# THE LIFE CYCLE OF *TRYPANOSOMA CRUZI*

K. M. Tyler, C. L. Olson and D. M. Engman
Departments of Microbiology-Immunology and Pathology
Feinberg Medical School of Northwestern University, Chicago, IL 60611

## ABSTRACT

Since the discovery of *Trypanosoma cruzi* as the parasite that causes Chagas disease, nearly a century ago, the details of the organism's life cycle have fascinated scientists. *T. cruzi* is a single-celled eukaryote with a complex life cycle alternating between reduviid bug vectors and vertebrate hosts. It is able to adapt via the process of cellular differentiation to replicate within the diverse environments represented of the insect's gut and host cell cytoplasm. These adaptive transformations take the form of coordinated changes in morphology, metabolism and cell cycle regulation. Different life cycle stages of *T. cruzi* show dramatically different protein and RNA profiles, which are the end result of unusual mechanisms for regulating gene expression. In recent years, new molecular techniques have been brought to bear on the life cycle dramatically increasing our knowledge of the strategies employed by the parasite to ensure its continued survival.

## INTRODUCTION

### Chagas disease

The etiologic agent of the chronic and often fatal Chagas disease is the American trypanosome, *Trypanosoma cruzi*, a flagellated protozoan of the order Kinetoplastida. The survival of *T. cruzi* is dependent on the successful transmission between, and the colonization of, two radically different environments: the midgut of the reduviid bug vector and the cytoplasm of the mammalian host cell. As is true of all infections, interruption of the pathogen's life cycle will lead to eradication of the disease. Strategies for interrupting the life cycle include minimizing human contact with the insect vector by improving public housing, reducing or eliminating the vector population, or by manipulation of the vector population to make it refractory to *T. cruzi* infection. These strategies would ideally being employed in tandem, together with treatment of infected individuals using curative chemotherapy. In recent years, such strategies have proven effective in South America, dramatically reducing or eliminating natural infection in most areas.

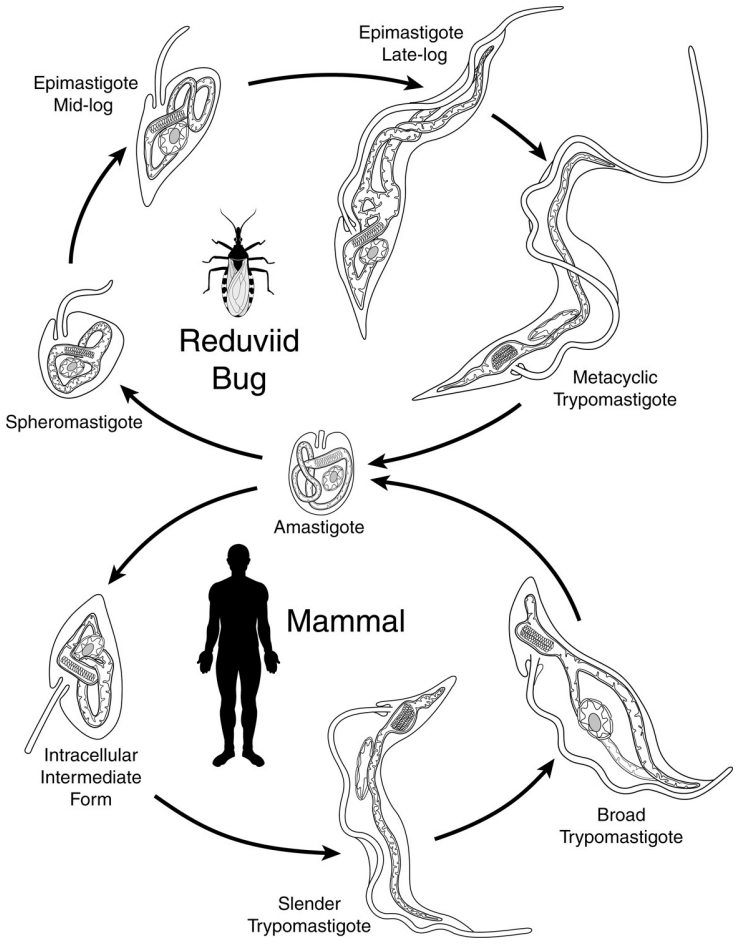


Figure 1. The life cycle of *Trypanosoma cruzi*. See text for details.

