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The developmental cell biology of *Trypanosoma brucei*

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Summary

Trypanosoma brucei provides an excellent system for studies of many aspects of cell biology, including cell structure and morphology, organelle positioning, cell division and protein trafficking. However, the trypanosome has a complex life cycle in which it must adapt either to the mammalian bloodstream or to different compartments within the tsetse fly. These differentiation events require stage-specific changes to basic cell biological processes and reflect responses to environmental stimuli and programmed differentiation events that must occur within a single cell.

The organization of cell structure is fundamental to the trypanosome throughout its life cycle. Modulations of the overall cell morphology and positioning of the specialized mitochondrial genome, flagellum and associated basal body provide the classical descriptions of the different life cycle stages of the parasite. The dependency relationships that govern these morphological changes are now beginning to be understood and their molecular basis identified. The overall picture emerging is of a highly organized cell in which the rules established for cell division and morphogenesis in organisms such as yeast and mammalian cells do not necessarily apply. Therefore, understanding the developmental cell biology of the African trypanosome is providing insight into both fundamentally conserved and fundamentally different aspects of the organization of the eukaryotic cell.

Keywords

Morphology; Cell division; Differentiation; Life cycle

Introduction

In multicellular organisms, the production of differentiated cells allows specialization and adaptation to a variety of functions or environmental conditions. These can be preprogrammed, generating differentiated tissues as part of normal development, or be responsive to signals as in the case of stem cells. By contrast, single-celled organisms need only respond to their environment to survive, at least under relatively stable conditions. However, in many cases, they also display programmed development as part of their life cycle. This enables survival in unstable conditions or dispersal.

The parasitic protozoa are excellent examples of organisms that display extreme adaptation to their environment, in many cases because they must evade the immune response of a mammalian host. They also exhibit complex life cycles, frequently being transmitted between mammalian hosts by arthropod vectors, in which they often also face similarly hostile conditions. The variety of different conditions encountered means that a single

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organism (or its offspring) must show both adaptability and the capacity for rigorously programmed differentiation. Occurring within a single cell, these developmental steps require coordinated modulation of many basic biological processes. Here, I discuss recent advances in our understanding of the basic aspects of the cell biology of the parasite *Trypanosoma brucei* and place these into the context of its life cycle.

Background

Trypanosoma brucei spp (comprising *Trypanosoma brucei brucei* and the human infective forms *T. b. rhodesiense* and *T. b. gambiense*) are parasites responsible for African sleeping sickness in 36 countries of sub-Saharan Africa, which are the poorest developing countries worldwide. Sleeping sickness has a devastating impact on human health and prosperity: >0.5 million cases and 70,000 deaths per year are a result of the parasite and the disease is always fatal unless treated. The parasite also infects cattle and game animals (where it causes the disease 'nagana'), thereby restricting agricultural development and significantly contributing to poverty in afflicted areas. In the past thirty years, failures in control measures and treatment regimes have caused the impact of this parasite to increase markedly, such that it is now the biggest killer in parts of Africa, surpassing HIV/AIDS in provinces of Angola, Congo and Southern Sudan (http://www.who.int/en/).

The trypanosome is transmitted between mammalian hosts by the tsetse fly, *Glossina* spp, in which it initially establishes in the midgut after a bloodmeal but then migrates to the salivary glands in preparation for transmission to a new mammalian host (Fig. 1). In mammals, the parasite survives free in the bloodstream, being able to evade antibody responses through antigenic variation (McCulloch, 2004; Pays et al., 2004). This entails the sequential expression of antigenically distinct variable surface glycoproteins (VSGs), which are linked to the surface membrane by a glycosylphosphatidylinositol (GPI) anchor. Trypanosomes proliferate in the mammalian bloodstream as morphologically slender forms, these being replaced by non-proliferative stumpy forms as parasite numbers increase (Matthews et al., 2004). This serves two purposes. First, the accumulation of division-arrested forms limits the increase in parasite numbers and thereby prolongs host survival (and hence the probability of disease transmission). Second, the uniform arrest of stumpy forms in G1 phase of the cell cycle ensures that the morphological changes that occur upon transmission to the tsetse fly can be coordinated with re-entry into the cell cycle. This is important because correct organelle positioning is crucial for successful completion of the cell cycle of tsetse midgut procyclic forms.

Upon uptake by the tsetse, bloodstream trypanosomes replace the VSG coat with a less-dense surface coat composed of procyclins, which are also GPI anchored (Roditi and Liniger, 2002). Energy generation also switches from being exclusively based on glycolysis to a mitochondrion-based respiratory system, which requires structural elaboration and metabolic activation of the organelle. After proliferation in the tsetse midgut, the parasite migrates to the salivary gland. The epimastigote forms generated there attach to the gland wall through elaborations of the flagellar membrane. After further multiplication, the parasite undergoes division arrest, reacquires a VSG coat and is released into the salivary gland lumen, in preparation for inoculation into a new mammalian host.

