J1, Tovar JA2, Moreno SN1, Orlando R3, Docampo R1.
Author information

Abstract
Acidocalcisomes are acidic organelles present in a diverse range of organisms from bacteria to human cells. In this study acidocalcisomes were purified from the model organism Trypanosoma brucei, and their protein composition was determined by mass spectrometry. The results, along with those that we previously reported, show that acidocalcisomes are rich in pumps and transporters, involved in phosphate and cation homeostasis, and calcium signaling. We validated the acidocalcisome localization of seven new, putative, acidocalcisome proteins (phosphate transporter, vacuolar H+-ATPase subunits a and d, vacuolar iron transporter, zinc transporter, polyamine transporter, and acid phosphatase), confirmed the presence of six previously characterized acidocalcisome proteins, and validated the localization of five novel proteins to different subcellular compartments by expressing them fused to epitope tags in their endogenous loci or by immunofluorescence microscopy with specific antibodies. Knockdown of several newly identified acidocalcisome proteins by RNA interference (RNAi) revealed that they are essential for the survival of the parasites. These results provide a comprehensive insight into the unique composition of acidocalcisomes of T. brucei, an important eukaryotic pathogen, and direct

evidence that acidocalcisomes are especially adapted for the accumulation of polyphosphate.

Elife. 2014 Dec 12;4. doi: 10.7554/eLife.05553.

Structural basis for ligand and innate immunity factor uptake by the trypanosome haptoglobin-haemoglobin receptor.

Lane-Serff H1, MacGregor P2, Lowe ED1, Carrington M2, Higgins MK1.
Author information

Abstract
The haptoglobin-haemoglobin receptor (HpHbR) of African trypanosomes allows acquisition of haem and provides an uptake route for trypanolytic factor-1, a mediator of innate immunity against trypanosome infection. In this study, we report the structure of Trypanosoma brucei HpHbR in complex with human haptoglobin-haemoglobin (HpHb), revealing an elongated ligand-binding site that extends along its membrane distal half. This contacts haptoglobin and the β-subunit of haemoglobin, showing how the receptor selectively binds HpHb over individual components. Lateral mobility of the glycosylphosphatidylinositol-anchored HpHbR, and a ~50(o) kink in the receptor, allows two receptors to simultaneously bind one HpHb dimer. Indeed, trypanosomes take up dimeric HpHb at significantly lower concentrations than monomeric HpHb, due to increased ligand avidity that comes from bivalent binding. The structure therefore reveals the molecular basis for ligand and innate immunity factor uptake by trypanosomes and identifies adaptations that allow efficient ligand uptake in the context of the complex trypanosome cell surface.

PLoS Pathog. 2014 Dec 4;10(12):e1004545. doi: 10.1371/journal.ppat.1004545. eCollection 2014.

SUMOylation by the E3 ligase TbSIZ1/PIAS1 positively regulates VSG expression in Trypanosoma brucei.

López-Farfán D1, Bart JM2, Rojas-Barros DI1, Navarro M1.
Author information

Abstract
Bloodstream form trypanosomes avoid the host immune response by switching the expression of their surface proteins between Variant Surface Glycoproteins (VSG), only one of which is expressed at any given time. Monoallelic transcription of the telomeric VSG Expression Site (ES) by RNA

polymerase I (RNA pol I) localizes to a unique nuclear body named the ESB. Most work has focused on silencing mechanisms of inactive VSG-ESs, but the mechanisms involved in transcriptional activation of a single VSG-ES remain largely unknown. Here, we identify a highly SUMOylated focus (HSF) in the nucleus of the bloodstream form that partially colocalizes with the ESB and the active VSG-ES locus. SUMOylation of chromatin-associated proteins was enriched along the active VSG-ES transcriptional unit, in contrast to silent VSG-ES or rDNA, suggesting that it is a distinct feature of VSG-ES monoallelic expression. In addition, sequences upstream of the active VSG-ES promoter were highly enriched in SUMOylated proteins. We identified TbSIZ1/PIAS1 as the SUMO E3 ligase responsible for SUMOylation in the active VSG-ES chromatin. Reduction of SUMO-conjugated proteins by TbSIZ1 knockdown decreased the recruitment of RNA pol I to the VSG-ES and the VSG-ES-derived transcripts. Furthermore, cells depleted of SUMO conjugated proteins by TbUBC9 and TbSUMO knockdown confirmed the positive function of SUMO for VSG-ES expression. In addition, the largest subunit of RNA pol I TbRPA1 was SUMOylated in a TbSIZ-dependent manner. Our results show a positive mechanism associated with active VSG-ES expression via post-translational modification, and indicate that chromatin SUMOylation plays an important role in the regulation of VSG-ES. Thus, protein SUMOylation is linked to active gene expression in this protozoan parasite that diverged early in evolution.

J Cell Sci. 2015 Jan 1;128(1):27-32. doi: 10.1242/jcs.150573. Epub 2014 Nov 6.

The exocyst is required for trypanosome invasion and the repair of mechanical plasma membrane wounds.