**Overview of the *T. cruzi* life cycle**:

For the purpose of this discussion we will begin our descriptions of the parasite life cycle with the infection of a mammalian host by metacyclic trypomastigotes present in the excreta of the blood-feeding reduviid bug vector (Figure 1). These are introduced into the host by contamination of the insect bite wound or a variety of mucosal membranes. The non-dividing metacyclic form is able to invade a wide range of phagocytic and non-phagocytic nucleated cells, initially entering a membrane bound (parasitophorous) vacuole. Upon entry, the parasite begins to differentiate to the amastigote form and escapes the vacuole into the cell cytoplasm where the dramatic morphologic transformation, including flagellar involution, is completed. The amastigote re-enters the cell cycle and proliferates until the cell fills with these forms. At this point the amastigotes elongate, reacquiring their long flagella, differentiating to the slender trypomastigote forms via an intracellular epimastigote intermediate. Slender trypomastigotes escaping the

cell can invade adjacent cells; alternatively, they can enter the blood and lymph and disseminate, in which case they may begin to differentiate extracellularly. Extracellular differentiation gives rise to the broad trypomastigotes and extracellular amastigotes. A mixture of these three forms may be present in the blood of infected individuals and can be taken up in the blood meal of a reduviid bug. In the bug midgut, remaining trypomastigotes differentiate into amastigotes. As a population, amastigotes first extend their flagella to become spheromastigotes, which then lengthen to become (mid-log) epimastigotes. These epimastigotes continue to elongate as nutrients from the blood meal are exhausted. Finally, after migration to the bug's hindgut, the elongate (late-log) epimastigotes attach to the waxy gut cuticle by their flagella and differentiate into infectious metacyclic trypomastigotes, completing the life cycle.

**The trypanosome cell**

*T. cruzi* has the classical features of a eukaryotic cell: membrane bound nucleus, plasma membrane, golgi apparatus and endoplasmic reticulum. However, in common with other members of the Kinetoplastida, *T. cruzi* has several peculiar features, such as a single mitochondrion, the DNA of which lies within a single unit, suborganellar structure - the kinetoplast. The kinetoplast DNA is a linked (catenated) network of hundreds of circular molecules, the minicircles and maxicircles. *T. cruzi* also compartmentalizes glycolysis in membrane bound vesicles called glycosomes, stores minerals in structures known as acidocalcisomes and sequesters membranes in vesicles named reservosomes (see chapter by De Souza, this volume for detailed accounts of the cell biology of this organism).

The cytoskeleton of *T. cruzi* is unusual, in that it is predominantly microtubular with no evidence of microfilament or intermediate filament systems. *T. cruzi* does not possess centrioles. The replicative stages undergo a "closed" mitosis, with a microtubule spindle arising from poorly defined structures in the nuclear membrane. The trypanosome's distinctive morphologies are dictated by a "pellicular" corset of microtubules which closely apposes the plasma membrane.

*T. cruzi* possesses a single flagellum subtended by a basal body and probasal body which lie within the cell. The basal body is the trypanosome's only defined microtubule organizing center. The flagellum varies in length during the life cycle from over 20 µm to less than 2 µm. The flagellar motor is a ciliary axonemal complex, with the typical 9 + 2 configuration of parallel microtubules. Once the axoneme exits the cell body, it is appended to an unusual semi-crystalline structure called the paraflagellar rod. It is believed that this structure provides support to the flagellar axoneme, increasing its rigidity and playing an essential role in motility. The exterior flagellum is surrounded by a specialized membrane which is rich in sterols and sphingolipids and which contains proteins that do not diffuse into other domains of the surface membrane.

Where the flagellum enters the cell there is a gap in the subpellicular corset, the junction between the pellicular plasma membrane and flagellar membrane at this point takes the form of an invagination known as the flagellar pocket. The majority of vesicular trafficking and nutrient uptake is believed to occur in this area and many receptors localize specifically to this region. A second, smaller invagination proximal to the flagellar pocket, the cytostome, has also been implicated in nutrient uptake.