Most studies on the trypanosome have focused on the bloodstream and procyclic forms of the parasite. This is because these stages can be readily cultured in vitro. They are also genetically tractable: knockouts can be generated through homologous recombination, and tetracycline-regulatable ectopic expression and RNA interference (RNAi) approaches are routine. Forward genetic approaches have been more limited since trypanosomes are diploid and the generation of sexual crosses is experimentally challenging; however, the recent

production of large-scale RNAi libraries (Morris et al., 2002) and the development of a mariner-based transposon mutagenesis strategy (coupled with frequent loss of heterozygosity; Leal et al., 2004) offer exciting prospects for informative mutant screens.

Trypanosome cell architecture

The trypanosome cell is elongated and has a highly polarized microtubule cytoskeleton (Fig. 2). This defines the cell shape and remains intact throughout the cell cycle, the daughter microtubule cytoskeleton being assembled and interdigitated between existing microtubules, such that cytoskeletal inheritance is semiconservative (Sherwin and Gull, 1989b). The microtubules show a uniform polarity: minus ends are anterior and plus ends are posterior (Robinson et al., 1995). The single-copy organelles in the trypanosome cell (i.e. the flagellar pocket, flagellum, kinetoplast, mitochondrion and nucleus) are precisely positioned within the cytoskeletal corset and are concentrated between the posterior end and the centre of the cell.

The most posterior structure is the mouth of the flagellar pocket. This is the exit point for the flagellum, which is tethered along the exterior length of the parasite. The flagellar pocket is the only site of endo- and exo-cytosis (Overath and Engstler, 2004); this is important in bloodstream forms, in which the surface membrane is densely packed with VSG to protect against the alternative pathway of complement activation (Ferrante and Allison, 1983) and to shield common antigenic determinants from immune recognition. This dense packing requires that the GPI-anchored VSG is significantly concentrated during trafficking from the endoplasmic reticulum (ER) to the surface (Grunfelder et al., 2002). Indeed, overall membrane uptake and VSG recycling at the flagellar pocket occur at a phenomenal rate in bloodstream cells, considering the restricted area of the flagellar pocket (the surface VSG pool turns over entirely within 12 minutes despite the flagellar pocket occupying only 5% of the cell surface area; Engstler et al., 2004). Endocytosis of the VSG and other molecules in the flagellar pocket is clathrin dependent: RNAi directed against the clathrin heavy chain causes rapid death of cells after massive enlargement of the flagellar pocket (Allen et al., 2003). A similar phenotype is seen when actin is targeted by RNAi in bloodstream forms, implicating this protein in endocytosis and intracellular trafficking (Garcia-Salcedo et al., 2004). Endocytosis in procyclic cells occurs at a lower rate, but is also clathrin dependent (Allen et al., 2003; Hung et al., 2004). Interestingly, however, RNAi directed against actin is not lethal in this life cycle stage (Garcia-Salcedo et al., 2004).

The small size of the trypanosome, its high rate of trafficking of GPI-anchored VSG to the surface and the concentration of the endocytic apparatus into the posterior end of the cell have made bloodstream forms an excellent system for analysis of protein trafficking using fluorescence and electron microscopy. Moreover, the single Golgi stack of the parasite and its restricted positioning between the nucleus and flagellar pocket has enabled *T. brucei* to become a model for the biogenesis of this organelle in eukaryotes. Thus, He et al. have derived a new marker for the *T. brucei* Golgi (TbGRASP) similar to mammalian GRASP-55 and used a photobleaching approach to demonstrate that, during cell division, the new Golgi forms de novo from material sourced directly from the old Golgi, rather than from the ER (He et al., 2004).

The motility of the trypanosome is dependent upon its single flagellum, which has a conventional axonemal structure plus an associated paraflagellar rod (Vaughan and Gull, 2003). This is a semi-rigid structure found in the kinetoplastids and euglenoids that contributes to parasite motility (Bastin et al., 1998), perhaps assisting trypanosome flagellar beat efficiency in the viscous mammalian bloodstream. Trypanin (Hutchings et al., 2002), a trypanosome protein related to a subunit of the dynein motor regulatory complex (PF2 in

Chlamydomonas and Gas11/Gas 8 in mammalian cells), also contributes to motility (Rupp and Porter, 2003). Trypanin contains a microtubule-binding domain and appears to stabilize the interaction between the flagellum and the subpellicular cytoskeleton, in a region called the flagellum-attachment zone (Kohl et al., 1999). This binding seems to impart directional motility on the parasite, since RNAi directed against trypanin causes uncontrolled tumbling, which contrasts with the paralysis induced by disruption of the paraflagellar rod. As well as assisting an understanding of motility, the study of flagellar proteins is also proving valuable for the dissection of the intraflagellar transport machinery, for which the tools available for gene function analysis make *T. brucei* an excellent model (Ersfeld and Gull, 2001; Kohl et al., 2003).