Fernandes MC1, Corrotte M1, Miguel DC1, Tam C1, Andrews NW2.
Author information

Abstract
The process of host cell invasion by Trypanosoma cruzi shares mechanistic elements with plasma membrane injury and repair. Both processes require Ca(2+)-triggered exocytosis of lysosomes, exocytosis of acid sphingomyelinase and formation of ceramide-enriched endocytic compartments. T. cruzi invades at peripheral sites, suggesting a need for spatial regulation of membrane traffic. Here, we show that Exo70 and Sec8 (also known as EXOC7 and EXOC4, respectively), components of the exocyst complex, accumulate in nascent T. cruzi vacuoles and at sites of mechanical wounding. Exo70 or Sec8 depletion inhibits T. cruzi invasion and Ca(2+)-dependent resealing of mechanical wounds, but does not affect the repair of smaller lesions caused by pore-forming toxins. Thus, T. cruzi invasion and mechanical lesion repair share a unique requirement for the exocyst, consistent with a dependence on targeted membrane delivery.

PLoS Pathog. 2014 Oct 30;10(10):e1004493. doi: 10.1371/journal.ppat.1004493. eCollection 2014.

Social motility of African trypanosomes is a property of a distinct life-cycle stage that occurs early in tsetse fly transmission.

Imhof S1, Knüsel S1, Gunasekera K2, Vu XL2, Roditi I2.
Author information

Abstract

The protozoan pathogen Trypanosoma brucei is transmitted between mammals by tsetse flies. The first compartment colonised by trypanosomes after a blood meal is the fly midgut lumen. Trypanosomes present in the lumen-designated as early procyclic forms-express the stage-specific surface glycoproteins EP and GPEET procyclin. When the trypanosomes establish a mature infection and colonise the ectoperitrophic space, GPEET is down-regulated, and EP becomes the major surface protein of late procyclic forms. A few years ago, it was discovered that procyclic form trypanosomes exhibit social motility (SoMo) when inoculated on a semi-solid surface. We demonstrate that SoMo is a feature of early procyclic forms, and that late procyclic forms are invariably SoMo-negative. In addition, we show that, apart from GPEET, other markers are differentially expressed in these two life-cycle stages, both in culture and in tsetse flies, indicating that they have different biological properties and should be considered distinct stages of the life cycle. Differentially expressed genes include two closely related adenylate cyclases, both hexokinases and calflagins. These findings link the phenomenon of SoMo in vitro to the parasite forms found during the first 4-7 days of a midgut infection. We postulate that ordered group movement on plates reflects the migration of parasites from the midgut lumen into the ectoperitrophic space within the tsetse fly. Moreover, the process can be uncoupled from colonisation of the salivary glands. Although they are the major surface proteins of procyclic forms, EP and GPEET are not essential for SoMo, nor, as shown previously, are they required for near normal colonisation of the fly midgut.

Cell Host Microbe. 2014 Oct 8;16(4):439-49. doi: 10.1016/j.chom.2014.09.003.

The Trypanosoma cruzi flagellum is discarded via asymmetric cell division following invasion and provides early targets for protective $CD8^+$ T cells.

Kurup SP1, Tarleton RL2.
Author information

Abstract

During invasion of host cells by Trypanosoma cruzi, the parasite that causes Chagas disease, the elongated, flagellated trypomastigotes remodel into oval amastigotes with no external flagellum. The underlying mechanism of this remodeling and the fate of the flagellum are obscure. We discovered that T. cruzi trypomastigotes discard their flagella via an asymmetric cellular division. The flagellar proteins liberated become among the earliest parasite proteins to enter the MHC-I processing pathway in infected cells. Indeed, paraflagellar rod protein PAR4-specific CD8(+) T cells detect infected host cells >20 hr earlier than immunodominant trans-sialidase-specific T cells. Overexpression of PAR4 in T. cruzi enhanced the subdominant PAR4-specific CD8(+) T cell response, resulting in improved control of a challenge infection. These results provide insights into previously unappreciated events in intracellular invasion by T. cruzi and highlight the importance of T cells that recognize infected host cells early in the infectious process, in the control of infections.

Mol Microbiol. 2014 Nov;94(3):625-36. doi: 10.1111/mmi.12783. Epub 2014 Sep 25.

Identification of Trypanosoma brucei components involved in trypanolysis by normal human serum.

Lecordier L1, Uzureau P, Tebabi P, Pérez-Morga D, Nolan D, Schumann Burkard G, Roditi I, Pays E.

Author information

Abstract

Normal human serum (NHS) confers human resistance to infection by the parasite Trypanosoma brucei owing to the trypanolytic activity of apolipoprotein L1 (APOL1), present in two serum complexes termed Trypanolytic Factors (TLF-1 and -2). In order to identify parasite components involved in the intracellular trafficking and activity of TLFs, an inducible RNA interference (RNAi) genomic DNA library constructed in bloodstream form T. brucei was subjected to RNAi induction and selection for resistant parasites under NHS conditions favouring either TLF-1 or TLF-2 uptake. While TLF-1 conditions readily selected the haptoglobin-haemoglobin (HP-HB) surface receptor TbHpHbR as expected, given its known ability to bind TLF-1, under TLF-2 conditions no specific receptor for TLF-2 was identified. Instead, the screen allowed the identification of five distinct factors expected to be involved in the assembly of the vacuolar proton pump V-ATPase and consecutive endosomal acidification. These data confirm that lowering the pH during endocytosis is required for APOL1 toxic activity.