## THE LIFE CYCLE

### In the mammalian host

Metacyclic trypomastigotes are able to parasitize a wide range of nucleated mammalian cells. Invasion occurs by one of three distinct mechanisms (Figure 2). The parasite may enter a cell under pressure from its own motility (Figure 2A); this is evidenced by the fact that even lightly fixing cells does not prevent invasion. Nevertheless, this mechanism is thought to be the least important mechanism of invasion. The best-studied entry mechanism is lysosome dependent. In this case, *T. cruzi* organizes the microtubule cytoskeleton of the host cell in order to direct recruitment of lysosomes to the point of parasite attachment. These lysosomes then fuse with the plasma membrane, first forming a junction with the parasite and then creating a vacuolar compartment in which the entering parasite transiently resides (Figure 2B). Finally, invasion may be facilitated by the host actin cytoskeleton. In this case, the parasitophorous vacuole is initially constructed from the plasma membrane of the host cell, which ruffles out along the parasite and encompasses it (Figure 2C). Once within the vacuole by any of these mechanisms, lysosomes continue to traffic to the parasitophorous vacuole. Fusion of lysosomes with the vacuole leads to acidification. This drop in pH serves a dual role in inducing the trypomastigote to differentiate rapidly to an amastigote and also activates a parasite derived porin like molecule - TcTox. This molecule mediates weakening of the membrane of the parasitophorous vacuole and permits escape of the parasite into the cytoplasm.

Flagellar and cell body shortening commences immediately following cell entry; it is a relatively rapid process and precedes rearrangement of the kinetoplast. The first cell division occurs only after a lag period, during this lag period the kinetoplast is rearranged, adopting its replicative morphology (Figure 3). After differentiating to the amastigote form, *T. cruzi* proliferates in the cell cytoplasm. At high density, amastigotes give rise to bloodstream trypomastigotes via a range of intermediate morphologies. The triggers for this differentiation are not yet defined, although glucose limitation and contact interaction are obvious candidates. The intermediate forms of this differentiation contain epimastigote forms. It has been suggested that these forms are a *bona fide*, but transient and somewhat cryptic life cycle stage. In differentiating to the trypomastigote form, the kinetoplast morphology is again a late marker of differentiation with some trypanosomes of clearly trypomastigote morphology possessing a replicative kinetoplast structure whilst still in the host cell.

The mechanisms the parasite has adopted for cell entry, which exploit the host cell trafficking machinery, are tremendously intriguing and apparently novel in biology (Yoshida, this volume). The trypanosome exploits host cellular behaviors that are clearly important, but poorly characterized to date. For this reason, such studies are of great general interest to cell biologists and research on *T. cruzi* invasion has led to great insights into cellular phenomena, such as the role of lysosomal exocytosis in cellular repair. In the past few years, new technologies have greatly facilitated the study of cellular invasion pathways and of trypanosome survival within living cells.