During cell division, the growing daughter flagellum precisely tracks the old flagellum, such that structural information is transferred from the old to the new flagellum through a novel example of cytotaxic inheritance (Moreira-Leite et al., 2001). This information is imparted through the flagellar connector, a mobile structure that connects the tip of the new flagellum with three of the doublet microtubules of the axoneme of the old flagellum (Briggs et al., 2004). Although it is apparently fundamental to the division of the procyclic form, there is no evidence for the existence of a flagellar connector in the bloodstream stage of *T. brucei*, nor is any related structure obvious in other kinetoplastids (Briggs et al., 2004). This surprising finding highlights very basic differences in cell-cycle control for different life cycle stages of trypanosomes. Perhaps the procyclic trypanosome population can afford fewer mistakes in cell division as it struggles to establish a foothold in the tsetse fly.

The trypanosome flagellum originates in a basal body that is, in turn, linked through the mitochondrial membrane to the mitochondrial genome, which comprises a mass of catenated DNA termed the kinetoplast. The kinetoplast and basal body are linked by a tripartite attachment complex that must traverse both the cell and the mitochondrial membranes (Fig. 3) (Ogbadoyi et al., 2003). This comprises a series of filaments providing guide ropes through which mitochondrial genome segregation is linked to replication and segregation of the basal body and flagellum. This, in turn, is linked to the microtubule cytoskeleton, such that drugs that disrupt the cytoskeleton prevent both basal body and kinetoplast segregation (reviewed by Gull, 2003).

The mitochondrion itself is a single elongated structure that runs from the posterior to the anterior of the cell. In bloodstream forms, the mitochondrion is a simple tubular structure devoid of cristae. This reflects the absence of mitochondrial respiration during this stage, energy generation being dependent on glycolytic reactions compartmentalized within specialized organelles termed glycosomes (Parsons, 2004). However, the procyclic form does not have the luxury of blood glucose as an abundant energy source and has a highly active mitochondrion. This is atypical in that an acetate:succinate CoA transferase and succinyl-CoA synthetase cycle is present (Bochud-Allemann and Schneider, 2002; van Weelden et al., 2003), in addition to the components of the Krebs cycle and the electron transport chain. Acetate:succinate CoA transferase, which also operates in the anaerobic mitochondria of some metazoa and anaerobic protists but not mammals, generates ATP by the conversion of acetyl coA to acetate. As in the case of other single-copy organelles, the mitochondrion must segregate with fidelity during cell division, and one contributor to this appears to be a dynamin-like protein (TbDLP), which is involved in the division of the mitochondrial membrane either directly or through recruitment of other effectors of membrane scission (Morgan et al., 2004). Interestingly, this protein is encoded by the only gene of this family identified in the *T. brucei* genome, which excludes a role for *T. brucei* dynamin-like proteins in intracellular trafficking – a common role for such GTPases in other eukaryotic cells.

Cell division and organelle positioning

The high degree of structural organization of *T. brucei* has significantly facilitated analysis of how its cell cycle and other processes are regulated. For example, the mitochondrial genome has a discrete periodic S phase and G2 phase that is coordinated with nuclear genome replication and segregation. This has meant that simple analysis of DAPI-stained cells allows the cell-cycle position of individual parasites to be easily mapped. Immunostaining using antibodies against structural elements of the cell can also be superimposed onto this, providing a higher level of definition (Sherwin and Gull, 1989a). Such assays have established several unusual features of the trypanosome cell cycle. The first is the strict regulation of organelle positioning during division. Thus, the kinetoplast-nuclear, and kinetoplast-posterior dimensions are fixed during the cell cycle of the procyclic form, cell growth at the posterior end occurring between the segregating basal bodies and being maintained throughout S, G2 and M phase (Robinson et al., 1995). The second is the observation that cytokinesis is not dependent upon the completion of nuclear mitosis: disruption of the mitotic spindle by drugs or genetic perturbation of trypanosome cyclin-dependent kinases (CDKs) generates cytoplasts that have a mitochondrial genome but no nucleus (these are termed zoids; Ploubidou et al., 1999; Hammarton et al., 2003; Li and Wang, 2003). Perhaps the temporal and spatial orchestration of organelle positioning by the cytoskeleton early during the cell cycle is incompatible with a delay in cytokinesis caused by a mitotic checkpoint.

