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Invited Review

The life cycle of *Trypanosoma cruzi* revisited

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Abstract

The basic features of the life cycle of *Trypanosoma cruzi* have been known for nearly a century. Various aspects of the life cycle, however, have been elucidated only recently, whilst others remain either controversial or unstudied. Here, we present a revised life cycle influenced by recent findings and specific questions that remain unresolved. © 2001 Published by Elsevier Science Ltd. on behalf of Australian Society for Parasitology Inc.

Keywords: Differentiation; Metacyclogenesis; Chagas

1. Introduction

Ostensibly, the life cycle of *Trypanosoma cruzi* has been elucidated for nearly a century (Chagas, 1909). Furthermore, the cell biology and life cycle of *T. cruzi* have been the focus of several excellent reviews (Brener, 1973; De Souza, 1984; Vickerman, 1985; Burleigh and Andrews, 1995). There remains conflict in the literature, however, with regard to some specific details. In recent years, several questions have been answered, but more have been raised. In this presentation, we aim to review recent findings in order to highlight aspects of the life cycle of *T. cruzi* that remain unresolved. Without initially reporting the often conflicting studies surrounding the descriptions of many of the life cycle stage transitions, this introduction (and Fig. 1) will provide a brief overview of our interpretation of the *T. cruzi* life cycle based on our own in vitro investigations and a review of the available literature.

In the peripheral blood of the mammalian host, *T. cruzi* trypomastigotes are a pleomorphic population made up of a mixture of two basic morphologies which are generally described as slender or broad. Consequently, the reduviid bug takes up a pleomorphic population of bloodstream trypomastigotes (and up to 10% amastigotes) in a blood meal from an infected mammal (Andrews et al., 1987; Ley et al., 1988), and parasites then pass into the reduviid bug midgut. Here, the trypomastigotes undergo differentiation to amastigote forms, the slender forms transforming via a broad (stout or stumpy) form intermediate. The amastigotes are generally 3–5 μm in diameter, replicative and, in the environment of the reduviid midgut, transform into epimastigotes, which are also replicative. Initially, the amastigotes swell and extend their flagella, which begin to beat visibly. At this stage, the forms are sometimes referred to as sphaeromastigotes. The cell body and flagellum of the sphaeromastigote elongate as cell density increases, giving rise to the classical epimastigote form. Although most consider epimastigotes to be a distinct life cycle stage, it is notable that they have a highly varied morphology which can become quite long (in excess of 30 μm). In vitro, the transformation from amastigote to elongate epimastigote appears to be reversible and is dependent on the concentration of glucose (Tyler and Engman, 2000). Since amastigotes, sphaeromastigotes and epimastigotes are proliferative, the transition from one of these forms to another appears to be a continuum, rather than a one-step differentiation event which co-ordinates gross cellular change with exit from or re-entry to the cell cycle.

Elongate epimastigotes attach hydrophobically to the waxy cuticle of the hindgut wall prior to differentiating into metacyclic forms (Bonaldo et al., 1988; Kleffmann et al., 1998). This process, metacyclogenesis, may be triggered by the hydrophobic interaction between the flagellum and the substrate to which it attaches (Bonaldo et al., 1988) and is a cAMP mediated process (Gonzales-Perdomo et al., 1988; Rangel-Aldao et al., 1988a,b). Once formed, metacyclics detach from the waxy cuticle and are excreted. Contamination of the reduviid bite wound or mucous membrane of a mammalian host with these excreta leads to infection.

Metacyclic trypomastigotes are able to parasitise a wide range of nucleated mammalian cells. Invasion (recently reviewed by Burleigh and Andrews, 1995) occurs by a unique parasitic mechanism in which the microtubule cytoskeleton of the mammalian cell is directed to recruit

