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Review

Specializations in a successful parasite: What makes the bloodstream-form African trypanosome so deadly?

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ABSTRACT

Most trypanosomatid parasites have both arthropod and mammalian or plant hosts, and the ability to survive and complete a developmental program in each of these very different environments is essential for life cycle progression and hence being a successful pathogen. For African trypanosomes, where the mammalian stage is exclusively extracellular, this presents specific challenges and requires evasion of both the acquired and innate immune systems, together with adaptation to a specific nutritional environment and resistance to mechanical and biochemical stresses. Here we consider the basis for these adaptations, the specific features of the mammalian infective trypanosome that are required to meet these challenges, and how these processes both inform on basic parasite biology and present potential therapeutic targets.

Contents

1. Introduction

The trypanosomatids, which formally include African trypanosomes, American trypanosomes and leishmania species, are causative agents of major human, livestock, wild animal and plant diseases. According to WHO statistics, together these organisms directly threaten the health of more than five hundred million people worldwide. African trypanosomes present a dual burden, both directly on human health where they are responsible for tens of thousands of deaths annually, but also on economic well-being through human morbidity and agricultural impact. This last aspect is currently unquantified but, given the frequency of trypanosome-mediated disease, it presents a major challenge for public health programs [1]. Moreover, current drugs are highly toxic, require complex dosing regimens and resistance is becoming prevalent [for a recent discussion see 2, for recent progress see 3]. For *Trypanosoma brucei* subspecies and *Trypanosoma congolense* specifically, advances over the last two decades in public health programs and monitoring have decreased the death toll substantially. However, the disease that these parasites cause, sleeping sickness or human African trypanosomiasis (HAT) is, as far as it is known, invariably fatal in the absence of therapeutic intervention [4]. The brutal consequence of untreated *T. brucei* infection in humans contrasts with trypanotolerant animal species

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such as *Bos indicus*, and tells us several things about the parasite. Firstly, *T. brucei* is able to survive and proliferate within many different mammalian hosts. Second, the trypanosome, at the population level, can evade the entirety of the host immune system. Third, there is differential interaction between parasite and host, such that in humans the disease is highly virulent, invariably progressing to death, while by contrast many mammals are able to modulate the infection and co-exist for a protracted period of months or even years, with apparently little negative impact to health [5].

T. brucei cycles between a mammalian and tsetse fly host. Substantial remodeling, of many cellular processes and morphology, accompanies differentiation between the major proliferative stages, the mammalian bloodstream form and the insect procyclic and epimastigote forms. These rapidly proliferating stages are interspersed with additional life cycle intermediates, where cell division appears to halt and essential pre-adaptations to the next host occur [6]. For example, on entering the mammalian host, the parasite must successfully respond to increased temperature, activate [or have activated, see 7] immune evasion mechanisms and be prepared for significant changes to the biochemical composition of the environment, triggering mechanisms required for acquisition of nutrients. Furthermore, the overall architecture of the cell and positioning of the organelles change on transitioning between life cycle stages, which is supported by major alterations in gene expression. Essentially this constitutes a 'wheel of death', i.e. a series of interlocking processes that ensure the demise of the human host. In this minireview we consider some of the specializations of the *T. brucei* bloodstream form, what underpins evolutionary selection for these changes and if there is potential for therapeutic exploitation based on these adaptations (Fig. 1).

2. Evasion of the acquired immune system

2.1. Surface coat remodeling

One of the most fundamental changes that occur between the insect vector and mammalian bloodstream forms of *T. brucei* is that to the parasite cell surface (Fig. 2). The procyclic form, present in the midgut of the tsetse fly, possesses a protease-resistant surface coat composed of the procyclin proteins, while the surface coat of epimastigote forms (a life cycle stage found in the fly salivary gland) consists mainly of an alanine-rich protein called BARP [8]. Ultimately, the bloodstream form expresses a single variant surface glycoprotein (VSG) over the entire plasma membrane. Upon inoculation into the mammalian host, both procyclic and epimastigote forms are sensitive to complement-mediated lysis, yet the bloodstream form is obviously able to survive and establish infection. This implies that there must be another life cycle stage in the fly that is pre-adapted against the host immune system. This crucial role is filled by the metacyclic form which develops and acquires a VSG coat while still in the fly salivary gland, although this surface coat is composed of one of a small selection of metacyclic-specific VSG genes. The metacyclic stage also has many of the morphological and biochemical characteristics of the bloodstream form, but it is non-proliferative. Once metacyclic parasites enter the mammalian host, a series of biochemical signals trigger their transformation into the typical slender form of a bloodstream trypanosome, which can proliferate.