The most obvious morphological difference between the different life cycle stages of the trypanosome is the position of the kinetoplast relative to the posterior end of the cell. In bloodstream forms, the kinetoplast lies close to the posterior end of the cell, and each daughter kinetoplast remains in this region throughout the cell cycle until cytokinesis. By contrast, the kinetoplast lies midway between the cell nucleus and posterior in procyclic forms; in epimastigote forms (in the tsetse salivary gland), the kinetoplast is anterior to the central nucleus (Fig. 4). The reasons for these shape changes during the life cycle are completely unknown, although they are clearly required to establish the cell architecture necessary for cell division of each life cycle stage. Perhaps an increased length of attached flagellum along the cell body assists motility in bloodstream forms, whereas the longer anterior flagellum aids substrate attachment of the epimastigote stage.

The mechanics of kinetoplast repositioning have been studied during differentiation between bloodstream and procyclic forms and have been found to comprise two components. Most importantly, the posterior end of the cell grows out by ~3 μm through polar extension of the microtubule cytoskeleton. The kinetoplast itself also moves towards the nucleus as DNA replicates during differentiation of nonproliferative stumpy forms to procyclic forms (Matthews et al., 1995).

Several molecules that can influence this aspect of the trypanosome cell in a stage-specific manner have been identified. The first, TbZFP2 (Hendriks et al., 2001), was identified as a member of a novel family of small proteins possessing a $CX_8CX_5CX_3H$ zinc finger, which is predicted to bind RNA. Although uniformly expressed during the life cycle, TbZFP2 generates a procyclic, stage-specific morphological phenotype – ‘nozzle formation’ – when ectopically overexpressed (Fig. 5). This displays expansion of the kinetoplast-posterior dimension of the cell, in some cases leading to cells twice their normal length. The cells also accumulate in G1 phase, either because TbZFP2 overexpression perturbs the cell-cycle machinery, or because the resulting cytoskeletal disruption prevents cell-cycle progression. Overexpression of a related small CCCH zinc-finger protein (TbZFP3) produces a similar procyclic-specific phenotype (A. Paterou and K.R.M., unpublished), as does RNAi directed against CYC2, a trypanosome cyclin (Hammarton et al., 2004; Li and Wang, 2003).

However, in these cases, the cell-cycle arrest is less stringent, and nozzled cells are able to segregate their kinetoplasts.

Although it is likely that some mutants demonstrating the nozzle phenotype are a consequence of cell-cycle defects or secondary perturbations in the cell, recent evidence suggests that there is a relationship between nozzle formation and the normal process of kinetoplast repositioning during differentiation. Thus, a third small CCCH molecule, TbZFP1, is transiently expressed during synchronous differentiation to procyclic forms when the kinetoplast is normally repositioned (Hendriks et al., 2001). Significantly, knocking out this gene specifically compromises kinetoplast repositioning during differentiation, whereas ectopic expression of a rescue copy restores it (E. Hendriks and K.R.M., unpublished). The TbZFP proteins are small molecules (<150 residues in length) that contain both RNA- and protein-binding motifs. It is expected, therefore, that these proteins act in concert with other (currently unidentified) proteins to govern mRNA abundance or translational capacity. Their stage-specific effects indicate a role in differential gene expression.

Stage-specific components of the cytoskeleton have also been identified, including cytoskeleton-associated protein (CAP)15, which is enriched in bloodstream forms, and CAP17 (Vedrenne et al., 2002) and CAP5.5 (Hertz-Fowler et al., 2001), which are both procyclic specific. These proteins do not label microtubules uniformly, each being absent on the flagellum, and CAP15 and CAP17 show reduced abundance at the posterior end of the cell, where the cytoskeleton is most dynamic. This might indicate an association with microtubule stability. Supporting this, expression of CAP15 in mammalian cells promotes resistance to microtubule depolymerization by nocodazole, whereas ectopic overexpression in trypanosomes perturbs normal cell structure and division. Molecules important in stage-specific morphology are therefore emerging, and it will be interesting to discover how their roles differ in distinct stages of the life cycle.

Complexity in two genomes

Analysis of the cell structure and division of trypanosomes emphasizes the central importance of the kinetoplast. As well as providing a single mitochondrial genome, whose replication is coordinated with that of the nucleus, the kinetoplast has many other unusual features. It is composed of two classes of circular DNA: maxicircles and minicircles (Klingbeil and Englund, 2004). The maxicircles (~50 copies/kinetoplast) contain the genes that encode mitochondrial proteins, whereas the minicircles (~10,000 copies/kinetoplast) encode short guide RNAs. These act as templates through which maxicircle transcripts are post-transcriptionally edited by the incorporation or deletion of uridines. Extensive RNA editing is a unique feature of kinetoplastid parasites that requires a multiprotein editing complex, the editosome (Madison-Antenucci et al., 2002). Interestingly, RNA editing is also developmentally regulated; for example, cytochrome oxidase subunit II mRNA is edited to correct a frame shift only in procyclic forms (Feagin and Stuart, 1988).