Nature. 2009 May 14;459(7244):213-7. doi: 10.1038/nature07997.

A surface transporter family conveys the trypanosome differentiation signal.

Dean S1, Marchetti R, Kirk K, Matthews KR.
Author information

Abstract
Microbial pathogens use environmental cues to trigger the developmental events needed to infect mammalian hosts or transmit to disease vectors. The parasites causing African sleeping sickness respond to citrate or cis-aconitate (CCA) to initiate life-cycle development when transmitted to their tsetse fly vector. This requires hypersensitization of the parasites to CCA by exposure to low temperature, conditions encountered after tsetse fly feeding at dusk or dawn. Here we identify a carboxylate-transporter family, PAD (proteins associated with differentiation), required for perception of this differentiation signal. Consistent with predictions for the response of trypanosomes to CCA, PAD proteins are expressed on the surface of the transmission-competent 'stumpy-form' parasites in the bloodstream, and at least one member is thermoregulated, showing elevated expression and surface access at low temperature. Moreover, RNA-interference-mediated ablation of PAD expression diminishes CCA-induced differentiation and eliminates CCA hypersensitivity under cold-shock conditions. As well as being molecular transducers of the differentiation signal in these parasites, PAD proteins provide the first example of a surface marker able to discriminate the transmission stage of trypanosomes in their mammalian host.

Cell Host Microbe. 2011 Apr 21;9(4):319-30. doi: 10.1016/j.chom.2011.03.011.

Imaging host cell-Leishmania interaction dynamics implicates parasite motility, lysosome recruitment, and host cell wounding in the infection process.

Forestier CL1, Machu C, Loussert C, Pescher P, Späth GF.
Author information

Abstract
Leishmania donovani causes human visceral leishmaniasis. The parasite infectious cycle comprises extracellular flagellated promastigotes that proliferate inside the insect vector, and intracellular nonmotile amastigotes that multiply within infected host cells. Using primary macrophages infected with virulent metacyclic promastigotes and high spatiotemporal resolution microscopy, we dissect the dynamics of the early infection process. We find that motile promastigotes enter macrophages in a polarized manner through their flagellar tip and are engulfed into host lysosomal compartments. Persistent intracellular flagellar activity leads to reorientation of the parasite flagellum toward the host cell periphery and results in oscillatory parasite movement. The latter is associated with local lysosomal exocytosis and host cell plasma membrane wounding. These findings implicate lysosome recruitment followed by lysosome exocytosis, consistent with parasite-driven

host cell injury, as key cellular events in Leishmania host cell infection. This work highlights the role of promastigote polarity and motility during parasite entry.

Cell, Volume 121, Issue 1, 8 April 2005, Pages 25–36

Telomeric Heterochromatin Propagation and Histone Acetylation Control Mutually Exclusive Expression of Antigenic Variation Genes in Malaria Parasites

Lucio H. Freitas-Junior[1, 4, 5], Rosaura Hernandez-Rivas[3, 4], Stuart A. Ralph[1], Dvorak Montiel-Condado[3], Omar K. Ruvalcaba-Salazar[3], Ana Paola Rojas-Meza[3], Liliana Mâncio-Silva[1, 6], Ricardo J. Leal-Silvestre[1, 6], Alisson Marques Gontijo[1], Spencer Shorte[2], Artur Scherf[1, ,]

Summary

Malaria parasites use antigenic variation to avoid immune clearance and increase the duration of infection in the human host. Variation at the surface of P. falciparum-infected erythrocytes is mediated by the differential control of a family of surface antigens encoded by var genes. Switching of var gene expression occurs in situ, mostly from telomere-associated loci, without detectable DNA alterations, suggesting that it is controlled by chromatin structure. We have identified chromatin modifications at telomeres that spread far into telomere-proximal regions, including var gene loci (>50 kb). One type of modification is mediated by a protein homologous to yeast Sir2 called PfSir2, which forms a chromosomal gradient of heterochromatin structure and histone hypoacetylation. Upon activation of a specific telomere-associated var gene, PfSir2 is removed from the promoter region and acetylation of histone occurs. Our data demonstrate that mutually exclusive transcription of var genes is linked to the dynamic remodeling of chromatin.

PLoS Biol. 2008 Jul 1;6(7):e161. doi: 10.1371/journal.pbio.0060161.

A histone methyltransferase modulates antigenic variation in African trypanosomes.

Figueiredo LM1, Janzen CJ, Cross GA.