The dense ~15 nm-thick VSG surface coat not only protects the bloodstream parasite against complement-mediated lysis, but also shields a great many invariant proteins from recognition by the host acquired immune system [9,10]. Each VSG molecule is composed of a large, alpha-helical N-terminal domain comprising the forefront of the immunological barrier [11,12], whereas a small C-terminal domain links the VSG dimer to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor [13,14]. The invariant surface proteins, i.e. those that are expressed by all *T. brucei* bloodstream variants, remain poorly characterized, but include the transferrin and the haptoglobin–haemoglobin receptors [15–17], families of nucleotide and sugar transporters [18], a repertoire of adenylate cyclases [19] and at least two families of type I *trans*-membrane domain proteins, designated invariant surface glycoproteins (ISG) 65 and 75 (due to their apparent molecular of 65 and 75 kDa, respectively) [20]. Expression of ISG65 and 75 is up-regulated upon differentiation to the bloodstream form and their trafficking and turnover is highly distinct to that of VSG [21], yet the roles they play in infection remain unknown.

While it is the conventional wisdom that the VSG coat forms a physical barrier around the parasite [11], experiments on live cells report antibody recognition to ISG65, followed by internalization and delivery of the ISG65–antibody complex to the endosomal system [22]. Despite these findings, prophylactic immunization of mice with recombinant ISGs does not appear to confer any significant degree of protection against subsequent infection [23]. More recently, studies demonstrated that antibodies could not contact epitopes beneath the N-terminal domain, preventing recognition of the VSG C-terminal region in live cells. This suggests that the dense packing of VSG molecules does indeed significantly modulate the ability of immunoglobulin to recognize many surface determinants [24]. However, these observations are restricted to the VSG itself, and the level of exposure of other surface molecules has yet to be accurately mapped. Tethering of VSGs to a single leaflet of the plasma membrane by a GPI anchor enables these antigenic molecules to be highly mobile without cytoskeletal interactions [25]. Therefore, it is possible that antibody binding at the parasite surface is significantly influenced by the fluidity of the VSG coat, whereby lateral diffusion of VSG molecules potentially exposes underlying invariant epitopes sufficiently to allow antibody recognition. Documenting the dynamic processes at the trypanosome surface is fundamental to understanding the mechanisms by which the mammalian immune system interacts with the parasite, and an area that deserves greater systematic enquiry.

2.2. Antigenic variation

Despite being the major determinant of immune evasion, the VSG itself is highly antigenic, such that eventually the host generates a sufficient antibody titre to eliminate the parasite. To survive, the parasite has evolved an exceptionally effective evasion mechanism whereby periodically it switches the single expressed VSG gene from a vast silent library to an immunologically distinct, novel one. There are about 2000 VSG and VSG-related genes and pseudogenes in the genome [26], and mosaic gene formation potentially allows an even greater repertoire of distinct antigenic forms of VSGs [27]. VSG switching is probably essentially stochastic and largely the result of homologous recombination into an active telomeric expression site (ES) [28]. Monoallelic transcription of the VSG occurs in a specialized region of the bloodstream-form nucleus called the expression body site or ESB [29]; all other VSG genes are either unassociated with a promoter or positioned within the nuclear periphery such that expression is repressed. There is remarkably low amino acid identity shared between VSG variants (~15%), but the secondary and tertiary structures remain very similar [12]. This is probably important to the role of VSG in immune evasion, as antigenically distinct VSGs must be able to alter many accessible amino acids if they are to avoid recognition by existing host immunity, yet also allow packing of VSG at the cell surface to maintain the protective coat.