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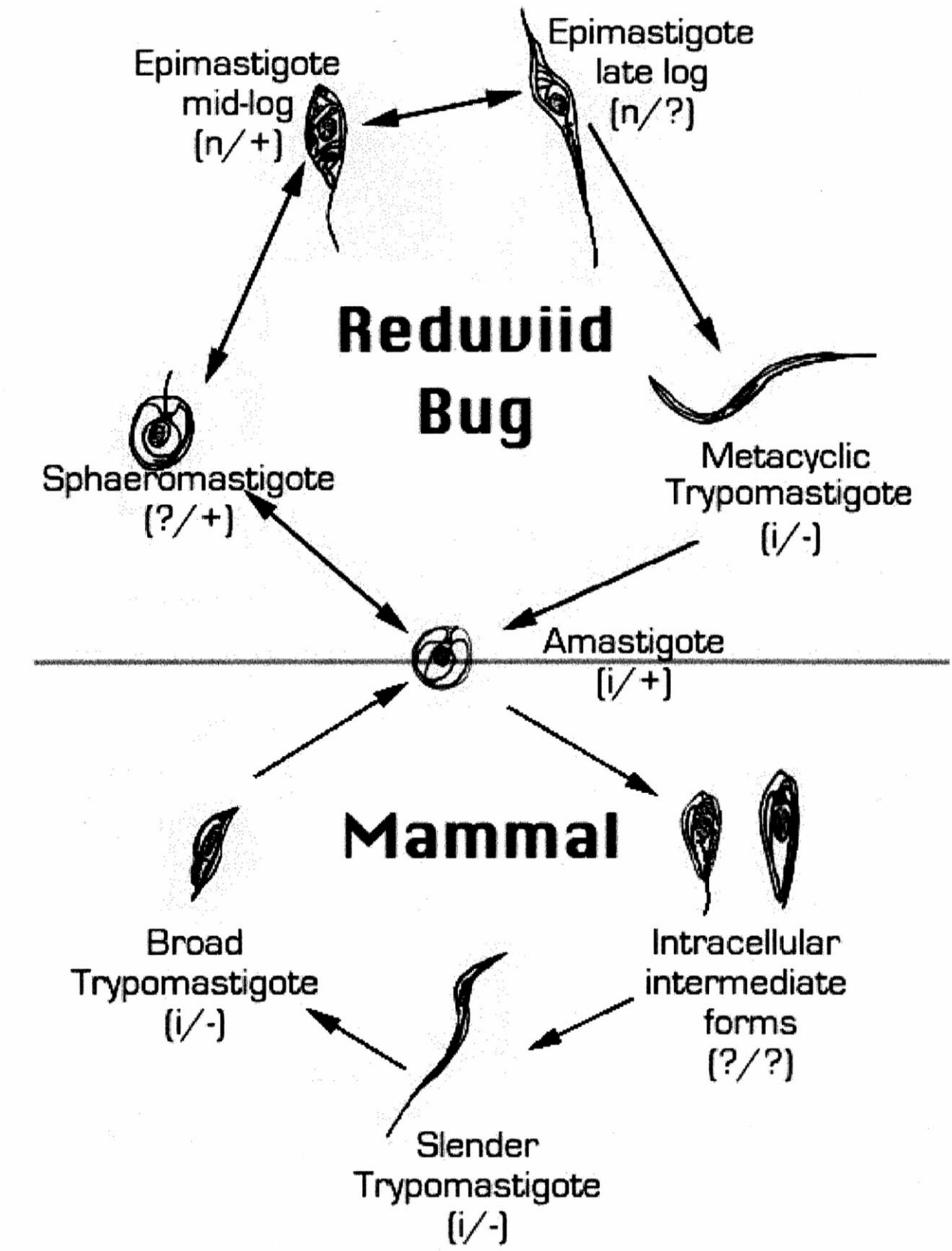

Fig. 1. The life cycle of *Trypanosoma cruzi*. These line drawings are approximations of the commonly seen morphologies in the vertebrate and invertebrate host and are not drawn to scale. The mitochondria are drawn in grey, and the flagellum and kinetoplast/basal body complex are in black; (i) is an infective form; (n) is non-infective; (+) represents a proliferative form; and (−) is non-proliferative.