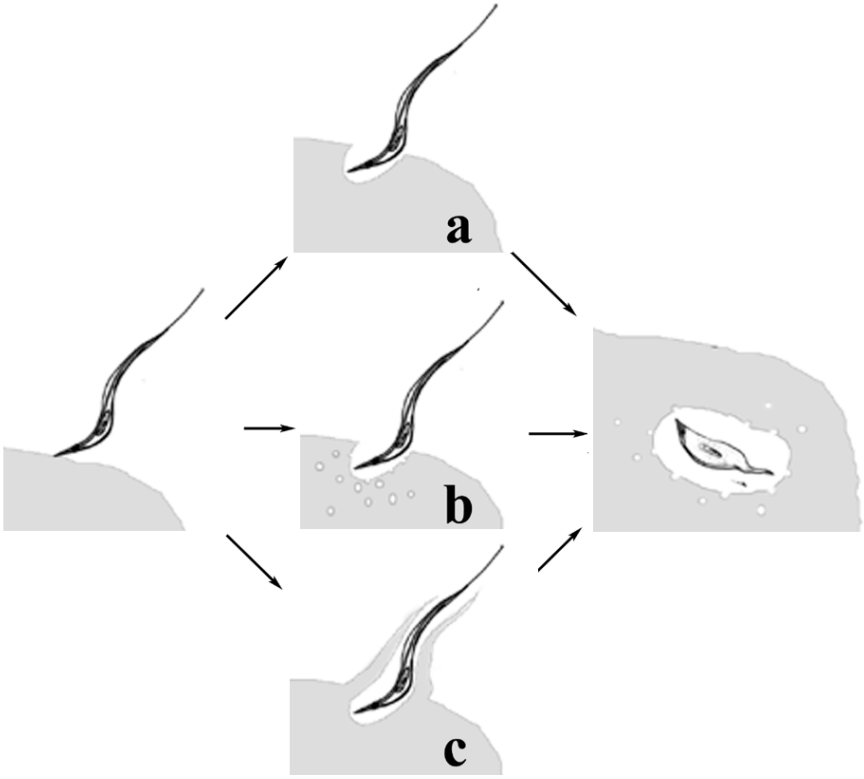


Figure 2. Mechanisms by which *T. cruzi* trypomastigotes can invade nucleated cells. a) **Host cell independent**. The trypanosome attaches to the host cell and, under pressure from its own motility, effects entry into the host cell. b) **Lysosome mediated**. The trypanosome utilizes the host microtubule cytoskeleton to traffic lysosomes to the attachment site, where the extra membrane facilitates entry into a parasitophorous vacuole. c) **Membrane ruffling**. An actin based mechanism akin to phagocytosis in which the cell is stimulated to extend processes along the parasite, facilitating entry. Regardless of mechanism or mechanisms (since they are not mutually exclusive) the endpoint is the parasite resident in a parasitophorous vacuole to which lysosomes traffic, and initiate differentiation and escape into the cytoplasm.

**From the mammalian bloodstream to the reduviid digestive tract**

Slender trypomastigotes are readily seen escaping from packed pseudocysts (infected cells). It is believed that these slender forms are committed to a program of differentiation to the amastigote form, and that this program will take place whether the parasite is present in the peripheral blood, cytoplasm or reduviid bug gut. The rate of this differentiation is acutely sensitive to pH. In the acid environment of the parasitophorous vacuole, differentiation is rapid, the cell body apparently shortening more rapidly than the flagellum is able, leading to club-shaped intermediate morphologies. At more neutral pH, in the blood, slower kinetics of differentiation are observed and broad (stout or stumpy) forms appear to be the intermediate morphologies *en route* to the amastigote form.

The trypomastigotes in the peripheral blood are pleomorphic, consisting of both slender and broad forms. The ratio of these forms varies from strain to strain, perhaps reflecting differences in the rate of