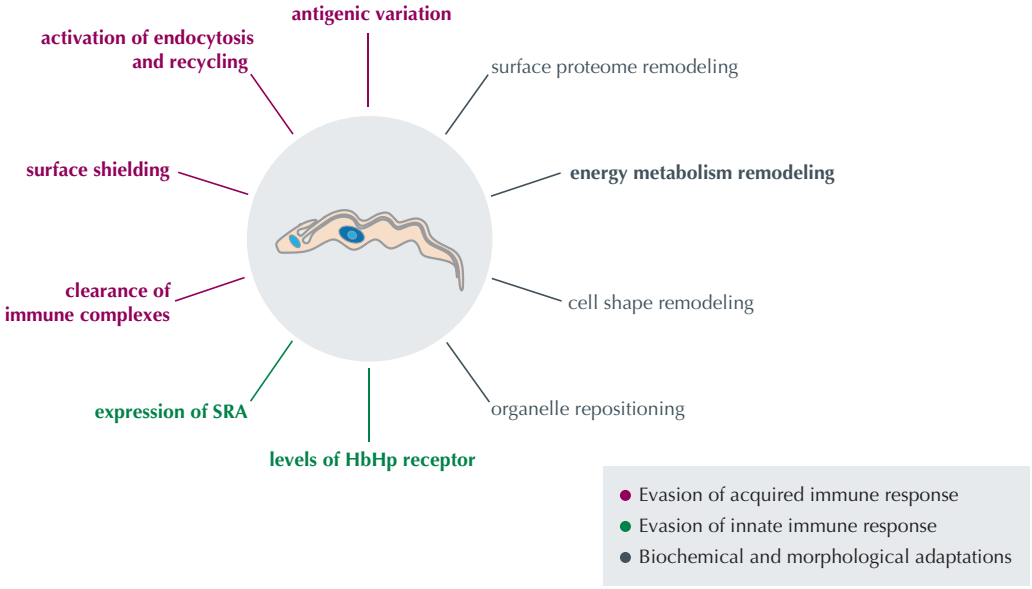

Fig. 1. The wheel of death and the multiple factors permitting trypanosome infection and pathology. At least three distinct overarching challenges in host–parasite interactions have to be met by African trypanosomes in order to survive in the mammalian host; (i) evading the acquired immune system (magenta), (ii) evading innate defense mechanisms (green) and (iii) adapting to the local biochemical and physiological environment (grey). Each can be subdivided into distinct mechanistic processes, where we have good evidence that the process is vital (bold), more circumstantial evidence or, presently, none at all. While potentially a teleological argument, the major changes that accompany the shift to the bloodstream form from the insect stages can, for the most part, be assumed to contribute in some manner to fitness, and hence will be selected for over a period of many generations, or arise through a direct and necessary consequence of such an adaptation; the precise reasons are not always fully clear. The trypanosome is shown as a schematic at the center, with the kinetoplast and nucleus in blue.

2.3. Antibody clearance

Antigenic variation is the major mechanism of chronic immune evasion but, as an important adjunct, the bloodstream parasite is pre-adapted to actively recycle the surface coat, facilitating rapid removal of VSG–antibody complexes, and presumably also ISG–antibody complexes [7]. The trypanosome flagellum and flagellar motility also contribute to this strategy – hydrodynamic forces generated by trypanosome motility result in surface drag. As this drag is increased by an immunoglobulin bound to a VSG, the VSG–antibody complexes are propelled towards the posterior of the cell [30]. This mechanism may explain why ablation of many flagellar proteins is lethal to the bloodstream form both *in vitro* [31] and in models of infection [32], but not to procyclic parasites. In fact, a recent genome wide study shows that up to half of knockdowns causing bloodstream-form growth defects are of genes associated with flagellar function [33]. Importantly, the very high rates of endocytic and recycling membrane transport in bloodstream form parasites are constitutive and do not require an antibody-mediated signal for activation, so the increased number of gene products required for normal proliferation uncovered by these studies most probably reflect this more active configuration of the transport system, rather than being a direct impact on antibody uptake. Whereas antigenic variation allows long-term survival of the infecting population but not of specific individuals, clearance of surface-bound immunoglobulin is an important contributor to the survival of individual parasites.

2.4. Activation of the endocytic system

Antibody–VSG complexes are internalized by clathrin-mediated endocytosis at the flagellar pocket [34], the sole site of endo- and