Ablation of one component of the editing complex, RNA ligase, reveals that RNA editing (and hence mitochondrial DNA) is required in both bloodstream and procyclic forms (Schnaufer et al., 2001). This was a surprising finding, since several bloodstream-form akinetoplastic *T. brucei* mutants have been selected by parasite growth in the presence of DNA-intercalating agents. Nonetheless, it is supported by the observation that RNAi directed against a component of the kinetoplast replication machinery, topoisomerase II, is lethal in bloodstream-form parasites (Timms et al., 2002) as well as procyclic forms (Wang and Englund, 2001). Other components of the mitochondrial DNA replication apparatus are

unique to kinetoplastids, including at least six DNA polymerases (Klingbeil et al., 2002) and a novel DNA ligase (Sinha et al., 2004).

The nuclear genome of trypanosomes shows an equivalent complexity: *T. brucei* contains 11 'megabase' chromosomes and >100 ~50 kb minichromosomes. These minichromosomes harbour a repertoire of *VSG* genes, each flanked by a 177 bp repeat sequence (Wickstead et al., 2004). Although the minichromosomes segregate with fidelity (Wickstead et al., 2003), this is not through association with the microtubule spindle via kinetochore attachment but rather results from tracking along the microtubules that run from pole to pole (Ersfeld and Gull, 1997). The megabase chromosomes have a more conventional organization and segregation, although their gene organization is not. The genes are arranged in polycistronic arrays that have distant upstream (as yet unidentified) promoters. These gene arrays are not organized into operons, but instead genes that are differentially expressed through the life cycle can be adjacent. Stage-regulated gene expression is almost exclusively controlled at the post-transcriptional level, through differential RNA processing (all genes are trans-spliced; only one gene that has a cis intron has been identified; Mair et al., 2000), mRNA stability and translation (Clayton, 2002).

Uniquely, the genes that encode the major surface antigens of the bloodstream and procyclic form are transcribed by RNA polymerase I (Gunzl et al., 2003). Moreover, *VSG* genes are always transcribed from expression sites at chromosome ends. Only one of these is active at any one time and this is apparently due to association of the telomere with an expression site body, a discrete and structurally stable RNA polymerase 1 transcription factory located at the nuclear envelope (Navarro and Gull, 2001). VSG expression control breaks down in stumpy forms as the trypanosome prepares for transmission to the procyclic form (Amiguet-Vercher et al., 2004). In the procyclic form, two forms of procyclin are expressed: EP and GPEET. These are named for the amino acid repeat that is contained within each protein and each is functionally distinct. GPEET is essential in procyclic forms, whereas EP knockouts are viable as procyclic culture forms but infect tsetse flies poorly. Both forms are rapidly induced during differentiation; however, GPEET expression is downregulated as the differentiating cells become established. This is mediated through a response element contained within secondary structure in the 3′-untranslated region of the GPEET mRNA. This regulates mRNA stability in response to glycerol and glucose, presumably through interaction with RNA-binding proteins (Vassella et al., 2000). Interestingly, this control operates through the activity of mitochondrial enzymes (Vassella et al., 2004), thereby linking developmental surface antigen expression with environment sensing and metabolic activity (Morris et al., 2002). Dissecting the chain of interactions between the external environment and gene expression will be key to understanding the control of the life cycle. The limited importance of transcription initiation and transcription factors in regulated gene expression suggests that there is a novel complexity to the post-transcriptional mechanisms governing developmental processes in trypanosomes.

The trypanosome life cycle: interconnections and integration of the whole cell

The changes in the cell biology of the parasite as it traverses from the mammalian bloodstream to the tsetse fly and back again must be highly regulated and interconnected. Mapping of the events that occur during synchronous transition from bloodstream stumpy forms to procyclic forms has revealed that the developmental programme of the parasite is temporally ordered. Thus, the insect-specific EP procyclin coat is gained after 2 hours, the VSG coat is lost after 4-5 hours, kinetoplast repositioning and DNA synthesis then occur between 6-12 hours after the initiation of differentiation, and the cells go back into a proliferative cell cycle and develop procyclic-specific mitochondrial activity after 14-24

hours (Matthews, 1999). Inevitably, this requires coordination between different events if a viable procyclic cell is to be generated. For example, the kinetoplast repositions prior to the segregation of this organelle and subsequent mitosis during the first cell cycle of the differentiating parasite. As highlighted earlier, this is important because inappropriate migration of the kinetoplast during or after segregation of that organelle would be likely to disrupt the highly orchestrated events required for successful cell division. Cross-talk between the regulatory mechanisms governing nuclear and mitochondrial gene expression must also exist to ensure coordinate regulation of multiprotein subunit complexes that contain components encoded in both organelles (respiratory components, for example) and it is not clear which genome is dominant. The mitochondrion and mitochondrial genome clearly have a central role in the development of the cell; this is evident from the importance of mitochondrial metabolism in surface antigen regulation (Vassella et al., 2004) and the observation that the development of dyskinetoplastid cells stalls after the initiation of differentiation but before re-entry into the cell cycle (Timms et al., 2002).