Abstract

To evade the host immune system, several pathogens periodically change their cell-surface epitopes. In the African trypanosomes, antigenic variation is

achieved by tightly regulating the expression of a multigene family encoding a large repertoire of variant surface glycoproteins (VSGs). Immune evasion relies on two important features: exposing a single type of VSG at the cell surface and periodically and very rapidly switching the expressed VSG. Transcriptional switching between resident telomeric VSG genes does not involve DNA rearrangements, and regulation is probably epigenetic. The histone methyltransferase DOT1B is a nonessential protein that trimethylates lysine 76 of histone H3 in Trypanosoma brucei. Here we report that transcriptionally silent telomeric VSGs become partially derepressed when DOT1B is deleted, whereas nontelomeric loci are unaffected. DOT1B also is involved in the kinetics of VSG switching: in DeltaDOT1B cells, the transcriptional switch is so slow that cells expressing two VSGs persist for several weeks, indicating that monoallelic transcription is compromised. We conclude that DOT1B is required to maintain strict VSG silencing and to ensure rapid transcriptional VSG switching, demonstrating that epigenetics plays an important role in regulating antigenic variation in T. brucei.

PLoS Negl Trop Dis. 2013 Oct 10;7(10):e2475. doi: 10.1371/journal.pntd.0002475. eCollection 2013.

Aquaporin 2 mutations in Trypanosoma brucei gambiense field isolates correlate with decreased susceptibility to pentamidine and melarsoprol.

Graf FE1, Ludin P, Wenzler T, Kaiser M, Brun R, Pyana PP, Büscher P, de Koning HP, Horn D, Mäser P.

Abstract

The predominant mechanism of drug resistance in African trypanosomes is decreased drug uptake due to loss-of-function mutations in the genes for the transporters that mediate drug import. The role of transporters as determinants of drug susceptibility is well documented from laboratory-selected Trypanosoma brucei mutants. But clinical isolates, especially of T. b. gambiense, are less amenable to experimental investigation since they do not readily grow in culture without prior adaptation. Here we analyze a selected panel of 16 T. brucei ssp. field isolates that (i) have been adapted to axenic in vitro cultivation and (ii) mostly stem from treatment-refractory cases. For each isolate, we quantify the sensitivity to melarsoprol, pentamidine, and diminazene, and sequence the genomic loci of the transporter genes TbAT1 and TbAQP2. The former encodes the well-characterized aminopurine permease P2 which transports several trypanocides including melarsoprol, pentamidine, and diminazene. We find that diminazene-resistant field isolates of T. b. brucei and T. b. rhodesiense carry the same set of point mutations in TbAT1 that was previously described from lab mutants. Aquaglyceroporin 2 has only recently been identified as a second transporter involved in melarsoprol/pentamidine cross-resistance. Here we describe two different kinds of TbAQP2 mutations found in T. b. gambiense field isolates: simple

loss of TbAQP2, or loss of wild-type TbAQP2 allele combined with the formation of a novel type of TbAQP2/3 chimera. The identified mutant T. b. gambiense are 40- to 50-fold less sensitive to pentamidine and 3- to 5-times less sensitive to melarsoprol than the reference isolates. We thus demonstrate for the first time that rearrangements of the TbAQP2/TbAQP3 locus accompanied by TbAQP2 gene loss also occur in the field, and that the T. b. gambiense carrying such mutations correlate with a significantly reduced susceptibility to pentam

Proc Natl Acad Sci U S A. 2008 Apr 1;105(13):5022-7. doi: 10.1073/pnas.0711014105. Epub 2008 Mar 26.

A mechanism for cross-resistance to nifurtimox and benznidazole in trypanosomes.

Wilkinson SR1, Taylor MC, Horn D, Kelly JM, Cheeseman I.

Author information

Abstract

Nifurtimox and benznidazole are the front-line drugs used to treat Chagas disease, the most important parasitic infection in the Americas. These agents function as prodrugs and must be activated within the parasite to have trypanocidal effects. Despite >40 years of research, the mechanism(s) of action and resistance have remained elusive. Here, we report that in trypanosomes, both drugs are activated by a NADH-dependent, mitochondrially localized, bacterial-like, type I nitroreductase (NTR), and that down-regulation of this explains how resistance may emerge. Loss of a single copy of this gene in Trypanosoma cruzi, either through in vitro drug selection or by targeted gene deletion, is sufficient to cause significant cross-resistance to a wide range of nitroheterocyclic drugs. In Trypanosoma brucei, loss of a single NTR allele confers similar cross-resistance without affecting growth rate or the ability to establish an infection. This potential for drug resistance by a simple mechanism has important implications, because nifurtimox is currently undergoing phase III clinical trials against African trypanosomiasis.

Lancet. 2009 Jul 4;374(9683):56-64. doi: 10.1016/S0140-6736(09)61117-X. Epub 2009 Jun 24.

Nifurtimox-eflornithine combination therapy for second-stage African Trypanosoma brucei gambiense trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial.