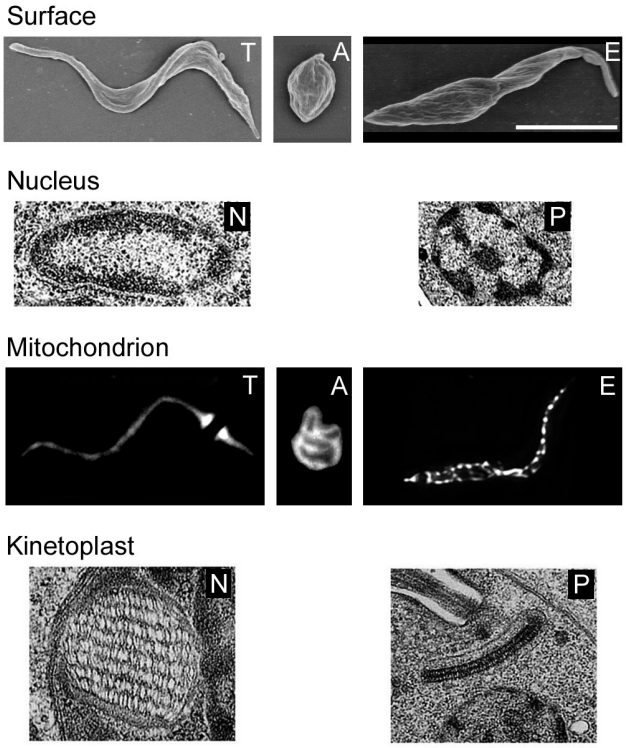


Figure 3. Coordinate changes in morphology and organellar structure during the life cycle. **Surface.** Scanning electron micrographs of the trypomastigote (T) amastigote (A) epimastigote (E). The left-handed helical twist of all forms is dictated by the trypanosome cytoskeleton. SEM images are taken at the same magnification. Scale bar provided is 5 µm. **Nucleus**. Nuclear structure is dramatically different between the non-proliferative (N) trypomastigote and the proliferative (P) amastigote and epimastigote stages. In contrast to the round nuclei of the proliferative stages, no nucleoli are visible in elongate trypomastigote nuclei and heterochromatin is more abundant. **Mitochondrion**. The morphology of the mitochondrial tubule and its complexity change during the life cycle. The linear tubule of the trypomastigote is transformed via the simple geometries of the amastigote and log phase epimastigote chondriome to a complex labyrinth in the stationary phase epimastigote form. **Kinetoplast.** Accompanying changes in the chondriome morphology, the kinetoplast morphology has a dramatically different structure in the trypomastigote forms (N) where it has an open "basket" like appearance compared with the proliferative stages (P) where the kinetoplast has a simple bilamellar appearance in cross-section. Although clearly linked, at a molecular level, the links between the changes in morphology, nuclear, chondriome and kinetoplast structures during the life cycle remain poorly characterized. TEMs kindly provided by Dr. C. Sterling, University of Arizona.

differentiation or in the invasiveness of the trypomastigotes in the different strains studied. When a pleomorphic population of bloodstream trypomastigotes (and up to 10% amastigotes) is ingested by a reduviid during a blood meal, parasites first pass into the bug midgut. Here, the trypomastigotes undergo differentiation to amastigote forms. The amastigote

forms (which are generally 3-5 µm in diameter) replicate and differentiate into epimastigotes, which are also able to replicate. Initially, the amastigote-like forms swell, roughly doubling in diameter, and elongate their flagella, which in turn begin to beat visibly. At this stage the forms are sometimes referred to as spheromastigotes. The cell body and flagellum of the spheromastigote elongate, giving rise to the classical epimastigote form which has a varied morphology and which can become quite long (in excess of 30 µm). The transformation from amastigote to elongate epimastigote appears to be reversible and dependent upon the concentration of free monosaccharides in the environment. Since amastigotes, spheromastigotes and epimastigotes are all proliferative forms, the transitions from one of these forms to another appear to lie in a continuum rather than being discrete steps.

The progression from slender trypomastigote to elongate epimastigote represents a progressive change from an environment rich in glucose (host bloodstream) to an environment which is extremely poor in monosaccharides (bug hindgut). In an apparent adaptation to this depletion of simple saccharides, an incremental biogenesis of the mitochondrion is readily observed (Figure 3). The single tubule of the slender trypomastigote mitochondrion splits into two tubules linked at either end in the broad form, which twist together in the amastigote mitochondrion often showing a figure 8-like appearance. As the cell body elongates into the epimastigote form, additional processes arise, but a simple geometry is initially maintained in shorter epimastigotes. As nutrients dwindle, mitochondrial complexity increases until, in the elongate epimastigote, multiple intertwined processes occupy much of the volume of the cell. Accompanying these changes is an increase in the expression of both key electron transport chain components and mitochondrial chaperones. Importantly, cytochrome reductase (complex III) is activated during the trypomastigote to amastigote transition, while dependence on cytochrome oxidase (complex IV), rather than the trypanosome's plant-like alternative oxidase, is developed only during the transition from short (mid-log) to elongate (late-log) epimastigote.

This progressive increase in mitochondrial complexity and activity parallels changes seen in *Trypanosoma brucei* during transition from mammalian host to its tsetse fly, insect vector. As with *T. brucei*, key enzymes associated with amino acid metabolism, the primary source of nutrients in the insect gut, are upregulated in the vector stages. Unlike *T. brucei*, Kreb's cycle components such as dihydrolipoamide dehydrogenase, appear to be constitutively active in all life cycle forms. Moreover, early spectophotometric studies showed that at least some cytochromes are present throughout the life cycle of *T. cruzi*. So it may be that some parts of the electron transport chain are constitutively active through the life cycle, working in concert with the trypanosome alternative oxidase.