Much less understood is the development of the trypanosome in the mammalian bloodstream and in the tsetse fly. Most experiments on bloodstream forms have focused on laboratory-adapted strains that grow uncontrolled in mammalian hosts (or in culture) without developing division-arrested stumpy forms. However, the transition between slender and stumpy forms poses many interesting problems of trypanosome biology that are still to be addressed. For example, cell division arrest and the transition from slender to stumpy forms are believed to be triggered by a parasite-derived signalling molecule, stumpy-induction factor (SIF; Vassella et al., 1997). However, although this is known to operate through a cyclic-AMP-dependent pathway, the identity of this molecule is unknown. Similarly, why do all trypanosomes not undergo irreversible transition to stumpy forms in the blood when the vast majority have been triggered to do so? This might reflect the fact that the bloodstream pool of trypanosomes is not uniformly exposed to SIF (for example, as a consequence of being sequestered in the tissues or lymphatic system) or that a subpopulation of parasites is refractory to SIF. These questions are interesting not only from the standpoint of trypanosome biology and the biology of cell fate and developmental decisions but also because understanding the generation of transmission-competent stumpy forms could provide new strategies for blocking trypanosome spread.

Conclusions and perspectives

The *T. brucei* genome is now complete (http://www.genedb.org/genedb/tryp/index.jsp), as are the genomes for related kinetoplastids, *Trypanosoma cruzi* and *Leishmania major*, providing a useful comparative resource for the triangulation of gene sequence and function. As always with such projects, the source material for sequencing the *T. brucei* genome was hotly debated in the field. However, a central argument for the selected line was that it be able to complete the life cycle through the tsetse fly. This will prove a good decision – for almost all aspects of trypanosome cell biology, understanding the differing requirements of the different life cycle stages will play a major part in understanding both constitutive and stage-specific processes. Through practical necessity, almost all work has focused on either bloodstream or in-vitro-cultured procyclic forms, or both. However, there remain huge gaps in our knowledge regarding what occurs in the tsetse midgut and salivary gland. For example, the existence of sexual exchange in the fly poses many interesting questions for cell biology, given the unusual genome and organellar organization of the parasite (Gibson, 2001). Likewise, the cytological and biochemical processes that occur in the salivary gland are challenging areas for trypanosome biology given their relative inaccessibility. These include organelle specialization (e.g. modification of the flagellum for substrate attachment), metabolic adaptations (the nutrient environment of the tsetse salivary gland is certainly different from that of the midgut) and changes in developmental programming and gene

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expression (the VSG system is reactivated in the metacyclic form as a pre-adaptation for invading a new mammalian host).

Clearly, *T. brucei* continues to provide interesting insights into the basic and unusual workings of an evolutionarily ancient eukaryote. However, its contributions to cell biology are not merely interesting oddities or evolutionary fossils of the processes found in higher eukaryotes. Rather, the currently available armoury of genetic tools, molecular markers and tractable biology makes these organisms an excellent model in their own right for addressing fundamental questions of broad interest and applicability. Notwithstanding this, the potential for using this knowledge to tackle a hugely important disease gives this work special relevance.

Acknowledgments

Through necessity, this commentary has focused on literature published in the past 2-3 years, and has not cited the huge contributions from earlier literature on which many of the recent developments are based. I apologize for this and refer the reader to several of the excellent review articles cited for more detailed source information. I also thank J. Bangs (University of Wisconsin, USA) for helpful comments on the manuscript and K. Gull (University of Oxford, UK) for permission to use images of the tripartite attachment complex. Work in the Matthews laboratory is funded by a programme grant from the Wellcome Trust (GR073358MA).

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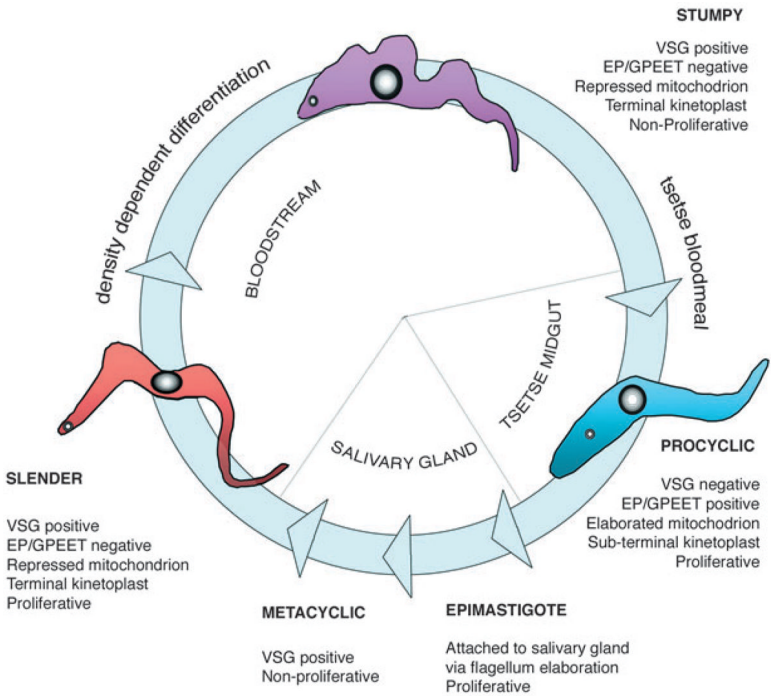