lysosomes to the point of parasite attachment (Tardieux et al., 1992; Rodriguez et al., 1996). These lysosomes fuse with the plasma membrane, first forming a junction with the parasites and then a vacuolar compartment allowing parasite entry (Rodriguez et al., 1997); the acidification of the parasitophorous vacuole resulting from lysosomal fusion also serves to activate a parasite secreted porin-like molecule, Tc-Tox, which facilitates exit from the vacuole (Andrews, 1993). The trypomastigote then escapes the vacuole and differentiates to the amastigote form. The amastigote proliferates in the cell cytoplasm to form a pseudocyst (Dvorak, 1976; Andrews, 1993). At high density, amastigotes give rise to bloodstream trypomastigotes via intermediate forms, some of which have an epimastigote morphology. This process is inhibited by clasto-lactacystin, indicating a role for proteosomes in this differentiation (Gonzalez et al., 1996). Once formed, trypomastigotes generally escape from the pseudocyst into the blood and lymph as slender forms, which can invade new cells in a manner essentially similar to metacyclic invasion (N. Andrews, personal communication). Slender forms which fail to invade a new cell undergo morphological change, first to the broad form and then to the amastigote. This default pathway of differentiation is presumably the cause of the observed pleomorphism in the trypomastigotes of the peripheral blood. Cells that are prematurely lysed may also release amastigotes, which are observed in the bloodstream during the acute phase of infection. These amastigotes can serve to propagate the infection since they too are able to infect cells, particularly phagocytic cells, albeit by a different mechanism than trypomastigotes (Ley et al., 1988). Finally, the mixture of trypomastigotes and amastigotes present in the blood of an infected mammal serves to complete the life cycle when taken up in a blood meal by a reduviid bug.

2. Remaining questions

It is nearly a century since Carlos Chagas first described the basic morphologies and life cycle of the American trypanosome (Chagas, 1909). Nevertheless, the study of cellular differentiation in this organism is hindered by the fact that some of the life cycle stage transitions remain unstudied or controversial. This presentation will focus on some of the key questions that remain unresolved. How many life cycle stages are there? How are metacyclic and bloodstream trypomastigotes similar and how do they differ? What is the significance of trypomastigote pleomorphism? What is a sphaeromastigote? Do intracellular amastigotes differentiate to trypomastigotes by way of an epimastigote intermediate? Are any *T. cruzi* life cycle stages multipotent, and if so, what defines which differentiation or de-differentiation pathway is followed?

3. Approach

In seeking to resolve some of these issues, we began, as have many others, by observing trypanosome morphologies in culture by using a mixture of differential interference contrast (DIC) and immunofluorescence microscopy. Using antibody to dihydrolipoamide dehydrogenase (DHLADH), a mitochondrial enzyme which is differentially regulated in *Trypanosoma brucei* (Vickerman, 1965; Tyler et al., 1997) but constitutively expressed in *T. cruzi* (Schoneck et al., 1997), we are able to illuminate the mitochondrial chondriome morphology at each life cycle stage (Fig. 2). Using antibody to flagellar calcium binding protein (FCaBP) to illuminate the trypanosome flagellum, we were further able to follow flagellar length as a tractable linear marker of morphology (Tyler and Engman, 2000).

4. Variation in chondriome morphology during the life cycle

Thick and thin section EM have been used to establish that there is only one mitochondrion/cell in *T. cruzi*, and that discoid cristae are present at each life cycle stage (Maria et al., 1972; Paulin, 1975, 1983; Newberry and Paulin, 1989). From such studies, it was established that slender trypomas-