The mitochondrial genome of *T. cruzi* encodes subunits of cytochrome reductase and cytochrome oxidase in its maxicircles. To make these subunits functional, the RNA must be transcribed from the maxicircle and amended post-transcriptionally by the addition and occasional deletion of uridine residues. This process, known as RNA editing, is mediated by machinery that includes the guide RNAs encoded by the kinetoplast minicircles. It would be surprising if the kinetoplast DNA (kDNA) encoded subunits were not coordinately regulated with their nuclear encoded counterparts as they are in *T. brucei*. In *T. brucei*, one level of control for mitochondrial encoded gene expression is stage specific editing of

constitutively produced transcripts. To date there is no evidence of stage specific RNA editing in *T. cruzi*. The observation of a massive and unexplained structural change in the kDNA of the trypomastigote may, however, indicate an entirely different mechanism for regulation of *T. cruzi* kinetoplast encoded proteins. It may be that transcription of the trypomastigote kinetoplast is essentially shut down when the kDNA adopts the basket like configuration (Figure 3). This would parallel gross morphological changes in the nucleus which have been correlated with pan-regulation of transcription between trypomastigote and replicative forms.

**Metacyclogenesis**

In the midgut of its triatomine bug vector, *T. cruzi* epimastigotes proliferate in the nutrient rich environment of a recent blood meal. As the meal is digested and the parasite density increases, the environment becomes nutrient poor and epimastigotes become more elongate. Eventually, epimastigotes reaching the insect rectum attach by their flagella and undergo metacyclogenesis to human infective trypomastigote forms. Metacyclogenesis occurs when epimastigotes from the nutrient poor hindgut adhere to the waxy cuticle of the reduviid bug rectum, initiating a dramatic morphological change. Once formed, metacyclics detach from the waxy cuticle and are excreted. Contamination of the reduviid bite wound of the mammalian host with these excreta leads to infection, completing the life cycle.

Metacyclogenesis can be described in two parts, the first enabling the second. First, the trypanosome senses loss of sugars from its environment and responds by activating its mitochondrion and by elongating its cell body and flagellum. The trypanosome flagellar membrane, which is sterol rich and more hydrophobic that the somatic membrane, is thus lengthened. Second, this flagellar lengthening permits the trypanosomes to adhere to a hydrophobic surface and it is this interaction which triggers metacyclogenesis. This trigger for metacyclogenesis is known to be cAMP mediated. Although the machinery involved in transducing the attachment signal has not been demonstrated it is presumably localized, at least in part, in the flagellar membrane. One family of adenylate cyclases has been discovered in trypanosomes and at least some members of this family are known to be resident in the flagellar membrane. Moreover, cAMP-regulated protein kinase (PKA) homolog is also believed to be resident in the flagellum - clearly these are candidates for metacyclogenesis control molecules.

As long as there are sufficient nutrients - particularly the exogenous sugars which must not dip below a critical level necessary for powering the differentiation - the hydrophobic interaction between the flagellum and the substrate to which it attaches is sufficient to trigger the differentiation process. Saccharide limitation and hydrophobic interaction with the flagellum seem necessary and sufficient to drive metacyclogenesis. *In vivo*, however, there is experimental support for the ability of a triatomine factor in haemolymph to induce metacyclogenesis and a role for accumulated parasite factors or excretory products in metacyclogenesis has not been ruled out. Metacyclogenesis involves coordination of an extreme morphological event with arrest in the cell cycle, changes in antigenicity, reduced mitochondrial activity and acquisition of infectivity. The manner in which these events are coordinated remains almost completely unstudied.