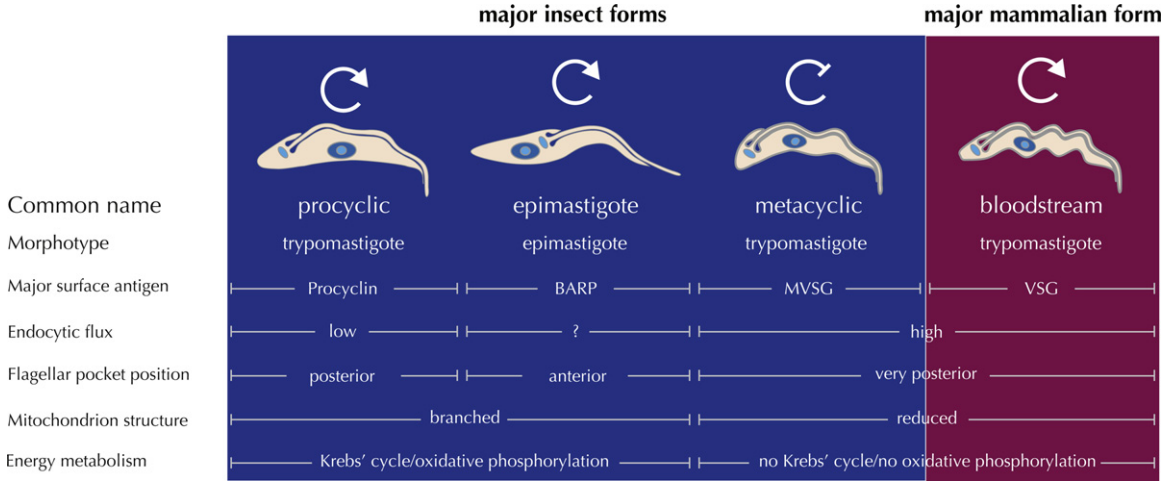

Fig. 2. Simplified schematic representation of some of the developmental forms found in the life cycle of *T. brucei*. A circular arrow above a developmental form indicates that this form is proliferative, while an interrupted circle accompanies a non-proliferative form. Features encompassed by horizontal grey lines represent some of the principal biochemical, cellular and morphological changes for each cell form.

exocytosis performed by the parasite. From Rab5-positive early endosomes, antibodies are targeted to the lysosome for degradation *via* Rab7-positive late endosomes, whereas VSG is efficiently recycled to the cell surface [35]. Assuming that this process is vital for survival within the host, it is most probably significant that the requirement for rapid capping and internalization of VSG-bound antibodies coincides with up-regulation of components of the endocytic pathway in metacyclic- and bloodstream-form parasites, especially as endocytosis imposes significant energy demands for the parasite [7,36]. Indeed, mRNA levels of clathrin and the recycling endosomal marker Rab11 are all increased in metacyclic and bloodstream forms when compared to the procyclic stage [7,37]. Further, the endocytic system seems to have a very specific pathway for VSG, as ISG65 and ISG75 are sorted away from VSG in a ubiquitin-dependent manner, which results in a significantly shortened half-life for these invariant proteins [22,38–40]. Additional adaptations are also evident in the trypanosome endocytic system, all of which may contribute to heightened efficiency for removal of material from the surface [10]. Importantly, this system may also account for the absence of a protective effect by immunization with ISG protein [23]; if the parasite is able to internalize ISG-specific antibodies efficiently, no protective effect would be observed despite recognition of the parasite surface and observations that such antibodies are produced by infected mammalian hosts *in vivo* [41].

Though these findings highlight a potentially essential contribution of the endocytic pathway to survival in the host, most studies have been on parasites in axenic culture, so the precise contribution to trypanosome infectivity remains to be directly interrogated. Recently, we used clathrin heavy chain hemizygotes and parasites expressing mutant forms of Rab5 and Rab11 to induce less potent impacts on endosomal trafficking than full functional ablation [42]. Surprisingly, clathrin hemizygotes, and the Rab5 and Rab11 mutants were able to establish infections in mice. However, using a low number of parasites to initiate infection, it was found that the Rab11 recycling pathway contributed to survival in mice, while similar effects were not observed for either clathrin or Rab5. While interpretation of these data is complex and should be treated with caution, one possible suggestion is that bloodstream-form parasites operate an endocytic system in excess of that required for survival in mice, but that impairment in recycling of VSG to the surface somehow leads to compromised survival. While this study represents the first attempt to directly analyze the role of endocytosis in an *in vivo* context, these experiments were also performed with hypervirulent trypanosomes and over very short periods of infection. Therefore, it is likely that the full scope of interactions between host immunity and the parasite endosomal system remain to be uncovered, and additional investigations are required to fully address the functions and modifications of the bloodstream form endocytic pathway in establishing chronic infections in mammals.

3. Evasion of innate immune responses

The last ten years or so have seen the characterization of an important and exciting aspect of bloodstream form biology, and one that has a major impact on the ability of trypanosomes to infect humans. A large number of wild mammalian species in Africa are natural trypanosome reservoirs, but many higher primates and humans are resistant to infection by some strains of *T. brucei*. The identity of at least one primate-specific trypanolytic factor has been demonstrated as the serum protein apolipoprotein L1 (ApoL1), present in a subset of HDL particles that also contain haptoglobin-related protein (Hpr) and ApoA1, and referred to as trypanosome lytic factor (TLF) [reviewed in 43, 44]. As trypanosomes are deficient in haem biosynthesis [26], they need to accumulate haem from the host using a surface receptor for the haptoglobin–haemoglobin (Hp–Hb) complex [17]. The Hp–Hb receptor (TbHpHbR) is attached to the membrane by a single GPI-anchor, and restricted to the flagellar pocket and associated endosomes. TbHpHbR recognizes Hpr–Hb complexes, which increases the efficiency of haem accumulation. In humans, however, uptake of Hpr-containing HDL particles also conveys ApoL1 to the cell interior. Once internalized, a pH-sensitive membrane-targeting domain in ApoL1 appears to direct the protein to the lysosomal membrane, where anionic pore-forming activity triggers Cl^- ion influx from the cytoplasm. The resulting osmotic imbalance leads to lysosomal swelling and eventual rupture [45–47].