Priotto G1, Kasparian S, Mutombo W, Ngouama D, Ghorashian S, Arnold U,

Ghabri S, Baudin E, Buard V, Kazadi-Kyanza S, Ilunga M, Mutangala W, Pohlig G, Schmid C, Karunakara U, Torreele E, Kande V.
Author information

Abstract
BACKGROUND:
Human African trypanosomiasis (HAT; sleeping sickness) caused by Trypanosoma brucei gambiense is a fatal disease. Current treatment options for patients with second-stage disease are toxic, ineffective, or impractical. We assessed the efficacy and safety of nifurtimox-eflornithine combination therapy (NECT) for second-stage disease compared with the standard eflornithine regimen.
FINDINGS:
One patient from the eflornithine group absconded after receiving the first dose, without any type of assessment done, and was excluded from all analyses. In the ITT population, 131 (91.6%) of 143 patients assigned to eflornithine and 138 (96.5%) of 143 patients assigned to NECT were cured at 18 months (difference -4.9%, one-sided 95% CI -0.3; p<0.0001). In the PP population, 122 (91.7%) of 133 patients in the eflornithine group and 129 (97.7%) of 132 in the NECT group were cured at 18 months (difference -6.0%, one-sided 95% CI -1.5; p<0.0001). Drug-related adverse events were frequent in both groups; 41 (28.7%) patients in the eflornithine group and 20 (14.0%) in the NECT group had major (grade 3 or 4) reactions, which resulted in temporary treatment interruption in nine and one patients, respectively. The most common major adverse events were fever (n=18), seizures (n=6), and infections (n=5) in the eflornithine group, and fever (n=7), seizures (n=6), and confusion (n=2) in the NECT group. There were four deaths, which were regarded as related to study drug (eflornithine, n=3; NECT, n=1).
INTERPRETATION:
The efficacy of NECT is non-inferior to that of eflornithine monotherapy. Since this combination treatment also presents safety advantages, is easier to administer (ie, infusion every 12 h for 7 days vs every 6 h for 14 days), and potentially protective against the emergence of resistant parasites, it is suitable for first-line use in HAT control programmes.

Nat Chem. 2014 Feb;6(2):112-21. doi: 10.1038/nchem.1830. Epub 2013 Dec 22.
Validation of N-myristoyltransferase as an antimalarial drug target using an integrated chemical biology approach.

Wright MH1, Clough B2, Rackham MD3, Rangachari K2, Brannigan JA4, Grainger M2, Moss DK2, Bottrill AR5, Heal WP6, Broncel M3, Serwa RA3, Brady D7, Mann DJ8, Leatherbarrow RJ9, Tewari R7, Wilkinson AJ4, Holder AA2, Tate EW1.
Author information

Abstract
Malaria is an infectious disease caused by parasites of the genus Plasmodium, which leads to approximately one million deaths per annum worldwide. Chemical validation of new antimalarial targets is urgently required in view of rising resistance to current drugs. One such putative target is the enzyme N-myristoyltransferase, which catalyses the attachment of the fatty acid myristate to protein substrates (N-myristoylation). Here, we report an integrated chemical biology approach to explore protein myristoylation in the major human parasite P. falciparum, combining chemical proteomic tools for identification of the myristoylated and glycosylphosphatidylinositol-anchored proteome with selective small-molecule N-myristoyltransferase inhibitors. We demonstrate that N-myristoyltransferase is an essential and chemically tractable target in malaria parasites both in vitro and in vivo, and show that selective inhibition of N-myristoylation leads to catastrophic and irreversible failure to assemble the inner membrane complex, a critical subcellular organelle in the parasite life cycle. Our studies provide the basis for the development of new antimalarials targeting N-myristoyltransferase.

Benznidazole biotransformation and multiple targets in Trypanosoma cruzi revealed by metabolomics.
Trochine A1, Creek DJ2, Faral-Tello P1, Barrett MP3, Robello C4.
Author information

Abstract
BACKGROUND:
The first line treatment for Chagas disease, a neglected tropical disease caused by the protozoan parasite Trypanosoma cruzi, involves administration of benznidazole (Bzn). Bzn is a 2-nitroimidazole pro-drug which requires nitroreduction to become active, although its mode of action is not fully understood. In the present work we used a non-targeted MS-based metabolomics approach to study the metabolic response of T. cruzi to Bzn.
METHODOLOGY/PRINCIPAL FINDINGS:
Parasites treated with Bzn were minimally altered compared to untreated trypanosomes, although the redox active thiols trypanothione, homotrypanothione and cysteine were significantly diminished in abundance post-treatment. In addition, multiple Bzn-derived metabolites were detected after treatment. These metabolites included reduction products, fragments and covalent adducts of reduced Bzn linked to each of the major low molecular weight thiols: trypanothione, glutathione, γ-glutamylcysteine, glutathionylspermidine, cysteine and ovothiol A. Bzn products known to be generated in vitro by the unusual trypanosomal nitroreductase, TcNTRI, were found within the parasites, but low molecular weight adducts of glyoxal, a proposed toxic end-product of NTRI Bzn metabolism, were not detected.
CONCLUSIONS/SIGNIFICANCE:
Our data is indicative of a major role of the thiol binding capacity of Bzn reduction products in the mechanism of Bzn toxicity against T. cruzi.

PLoS Pathog. 2014 Jun 12;10(6):e1004178. doi: 10.1371/journal.ppat.1004178. eCollection 2014.

A genome-wide tethering screen reveals novel potential post-transcriptional regulators in Trypanosoma brucei.

Erben ED1, Fadda A1, Lueong S2, Hoheisel JD2, Clayton C1.