## TRYPANOSOME DIFFERENTIATION

*T. cruzi* employs cellular differentiation as a strategy for adapting to the diverse environments represented by its host and vector. Consequently, the study of cellular differentiation in this organism is synonymous with the study of its life cycle. Differentiation is highly controlled, affecting many fundamental processes within the cell. It is characterized by profound changes in cellular morphology, motility and metabolism, typically in response to external stimuli. Differentiation normally involves a change in the pattern of gene expression, such that the gene products impact in a coordinated fashion on the cellular processes involved. One established approach to understanding such a complex phenomenon, in any given cell type, involves first identifying what is changing during the course of differentiation and then determining the mechanisms by which such change is brought about.

During life cycle transitions *T. cruzi* is known to regulate several key areas of its cell biology:

1) Cell surface - allowing the parasite to interact productively with successive environments
2) Cytoskeleton - both pellicular and flagellar, affecting morphology and motility
3) Nutrient uptake and metabolism - notably mitochondrial structure and activity and the presence or absence of a cytostome, but also regulation of some glycosomal and cytosolic enzymes
4) Cell cycle - with the invasive forms being non-proliferative and reentering the cell cycle upon reaching a stable environment
5) Defense - since the immune responses of the human host and bug gut are immensely different, different molecules are required by the trypanosome to survive them.

### The signal to differentiate

Cellular differentiation is a cascade linking the impetus to differentiate with the multiple effects of differentiation. Signaling cascades are the focus of intense research in *T. cruzi* research, in both the parasite (DoCampo, this volume) and the parasitized host cell (Yoshida, this volume).

The functions of cAMP and inositol metabolites are often antagonistic and it is clear that the regulation of cAMP levels and inositide metabolism is critical to the control of *T. cruzi* differentiation. A role for cAMP in differentiation from dividing epimastigotes to non-dividing metacyclic forms has been directly demonstrated using lipophilic analogs of cAMP which induce metacyclogenesis directly. In contrast, inositide metabolism has been shown not to affect metacyclogenesis, but rather to promote trypanosome proliferation and to be critically involved (through phospholipase C) in differentiation from the trypomastigote to amastigote form. This differentation involves re-entry to the proliferative cell cycle and has also been shown to be accelerated by inhibition of type I protein phosphatases. Interestingly, signaling proteins are increasingly being localized to the sterol rich environment of the flagellar membrane and to the flagellar cytoskeleton. Notably, these proteins include calcium binding proteins and adenylate cyclases, which has led to the idea of the flagellum being regarded as an organelle not just for motility and adhesion but also as a sensory center. It is likely that components of the signaling relays regulating flagellar beat and differentiation are both residents of lipid rafts in the trypanosome flagellum.

**Modulation of gene expression in *T. cruzi***

Once the stimulus to differentiate has been transduced into the cell, the machinery responding to the call for differentiation falls readily into two parts: the manufacture of new biological molecules and assemblies, and the breakdown of old ones.

**Stage specific protein turnover**

Proteins targeted for destruction can be tagged (by ubiquitin ligases) with ubiquitin. Once tagged, these molecules are selectively degraded by large assemblies know as proteosomes. Recent evidence using chemical inhibitors of proteosomes suggest that life cycle differentiations in *T. cruzi* are dependent on proteosomal degradation. Support for this view comes from *T. brucei*, where two molecules with ubiquitin ligase homology have been shown to participate directly in control of morphological change during differentiation. It is also important to note that cell cycle control is modulated by proteosomal degradation of ubiquitinated histones, emphasizing a link between cell cycle and morphology in trypanosomes. Although the proteosome is undoubtedly a key player in differentiation, other proteases such as the major cysteine protease of *T. cruzi* (cruzipain/cruzain) have also been suggested to play an enabling role in *T. cruzi* differentiation, although its precise role and targets have not yet been discovered.

**Control of new protein synthesis**

The polymerase II of *T. cruzi* transcribes coding sequences as large polycistronic units and, to date, promoters or transcriptional start sites have proven difficult to detect. This is in contrast to most metazoa, in which transcription initiation is a major point of regulation. Consequently, it appears that *T. cruzi* modulates protein synthesis during its life cycle by controlling RNA maturation and by employing a range of post-transcriptional mechanisms.