Therefore, ApoL1 represents an innate immune mechanism rendering several higher primate species resistant to *T. b. brucei* infection, and which exploits an essential parasite requirement to scavenge host haem. However, two *T. brucei* subspecies, *T. b. rhodesiense* (causing acute HAT in southern and eastern Africa) and *T. b. gambiense* (causing chronic HAT in western and central Africa) are resistant to ApoL1-mediated lysis, raising the question of how the bloodstream stages of these parasites evolved mechanisms facilitating human infection.

T. b. rhodesiense is genetically identical to *T. b. brucei*, except for the presence of a serum-resistance associated (SRA) gene [48]. SRA is a very divergent VSG-related molecule which lacks much of the N-terminal surface loops. A current model proposes that SRA interacts with the C-terminal region of ApoL1 and neutralizes the pore-forming activity in the endosome, prior to ApoL1 reaching the lysosome [46,49,50]. Importantly, the absence of significant sequence variation in SRA genes from different *T. b. rhodesiense* strains indicates that SRA is a recent acquisition, and may broadly correlate with the origins of humans and related primates in Africa. Unlike humans, baboons and several other primates including mandrills and sooty mangabeys, are not susceptible to infection by *T. b. rhodesiense* [51,52]. It appears that two specific lysine residues at the C-terminus of baboon ApoL1, that are absent from the human ortholog, prevent interaction between ApoL1 and SRA, such that baboon ApoL1 is able to kill *T. b. rhodesiense* [53]. Significantly, when expressed in mice, baboon ApoL1 can confer protection against *T. b. rhodesiense* [53], showing the central importance of the ApoL1-SRA interaction to innate resistance to *T. b. rhodesiense*.

In sharp contrast, human-infective *T. b. gambiense* lacks an SRA gene. Hence the mechanism of avoidance of TLF killing must lie elsewhere. Recently it was demonstrated that resistance to human serum-mediated lysis is a consequence of reduced expression and mutations to the *T. b. gambiense* TbHpHbR gene [54]. Therefore, by having lower copy number and/or an altered receptor on its surface, *T. b. gambiense* reduces internalization of Hpr/ApoL1-containing HDL particles, so avoiding lysis [54,55]. Given that trypanosomes rely on host haem for optimal growth and protection against oxidative damage by host macrophages, reduced Hp/Hb uptake may incur a fitness cost to the parasite [54,55]. Alternatively, additional mechanisms for haem uptake may be present in *T. b. gambiense*.

Interestingly, two rare alleles of APOL1 are common in North Americans of African descent, the presence of either confers resistance to both *T. b. rhodesiense* and *T. b. brucei*, but is also linked to an increased risk of renal disease [56]. This situation is very reminiscent of the association between resistance to severe malaria infection and the sickle cell haemoglobin allele [57]. It is likely that the defective protein prevents SRA from binding and neutralizing the trypanolytic action of these rare ApoL1 variants. Moreover, the fact that the defective gene only occurs in individuals of African origin suggests that natural selection might have fixed APOL1 mutants to counteract against parasite infection [56]. The exploitation of these natural ApoL1 variants as well as baboon ApoL1 offers great potential for the development of both human and animal-targeted