Abstract

In trypanosomatids, gene expression is regulated mainly by post-transcriptional mechanisms, which affect mRNA processing, translation and degradation. Currently, our understanding of factors that regulate either mRNA stability or translation is rather limited. We know that often, the regulators are proteins that bind to the 3'-untranslated region; they presumably interact with ribonucleases and translation factors. However, very few such proteins have been characterized in any detail. Here we describe a genome-wide screen to find proteins implicated in post-transcriptional regulation in Trypanosoma brucei. We made a library of random genomic fragments in a plasmid that was designed for expression of proteins fused to an RNA-binding domain, the lambda-N peptide. This was transfected into cells expressing mRNAs encoding a positive or negative selectable marker, and bearing the "boxB" lambda-N recognition element in the 3'-untranslated region. The screen identified about 300 proteins that could be implicated in post-transcriptional mRNA regulation. These included known regulators, degradative enzymes and translation factors, many canonical RNA-binding proteins, and proteins that act via multi-protein complexes. However there were also nearly 150 potential regulators with no previously annotated function, or functions unrelated to mRNA metabolism. Almost 50 novel regulators were shown to bind RNA using a targeted proteome array. The screen also provided fine structure mapping of the hit candidates' functional domains. Our findings not only confirm the key role that RNA-binding proteins play in the regulation of gene expression in trypanosomatids, but also suggest new roles for previously uncharacterized proteins.

PMID: 24945722 [PubMed - in process] PMCID: PMC4055773

Cell. 2007 Nov 2;131(3):505-15.

Hydrodynamic flow-mediated protein sorting on the cell surface of trypanosomes.

Engstler M1, Pfohl T, Herminghaus S, Boshart M, Wiegertjes G, Heddergott N, Overath P.
Author information

Abstract
The unicellular parasite Trypanosoma brucei rapidly removes host-derived immunoglobulin (Ig) from its cell surface, which is dominated by a single type of glycosylphosphatidylinositol-anchored variant surface glycoprotein (VSG). We have determined the mechanism of antibody clearance and found that Ig-VSG immune complexes are passively sorted to the posterior cell pole, where they are endocytosed. The backward movement of immune complexes requires forward cellular motility but is independent of endocytosis and of actin function. We suggest that the hydrodynamic flow acting on swimming trypanosomes causes directional movement of Ig-VSG immune complexes in the plane of the plasma membrane, that is, immunoglobulins attached to VSG function as molecular sails. Protein sorting by hydrodynamic forces helps to protect trypanosomes against complement-mediated immune destruction in culture and possibly in infected mammals but likewise may be of functional significance at the surface of other cell types such as epithelial cells lining blood vessels.

Nat Methods. 2013 Feb;10(2):125-7. doi: 10.1038/nmeth.2301. Epub 2012 Dec 23.

Conditional genome engineering in Toxoplasma gondii uncovers alternative invasion mechanisms.

Andenmatten N1, Egarter S, Jackson AJ, Jullien N, Herman JP, Meissner M.
Author information

Abstract
We established a conditional site-specific recombination system based on dimerizable Cre recombinase-mediated recombination in the apicomplexan parasite Toxoplasma gondii. Using a new single-vector strategy that allows ligand-dependent, efficient removal of a gene of interest, we generated three knockouts of apicomplexan genes considered essential for host-cell invasion. Our findings uncovered the existence of an alternative invasion pathway in apicomplexan parasites.\

Nature. 2014 Jul 31;511(7511):587-91. doi: 10.1038/nature13555. Epub 2014 Jul 16.

PTEX is an essential nexus for protein export in malaria parasites.

Elsworth B1, Matthews K2, Nie CQ3, Kalanon M4, Charnaud SC5, Sanders PR3, Chisholm SA4, Counihan NA4, Shaw PJ6, Pino P7, Chan JA3, Azevedo MF3, Rogerson SJ8, Beeson JG9, Crabb BS10, Gilson PR1, de Koning-Ward TF2.
Author information

Abstract
During the blood stages of malaria, several hundred parasite-encoded proteins are exported beyond the double-membrane barrier that separates the parasite from the host cell cytosol. These proteins have a variety of roles that are essential to virulence or parasite growth. There is keen interest in understanding how proteins are exported and whether common machineries are involved in trafficking the different classes of exported proteins. One potential trafficking machine is a protein complex known as the Plasmodium translocon of exported proteins (PTEX). Although PTEX has been linked to the export of one class of exported proteins, there has been no direct evidence for its role and scope in protein translocation. Here we show, through the generation of two parasite lines defective for essential PTEX components (HSP101 or PTEX150), and analysis of a line lacking the non-essential component TRX2 (ref. 12), greatly reduced trafficking of all classes of exported proteins beyond the double membrane barrier enveloping the parasite. This includes proteins containing the PEXEL motif (RxLxE/Q/D) and PEXEL-negative exported proteins (PNEPs). Moreover, the export of proteins destined for expression on the infected erythrocyte surface, including the major virulence factor PfEMP1 in Plasmodium falciparum, was significantly reduced in PTEX knockdown parasites. PTEX function was also essential for blood-stage growth, because even a modest knockdown of PTEX components had a strong effect on the parasite's capacity to complete the erythrocytic cycle both in vitro and in vivo. Hence, as the only known nexus for protein export in Plasmodium parasites, and an essential enzymic machine, PTEX is a prime drug target.