In the monocistronic transcription of metazoan protein encoding genes, the pre-mRNA is processed by addition of a poly (A) tail to the 3' end and by co-transcriptional capping of the 5' end. These modifications confer stability to the mature mRNA and allow its recognition by the ribosomes for translation. Polycistronic transcripts from trypanosomes cannot be capped co-transcriptionally. Instead, a stabilizing 5' terminus (spliced leader sequence) with a cap is added to all protein-coding mRNAs by the process of trans-splicing. Generally, trans-splicing of the 5' end of one trypansome mRNA is linked directly to the polyadenylation of the 3' end of the upstream mRNA.

The mRNAs of *T. cruzi* stage regulated proteins may show entirely different patterns of mRNA abundance than mRNAs encoded by adjacent genes, even though both genes are constitutively transcribed as the same polycistronic transcript. One such example is the amastigote specific amastin protein that is encoded by genes in alternating tandem array with the constitutively expressed tuzin protein. The profile of mRNA abundance for these two genes is dramatically different through the life cycle. In this case and in most others that have been investigated, *T. cruzi* mRNA abundance is dictated by the longevity of the mRNA and this, in turn, is dictated by sequences within the 3' untranslated region (UTR). These UTR sequences are thought to specifically bind regulatory proteins that can either stabilize or destabilize them. The first such UTR binding proteins have now been discovered and putatively linked to mRNA stability. Other co-transcriptional

and post-transcriptional mechanisms may also play a role in determining mRNA abundance, but are less well worked out. Sometimes protein expression does not correlate with RNA levels in trypanosomes; in such cases control is thought to lie at the level of translation. Finally, it has been observed that both the nucleus (chromatin) and kinetoplast (kDNA) structures vary dramatically during the life cycle, between replicative and non replicative forms, affecting not only their overall morphology, but also accessibility of their DNAs to digestive (and other) enzymes (Figure 3). It has been suggested that the structural changes observed correlate directly with a net down regulation of transcription in the non-proliferative forms. To date, however, it is not clear whether direct chemical modification of the DNA has a role to play as it does in transcriptional silencing of metazoan cells.

## Further reading

Brener Z. 1973. Biology of *Trypanosoma cruzi*. Annu Rev Microbiol 27:347-82.
Burleigh BA, Andrews NW. 1998. Signaling and host cell invasion by *Trypanosoma cruzi*. Curr Opin Microbiol 1:461-5.
De Souza W. 1984. Cell biology of *Trypanosoma cruzi*. Int Rev Cytol 86:197-283.
Gull K. 1999. The cytoskeleton of trypanosomatid parasites. Annu Rev Microbiol 53:629-55.
Hecker H, Betschart B, Bender K, Burri M, Schlimme W. 1994. The chromatin of trypanosomes. Int J Parasitol 24:809-19.
Matthews KR. 1999. Developments in the differentiation of *Trypanosoma brucei*. Parasitol Today 15:76-80.
Naula C, Seebeck T. 2000. Cyclic AMP signaling in trypanosomatids. Parasitol Today 16:35-8.
Parsons M, Ruben L. 2000. Pathways involved in environmental sensing in trypanosomatids. Parasitol Today 16:56-62.
Priest JW, Hajduk SL. 1994. Developmental regulation of mitochondrial biogenesis in *Trypanosoma brucei*. J Bioenerg Biomembr 26:179-91.
Kollien AH, Schaub GA. 2000. The development of *Trypanosoma cruzi* in Triatominae. Parasitol Today 16:381-7.
Elias MC, Marques-Porto R, Freymuller E, Schenkman S. 2001. Transcription rate modulation through the *Trypanosoma cruzi* life cycle occurs in parallel with changes in nuclear organisation. Mol Biochem Parasitol 112:79-90.
Shapiro TA, Englund PT. 1995. The structure and replication of kinetoplast DNA. Annu Rev Microbiol 49:117-43.
Aikawa M, Sterling CR. 1974. Intacellular parasitic protozoa. Academic Press.
Tyler KM, Engman DM. 2001. The life cycle of *Trypanosoma cruzi* revisited. Int J Parasitol 31:472-81.
Vickerman K. 1985. Developmental cycles and biology of pathogenic trypanosomes. Br Med Bull 41:105-14.