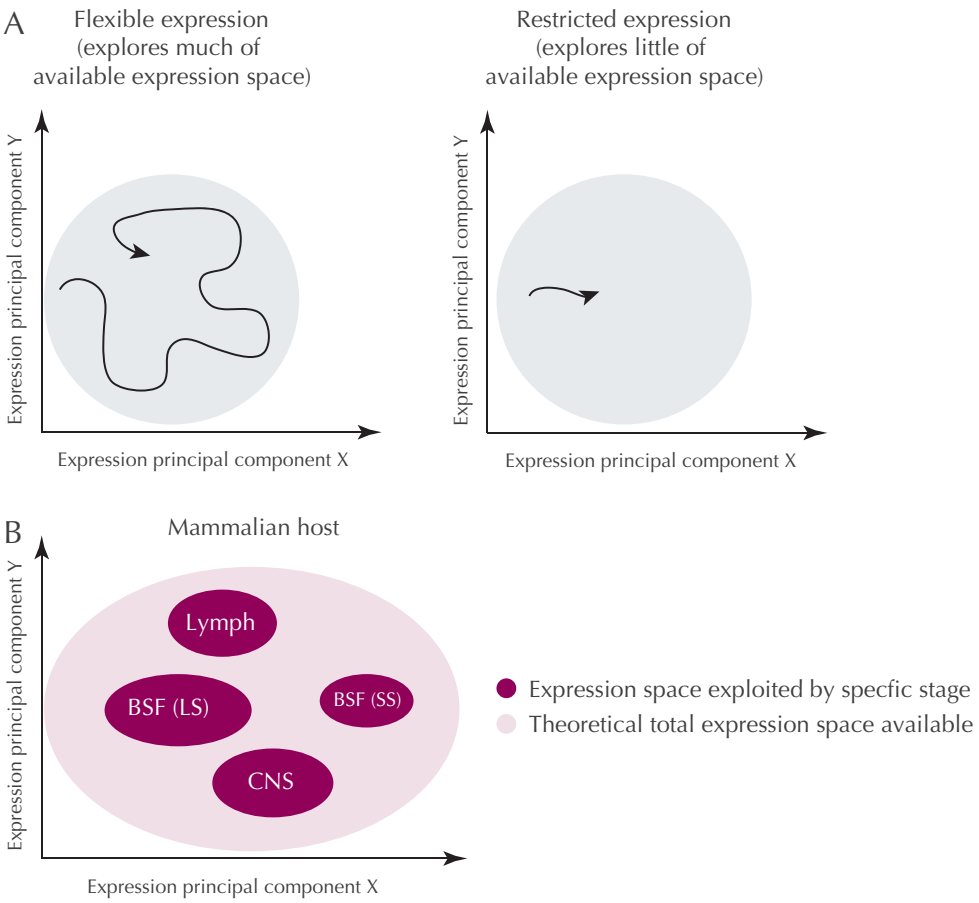

Fig. 3. Inflexible gene expression and multiple life stages. Panel A; in a flexible expression system an organism modulates the expression of its genes over a wide range, covering much of the permissible space that is allowed by the environment within which it dwells. By contrast, under a restricted program an organism does not alter gene expression significantly, which may reflect an adaptation to a constant environment or one where responses to changes to the environment provide no selective advantage. Panel B; conceptual model for multi-dimensional trypanosome expression profiles, reduced to an *x*, *y* two-dimensional principal component surface. In dark magenta are shown as lozenges mammalian stages, where expression is restricted to distinct areas for each form, but importantly in each case is a much smaller area than the total theoretically permissible. This is embedded within a larger lozenge representing the total expression area theoretically available. It is unclear if the restricted expression profiles would result from environmental or immunological pressures, such that the true available area to the parasite is in fact much smaller than theoretically possible, for example requiring continual expression of VSG genes in bloodstream stages, or from secondary loss of stress responses due to an absence of a selective advantage for retaining these mechanisms.

therapeutics, and underscores how a study of basic immune mechanisms can lead to very real practical potential [58].

4. Biochemical and morphological adaptations

Immune effectors aside, the bloodstream of the mammalian host is also a very distinct environment to that of the tsetse fly in terms of nutrients, pH, temperature and mechanical stress. In order to meet this challenge, the parasite remodels its surface coat but also triggers large-scale biochemical adjustments to facilitate pre-adaptation. Among these, probably the most intensely studied are those changes that occur in energy metabolism. Upon injection of metacyclic parasites from the fly salivary gland into the mammalian bloodstream there is a switch from performing oxidative phosphorylation using the mitochondrion to relying entirely on glycolysis for ATP production. This is reflected in substantial changes in mitochondrial morphology; in contrast to the highly branched, cisternae-rich organelle of the insect form, a bloodstream-form cell possesses a less developed mitochondrion with substantially fewer branches [59,60]. In the glucose-rich environment that is the mammalian bloodstream, and with an abundance of glucose transporters on the surface of the parasite [61], shutting down mitochondrial metabolic function allows a reduction in protein biosynthetic requirements. This may not only conserve energy but also substantially reduce oxidative stress resulting from the flux of electrons through the transport chain at the mitochondrial inner membrane, and might convey a further selective advantage to the trypanosome.

Another well-studied biochemical pre-adaptation relates to the biosynthesis of large poly-N-acetylactosamine (pNAL) N-glycan moieties [62], which are present only in the bloodstream form, and associated with proteins of the flagellar pocket and endosomes [62,63]. As these large N-linked glycans are not detected in other regions of the plasma membrane or other intracellular compartments, these moieties have been suggested to play a role in determining protein localization in the cell, in particular in endosomal targeting [63]. Loss of the pNAL modification due to ablation of oligosaccharyltransferases can be tolerated in culture, leading to only a moderate proliferative defect [64]. However, these enzymes are required for parasite survival *in vivo* [64], suggesting that the pNAL glycans, while not required for basic viability, may make an essential contribution in infection; however it is unclear what this mechanism may be.