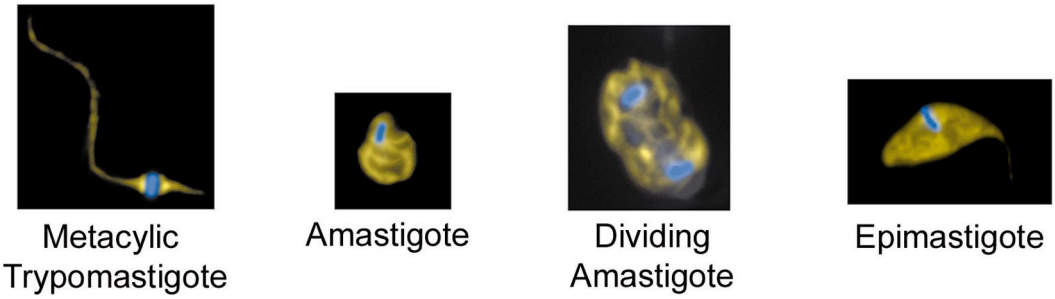

Fig. 2. Mitochondrial structure of three life cycle stages of *Trypanosoma cruzi*. The chondriome (green) is visualised by immunofluorescence assay for the constitutively expressed Kreb's cycle enzyme, dihydrolipoamide dehydrogenase. The complexity of structure increases from trypomastigote, which is linear, to amastigote, which is essentially a twisted loop, to epimastigote, in which the chondriome can be convoluted and complex. In the proliferative amastigote and epimastigote stages, the structure appears to be dynamic and varies both with the cell density of the population and with the position of the cell cycle occupied.

tigotes and metacyclic trypomastigotes have a single tubule which extends the length of the non-undulating side of the cell in a manner that is reminiscent of the slender form of *T. brucei*. In broad trypomastigotes, two linked tubules are present (Maria et al., 1972), but in amastigotes and epimastigotes, the existing literature suggests simple horseshoe and loop structures of a single tubule (Paulin, 1975; Newberry and Paulin, 1989). Fluorescence analysis (using mitochondrially directed GFP, mitotracker, or DHLADH immunofluorescence) on fields of epimastigotes and amastigotes reveals a somewhat more complex set of structures than suggested by electron microscopy. Amastigotes predominantly have a double loop or 'figure 8' structure akin to a twisted rubber band, but display more complexity in 'dividing-forms' which are late in the cell cycle (Fig. 2 and unpublished data). Epimastigote mitochondria have dynamic structures, which appear to vary in complexity with both the density of the population and with the cell cycle.

An increase in chondriome complexity from the slender trypomastigote through the broad trypomastigote to the amastigote and then the epimastigote is analogous to that seen during the transition from the slender to the stumpy to the procyclic form of *T. brucei*. In *T. brucei*, this set of transitions is accompanied by increases in enzyme activity, as first Kreb's cycle is partially activated in the stumpy form, and then, electron transport chain mediated oxidative phosphorylation becomes fully active (Vickerman, 1965; Priest and Hajduk, 1994). These transitions have attracted much attention, in part, because kinetoplast-encoded mRNAs are differentially edited during this process (Feagin and Stuart, 1988). We have been able to show that, while Kreb's cycle components like DHLADH are constitutively active during the life cycle of *T. cruzi*, some nucleus-encoded components of the electron transport chain and mitochondrial chaperones are life cycle stage regulated, showing low levels of expression in metacyclic and slender form trypomastigotes (Tyler and Engman, unpublished). This leads to a prediction that kinetoplast-encoded proteins will be co-ordinately regulated. Others have looked for evidence of differential editing in *T. cruzi* and found none, although the studies were done with mixed populations of pleomorphic trypomastigotes, and so, may not be conclusive (Kim et al., 1994). The observation of a massive and unexplained structural change in the kinetoplast DNA of the trypomastigote (Brack, 1968; De Souza, 1984) may, however, indicate an entirely different mechanism for regulation of *T. cruzi* kinetoplast-encoded proteins. It is well established that transcription can be regulated by changes in chromatin structure (Paranjape et al., 1994), and it is possible that changes in chromatin structure, packing and condensation state lead to a generalised inhibition of transcription from the kinetoplast when the kinetoplast DNA adopts the basket-like configuration. This would be analogous to transcriptional repression observed during nuclear mitosis. Certainly, this possibility warrants further investigation.

5. Life cycle in the mammalian host and the importance of trypomastigote pleomorphism

Trypanosoma cruzi replicates as an amastigote in the cytoplasm of the nucleated cells of its vertebrate host, forming a pseudocyst. Only trypomastigotes and amastigotes are observed in the peripheral blood of the host, and slender trypomastigotes are readily seen escaping from packed pseudocysts in culture. The trypomastigotes in the peripheral blood are pleomorphic, consisting of both slender and broad forms. The ratio of these forms varies depending on the parasite strain, and there are reports in the literature both that slender forms precede broad forms and that broad forms precede slender forms (Brener, 1969, 1973). Intriguingly, there are also periodic and longstanding reports that a transient epimastigote form is seen in the pseudocyst which gives rise to the trypomastigote forms (Wenyon, 1926; Mehlhorn et al., 1977; Faucher et al., 1995; Almeida-de-Faria et al., 1999).