Curr Biol. 2014 Jan 20;24(2):181-6. doi: 10.1016/j.cub.2013.11.044. Epub 2014 Jan 2.
Meiosis and haploid gametes in the pathogen Trypanosoma brucei.

Peacock L1, Bailey M2, Carrington M3, Gibson W4.
Author information

Abstract
In eukaryote pathogens, sex is an important driving force in spreading genes for drug resistance, pathogenicity, and virulence. For the parasitic trypanosomes that cause African sleeping sickness, mating occurs during transmission by the tsetse vector and involves meiosis, but haploid gametes have not yet been identified. Here, we show that meiosis is a normal part of

development in the insect salivary glands for all subspecies of Trypanosoma brucei, including the human pathogens. By observing insect-derived trypanosomes during the window of peak expression of meiosis-specific genes, we identified promastigote-like (PL) cells that interacted with each other via their flagella and underwent fusion, as visualized by the mixing of cytoplasmic red and green fluorescent proteins. PL cells had a short, wide body, a very long anterior flagellum, and either one or two kinetoplasts, but only the anterior kinetoplast was associated with the flagellum. Measurement of nuclear DNA contents showed that PL cells were haploid relative to diploid metacyclics. Trypanosomes are among the earliest diverging eukaryotes, and our results support the hypothesis that meiosis and sexual reproduction are ubiquitous in eukaryotes and likely to have been early innovations.

Science. 2012 Dec 7;338(6112):1352-3. doi: 10.1126/science.1229641.
Developmental progression to infectivity in Trypanosoma brucei triggered by an RNA-binding protein.
Kolev NG1, Ramey-Butler K, Cross GA, Ullu E, Tschudi C.
Author information

Abstract
Unraveling the intricate interactions between Trypanosoma brucei, the protozoan parasite causing African trypanosomiasis, and the tsetse (Glossina) vector remains a challenge. Metacyclic trypanosomes, which inhabit the tsetse salivary glands, transmit the disease and are produced through a complex differentiation and unknown program. By overexpressing a single RNA-binding protein, TbRBP6, in cultured noninfectious trypanosomes, we recapitulated the developmental stages that have been observed in tsetse, including the generation of infective metacyclic forms expressing the variant surface glycoprotein. Thus, events leading to acquisition of infectivity in the insect vector are now accessible to laboratory investigation, providing an opening for new intervention strategies.

Science. 2011 Jan 28;331(6016):473-7. doi: 10.1126/science.1199284. Epub 2010 Dec 23.
Intramembrane cleavage of AMA1 triggers Toxoplasma to switch from an invasive to a replicative mode.

Santos JM1, Ferguson DJ, Blackman MJ, Soldati-Favre D.
Author information

Abstract
Apicomplexan parasites invade host cells and immediately initiate cell division. The extracellular parasite discharges transmembrane proteins onto its surface to mediate motility and invasion. These are shed by intramembrane cleavage, a process associated with invasion but otherwise poorly understood. Functional analysis of Toxoplasma rhomboid 4, a surface

intramembrane protease, by conditional overexpression of a catalytically inactive form produced a profound block in replication. This was completely rescued by expression of the cleaved cytoplasmic tail of Toxoplasma or Plasmodium apical membrane antigen 1 (AMA1). These results reveal an unexpected function for AMA1 in parasite replication and suggest that invasion proteins help to promote parasite switch from an invasive to a replicative mode.

FASEB J. 2011 Feb;25(2):515-25. doi: 10.1096/fj.10-157529. Epub 2010 Oct 15.

Multiple levels of gene regulation mediate differentiation of the intracellular pathogen Leishmania.

Lahav T1, Sivam D, Volpin H, Ronen M, Tsigankov P, Green A, Holland N, Kuzyk M, Borchers C, Zilberstein D, Myler PJ.

Author information

Abstract

For many years, mRNA abundance has been used as the surrogate measure of gene expression in biological systems. However, recent genome-scale analyses in both bacteria and eukaryotes have revealed that mRNA levels correlate with steady-state protein abundance for only 50-70% of genes, indicating that translation and post-translation processes also play important roles in determining gene expression. What is not yet clear is whether dynamic processes such as cell cycle progression, differentiation, or response to environmental changes change the relationship between mRNA and protein abundance. Here, we describe a systems approach to interrogate promastigote-to-amastigote differentiation in the obligatory intracellular parasitic protozoan Leishmania donovani. Our results indicate that regulation of mRNA levels plays a major role early in the differentiation process, while translation and post-translational regulation are more important in the latter part. In addition, it appears that the differentiation signal causes a transient global increase in the rate of protein synthesis, which is subsequently down-regulated by phosphorylation of α-subunit of translation initiation factor 2. Thus, Leishmania dynamically changes the relationship between mRNA and protein abundance as it adapts to new environmental circumstances. It is likely that similar mechanisms play a more important role than previously recognized in regulation of gene expression in other organisms.