Modulation of the overall cell morphology and positioning of internal organelles critical for parasitism are also pre-adaptations of trypanosomes [65]. For example, the flagellar pocket is repositioned towards the posterior end of the parasite upon differentiation from the epimastigote to the metacyclic insect

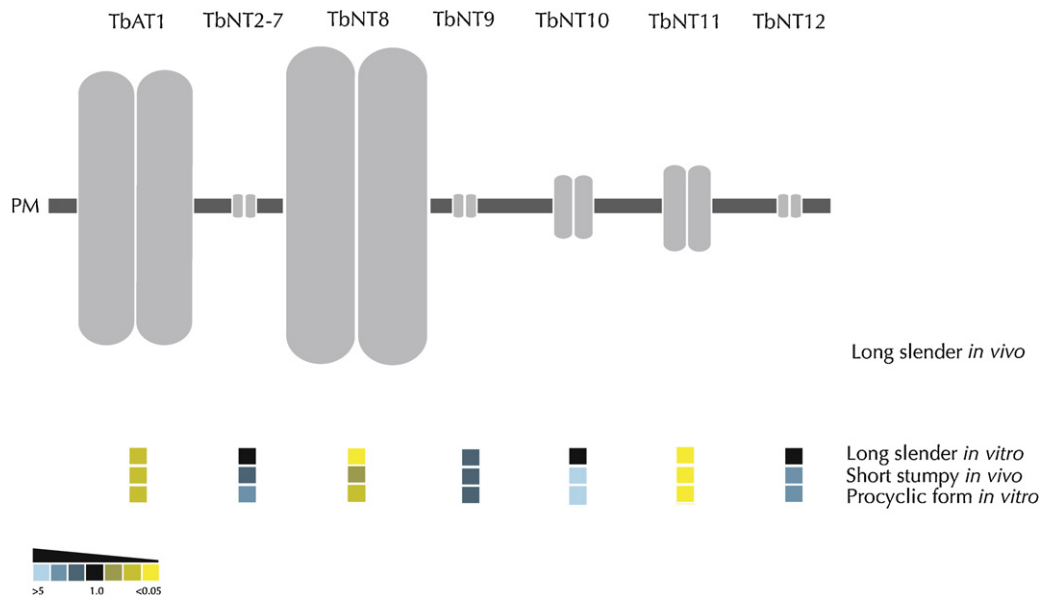

Fig. 4. Changes in mRNA levels for members of the equilibrative nucleoside transporter (ENT) family as an example of environmental remodeling. mRNA levels in *T. brucei* from long slender forms grown *in vivo* for the seven identified members of the ENT family of transporters are represented by area. TbNT2-7 transporters share a high percentage of sequence similarity and related transporter properties and, therefore, have been collapsed into one single group. Fold increase (blue) or fold decrease (yellow) in mRNA levels compared to that of long slender forms *in vivo* is shown below each transporter, for long slender *in vitro*, short stumpy *in vivo* and procyclic *in vitro* cells. Black represents equivalent mRNA levels. There is significant differential regulation of mRNA for this family of transporters, which may suggest modifications to facilitate adaptation to distinct environments within the different hosts or culture conditions. Representation of mRNA levels is based on qRT-PCR data (not shown).

stages [66]. It is not quite clear how that might contribute to the success of the parasite in the mammalian bloodstream, although it is tempting to speculate that it may facilitate VSG-bound antibody clearance from the surface of the parasite by hydrodynamic drag [30]. Mathematical models in which the flagellar pocket is repositioned more distal from the posterior of the cell suggest that the delivery of surface molecules to the flagellar pocket becomes less efficient, consistent with the concept that the posterior positioning of the organelle is a selective advantage and hence an important contributor to the immune evasion mechanism (MCF and Alain Pumir, unpublished data). It is unclear if this represents an essential component of the virulence system, simply offers a selective advantage or is a fortuitous consequence of a morphological transition that occurs for other reasons, what is colloquially known as an evolutionary spandrel.

Finally, in this regard, the structural appearance of the nucleus also changes during the trypanosome life cycle, showing increased electron dense material likely corresponding to chromatin condensation in the bloodstream form when compared to the procyclic insect stage [67,68]. A differential level of nuclear compaction is generally associated with transcriptional status, and it is possible that the heterochromatin-rich bloodstream nucleus correlates with a default mechanism for switching off all inactive VSG genes. At this time the only very clear example of compartmental specialization that has been documented for the bloodstream nucleus is the presence of the ESB [29], but it is unlikely that this is the sole aspect.