Fig. 1. The life cycle of *Trypanosoma brucei*

Trypanosomes proliferate in the bloodstream of mammalian hosts as morphologically slender forms. These cells express the bloodstream-stage-specific VSG coat to evade the mammalian immune response. The kinetoplast (the mitochondrial genome of the parasite) is located at the posterior end of the cell and mitochondrial activity is relatively repressed. As parasite numbers increase in the bloodstream, differentiation to morphologically stumpy forms occurs. These are division-arrested forms pre-adapted for transmission to tsetse flies. Upon uptake in a tsetse bloodmeal, procyclic forms are generated, these being proliferative in the fly midgut. Procyclic forms express a surface coat distinct from that of bloodstream forms, the VSG being lost and replaced by a coat composed of EP and GPEET procyclins. The kinetoplast is also repositioned to a sub-terminal position. After establishment in the fly midgut, trypanosomes arrest in division and then migrate to the tsetse salivary gland, where they attach as epimastigote forms. These are proliferative and attached through elaboration of their flagellum. Eventually, these generate non-proliferative metacyclic forms, which have re-acquired a VSG coat in preparation for transmission to a new mammalian host. Arrowheads represent differentiation events in the trypanosome life cycle.

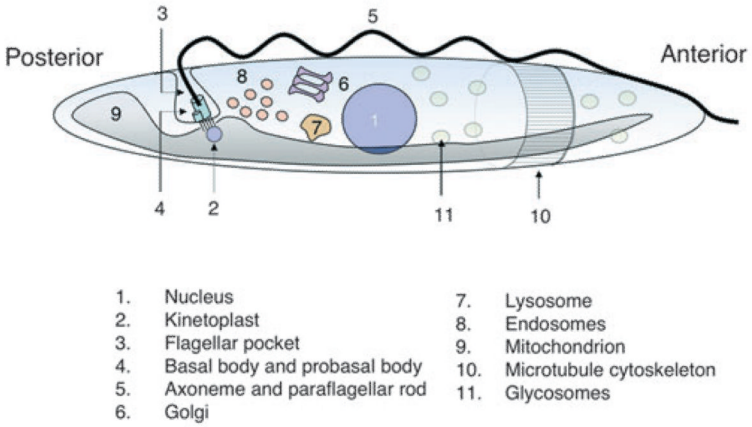

Fig. 2. Trypanosome cell architecture

A simplified representation of the location of the major structural features of the trypanosome cell. A cutaway section towards the anterior of the cell shows the microtubule cytoskeleton underlying the cell membrane. For more detailed images of the trypanosome cell, the reader is referred to recent articles (Grunfelder et al., 2003; Overath and Engstler, 2004; Vaughan and Gull, 2003).

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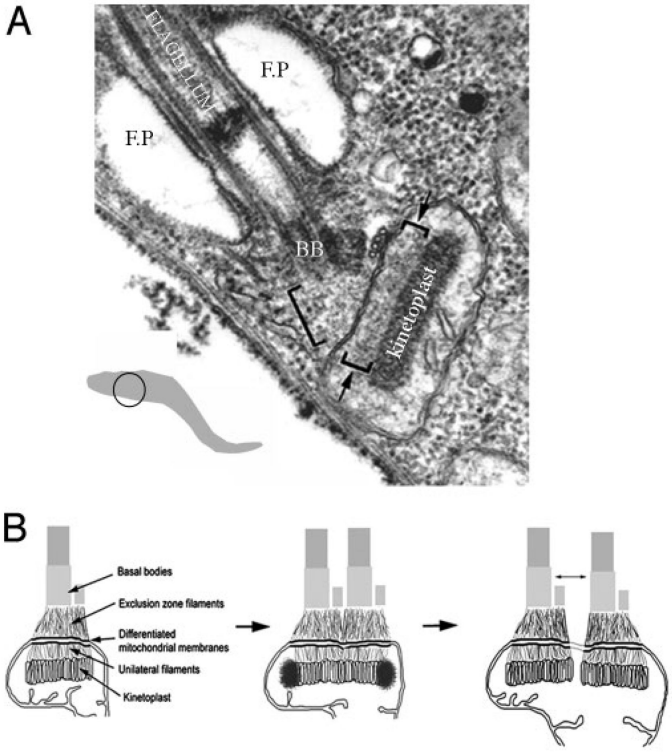