Additional Papers for Student Assignments

Sci Signal. 2009 Nov 17;2(97):ra74. doi: 10.1126/scisignal.2000374.

Trypanosoma cruzi targets Akt in host cells as an intracellular antiapoptotic strategy.

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Abstract
The parasite Trypanosoma cruzi, which causes Chagas' disease, differentiates in the cytosol of its host cell and then replicates and spreads infection, processes that require the long-term survival of the infected cells. Here, we show that in the cytosol, parasite-derived neurotrophic factor (PDNF), a trans-sialidase that is located on the surface of T. cruzi, is both a substrate and an activator of the serine-threonine kinase Akt, an antiapoptotic molecule. PDNF increases the expression of the gene that encodes Akt while suppressing the transcription of genes that encode proapoptotic factors. Consequently, PDNF elicits a sustained functional response that protects host cells from apoptosis induced by oxidative stress and the proinflammatory cytokines tumor necrosis factor-alpha and transforming growth factor-beta. Given that PDNF also activates Akt by binding to the neurotrophic surface receptor TrkA, we propose that this protein activates survival signaling both at the cell surface, by acting as a receptor-binding ligand, and inside cells, by acting as a scaffolding adaptor protein downstream of the receptor.

Sci Transl Med. 2014 Apr 30;6(234):234ra56. doi: 10.1126/scitranslmed.3008222.

Modular multiantigen T cell epitope-enriched DNA vaccine against human leishmaniasis.

Das S1, Freier A, Boussoffara T, Das S, Oswald D, Losch FO, Selka M, Sacerdoti-Sierra N, Schönian G, Wiesmüller KH, Seifert K, Schroff M, Juhls C, Jaffe CL, Roy S, Das P, Louzir H, Croft SL, Modabber F, Walden P.

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Abstract

The leishmaniases are protozoal diseases that severely affect large populations in tropical and subtropical regions. There are only limited treatment options and preventative measures. Vaccines will be important for prevention, control and elimination of leishmaniasis, and could reduce the transmission and burden of disease in endemic populations. We report the development of a DNA vaccine against leishmaniasis that induced T cell-based immunity and is a candidate for clinical trials. The vaccine antigens were selected as conserved in various Leishmania species, different endemic regions, and over time. They were tested with T cells from individuals cured of leishmaniasis, and shown to be immunogenic and to induce CD4(+) and CD8(+) T cell responses in genetically diverse human populations of different endemic regions. The vaccine proved protective in a rodent model of infection. Thus, the immunogenicity of candidate vaccine antigens in human populations of endemic regions, as well as proof of principle for induction of specific immune responses and protection against Leishmania infection in mice, provides a viable strategy for T cell vaccine development.

MBio. 2017 Jul 11;8(4). pii: e00729-17. doi: 10.1128/mBio.00729-17.

CRISPR/Cas9 Genome Editing Reveals That the Intron Is Not Essential for var2csa Gene Activation or Silencing in Plasmodium falciparum.

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Abstract

Plasmodium falciparum relies on monoallelic expression of 1 of 60 *var* virulence genes for antigenic variation and host immune evasion. Each *var* gene contains a conserved intron which has been implicated in previous studies in both activation and repression of transcription via several epigenetic mechanisms, including interaction with the *var*promoter, production of long noncoding RNAs (lncRNAs), and localization to repressive perinuclear sites. However, functional studies have relied primarily on artificial expression constructs. Using the recently developed *P. falciparum*clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system, we directly deleted the *var2csa P. falciparum* 3D7_1200600 (Pf3D7_1200600) endogenous intron, resulting in an intronless *var* gene in a natural, marker-

free chromosomal context. Deletion of the *var2csa* intron resulted in an upregulation of transcription of the *var2csa* gene in ring-stage parasites and subsequent expression of the PfEMP1 protein in late-stage parasites. Intron deletion did not affect the normal temporal regulation and subsequent transcriptional silencing of the *var* gene in trophozoites but did result in increased rates of *var* gene switching in some mutant clones. Transcriptional repression of the intronless *var2csa* gene could be achieved via long-term culture or panning with the CD36 receptor, after which reactivation was possible with chondroitin sulfate A (CSA) panning. These data suggest that the *var2csa* intron is not required for silencing or activation in ring-stage parasites but point to a subtle role in regulation of switching within the *var* gene family.**IMPORTANCE***Plasmodium falciparum* is the most virulent species of malaria parasite, causing high rates of morbidity and mortality in those infected. Chronic infection depends on an immune evasion mechanism termed antigenic variation, which in turn relies on monoallelic expression of 1 of ~60 *var* genes. Understanding antigenic variation and the transcriptional regulation of monoallelic expression is important for developing drugs and/or vaccines. The *var* gene family encodes the antigenic surface proteins that decorate infected erythrocytes. Until recently, studying the underlying genetic elements that regulate monoallelic expression in *P. falciparum* was difficult, and most studies relied on artificial systems such as episomal reporter genes. Our study was the first to use CRISPR/Cas9 genome editing for the functional study of an important, conserved genetic element of *var* genes-the intron-in an endogenous, episome-free manner. Our findings shed light on the role of the *var* gene intron in transcriptional regulation of monoallelic expression.