5. Gene expression changes – a whole genome view

Transcriptome analysis, i.e. measurement of levels of all mRNA in a cell at a given time, has the potential to allow very precise definition of pathways and processes altering during life cycle adaptation. Transcriptome analysis is very much influenced by the minor role of promoters in trypanosomes for control of individual transcript levels, with the result that most mRNA are post-transcriptionally regulated [69] (Fig. 3). Early studies identified limited changes to mRNA levels between bloodstream and procyclic stages [70,71], suggesting that significant changes to a small cohort of genes were sufficient for life cycle remodeling. Subsequent studies have indicated more extensive regulation [21,72]. The latter study demonstrated that >90% of predicted ORFs are transcribed, and detected very limited mRNA level variance between proliferative and non-proliferative bloodstream forms. The most highly expressed genes include those involved in the cytoskeleton, translation and several other core processes, but with a significant number of uncharacterized gene products. This suggests that alterations to cell morphology and much of the basic cell biology of bloodstream forms arises *via* regulatory proteins, present at lower copy number, rather than direct remodeling of the high abundance structural proteins, and hence the structures, themselves. Where variable expression between life stages is most notable is in primary metabolism, DNA metabolism and translation. By contrast, ubiquitin pathways and chromatin-remodeling factors are comparatively invariant, consistent with our present understanding of these processes [72].

Investigations of stress conditions suggest a rather static and inflexible transcriptional system in bloodstream-form parasites, where there may be alterations at the protein level that are not reflected at the mRNA level [21] (Fig. 3). This may be partly due to the absence of pathways associated with modulation of mRNA due to stress that are present in most other organisms, including the unfolded protein response, activated in response to chemical insults such as dithiotreitol and tunicamycin, both of which interfere with protein folding [21]. We suggest that this phenomenon is likely a secondary loss, and a similar phenomenon is also observed for intra-erythrocytic stages of *Plasmodium falciparum* where conditions within the host bloodstream are normally invariant [73].

An interesting potential example of subtle modulation of expression to adapt to distinct environments is provided by the purine scavenge pathway. Similar to most protozoan parasites, trypanosomes are auxotrophic for purines and therefore rely entirely on salvage from the host environment for their purine supply [74]. A family of polytopic transporters, the equilibrative nucleoside transporters (ENTs), present at the cell surface, are responsible

for nucleoside uptake, as well as several trypanocidal compounds [75]. There are significant differences to the expression of purine permeases of the ENT family during progression through the life cycle (summarized in Fig. 4), with alterations in the levels of mRNA for several family members between life stages. We speculate that this modulation of ENT mRNA levels may be required to accommodate the likely distinct nucleoside compositions of the mammalian bloodstream and the tsetse fly gut. Similarly, two isoforms of the *T. brucei* glucose transporters, trypanosome hexose transporter one and two (THT1 and THT2) are found at the cell surface. THT1 is most abundantly expressed in bloodstream forms and facilitates a low affinity, high capacity glucose uptake system [76]. Therefore, unique biological roles for these transporters could promote the viability of the parasite in its natural environment.

In summary, there is clear variance in steady state levels of mRNAs between life stages. Indications of where these differences lie provide important clues to how the parasite remodels for survival in the bloodstream. However, discrepancies between datasets, arising from biological and analytical differences, highlight a need to standardize conditions, strains and protocols.

6. Perspectives

The African trypanosome is among the deadliest of human pathogens, with a fatality rate approaching 100% for untreated infections. The high virulence of the East African trypanosome likely represents the fact that it is more often a parasite of non-human mammals. This is supported by the apparently recent evolution of SRA by *T. b. rhodesiense* as a resistance mechanism against TLF. *T. b. gambiense* infection is also invariably fatal but does not cause such acute illness. For these and other trypanosomes, multiple evolutionary pressures have been exerted, encompassing mechanisms for evading both the host acquired and innate immune attacks, as well as adapting to the biochemical and physical environment in the bloodstream. These mechanisms have clearly arisen at different times during the evolution of the trypanosomes and related species: (i) the overall cellular architecture is an ancient feature shared by all trypanosomatids, and present in insect, plant and mammalian-infective lineages; (ii) antigenic variation of the form exploited by *T. brucei* is restricted to African and closely related Asian trypanosomes and; (iii) SRA represents a specific and most recent adaptation, and is likely associated with the crossing to higher primate hosts. Our understanding of the molecular basis for many of these processes has advanced greatly in the past decade and, together with some resurgence in development of small molecule therapeutics against trypanosomes, this knowledge is showing genuine promise for breaking the bloodstream wheel of death.

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