Fig. 3. The tripartite attachment complex

(A) An electron micrograph of the region of the flagellar pocket (labelled F.P). The basal body (BB) is shown connected to the kinetoplast through a series of unilateral filaments, which link the kinetoplast to the inner mitochondrial membrane (these are indicated by the small brackets). The exclusion zone filaments (indicated by a large bracket) link the mitochondrial outer membrane and the basal body. The inset shows a schematic representation of the region of the trypanosome cell shown in the electron micrograph. (B) A representation of the flagellar/basal bodies/kinetoplast region and its replication during the trypanosome cell cycle. The left-hand image is the organization of the tripartite attachment complex in G1 phase; the middle image represents S phase, when kinetoplast replication is underway, and when the probasal body has matured and two new probasal bodies have formed. The right-hand image shows segregation of the basal bodies and concomitant kinetoplast DNA segregation. Figure adapted with kind permission from K. Gull (University of Oxford, UK) and The American Society for Cell Biology (Ogbadoyi et al., 2003).

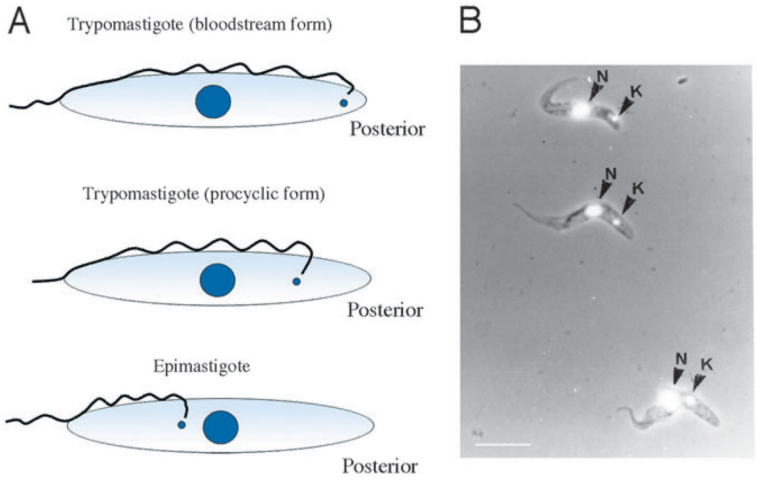

Fig. 4. The kinetoplast changes position during the trypanosome life cycle

(A) The relative position of the kinetoplast in trypomastigote bloodstream and procyclic forms and in epimastigote forms with respect to the nucleus and posterior end of the trypanosome. (B) A phase contrast image of cells undergoing differentiation between bloodstream stumpy forms and procyclic forms. The cells have been counter-stained with DAPI to reveal the position of the nucleus (N) and kinetoplast (K). In the three cells shown, the kinetoplast is progressively repositioned, being earliest in this process in the top cell and latest in the bottom cell. Panel B is reproduced from Matthews et al. (Matthews et al., 1995). Bar, 20 μm.

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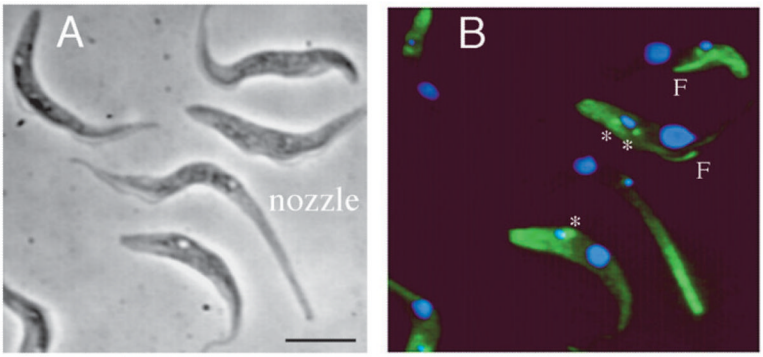

Fig. 5. The morphology of procyclic-form trypanosomes induced by ectopic overexpression of TbZFP2

(A) A phase contrast image of procyclic forms, one of which shows a greatly extended posterior end (nozzle). (B) A composite of the DNA of the trypanosome cell (labelled with DAPI and pseudocoloured blue; each cell contains a central nucleus and posterior kinetoplast) and staining with an antibody (YL1/2; Kilmartin et al., 1982) against tyrosinated α-tubulin, which labels dynamic microtubules in the trypanosome cytoskeleton (pseudocoloured green). Note that the posterior end of each cell shows staining, as does the newly growing daughter flagellum (labelled F) and the basal bodies (labelled in two of the cells with an asterisk). The nozzle cell shows an extended region of staining at the posterior end of the cell, indicating microtubule extension in this region. Bar, 8 μm.

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