In order to resolve some of these issues, I began by using DIC and immunofluorescence microscopy to examine a population of trypanosomes which I obtained by breaking open infected myocytes mechanically. The infected myocytes contained pseudocysts packed with trypanosomes in different stages of differentiation from amastigote to trypomastigote. Five distinct morphologies were observed in this population – amastigote, epimastigote, and three morphologies of trypomastigote: slender, broad and lozenge-shaped (Fig. 3). Interestingly, all of these morphologies have been previously described in the drawings of Wenyon (1926) of the forms present in a mouse heart. The lozenge-shaped form was distinct from the broad form in that the shape was very characteristic and regular and the chondriome structure was entirely different, appearing to be more peripheral than the split tubule of the broad form. It seemed likely that the intracellular epimastigotes and lozenge-shaped trypomastigote cells were the sequentially arising intracellular intermediate forms leading to the formation of the slender trypomastigote. We therefore looked directly at fixed pseudocysts by immunofluorescence using a deconvolution microscope. The differentiating forms in the pseudocysts appeared to contain both epimastigotes and the lozenge-shaped forms observed. Since the time from myocyte disruption to processing was only minutes, it is unlikely that the epimastigotes were generated during the processing (see below).

By analogy with work on *T. brucei*, where it has been known for some years that the slender form gives rise to the stumpy form (Vickerman, 1985), it seemed intuitive that the slender form precedes the broad form in *T. cruzi* also. A simple experiment confirmed this: culture trypomastigotes transform to the amastigote form readily in vitro under established conditions. By sampling and counting the various morphologies at each time point, it was shown that slender forms are the first to be lost from the population, followed, after a transient increase, by the broad forms. As

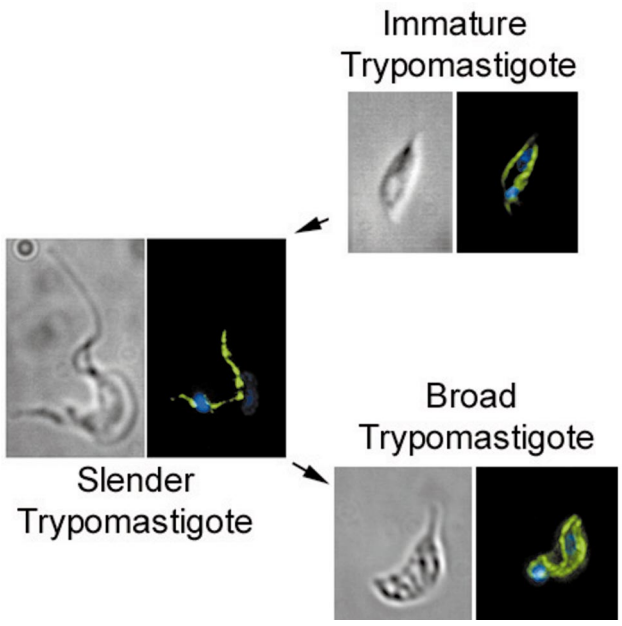

Fig. 3. Bloodstream trypomastigotes are pleomorphic. Differential interference contrast images (left). Fluorescence images (right): the chondriome (green), kinetoplast and nucleus (blue) are shown. Most often, slender and broad trypomastigotes are observed. Note how the single chondriome of the slender form gives rise to a twisted loop reminiscent of the amastigote mitochondrion in the 'broad form' which retains some free flagellum. The immature trypomastigote, more often seen in the pseudocyst, has a distinctive lozenge shape and has no visible free flagellum.

trypomastigote numbers fall, amastigote forms accumulate, and differentiation is complete by 48 h (Fig. 4A). This is consistent with the broad form serving as an intermediate between the slender form and the amastigote.

In contrast, a monomorphic population of metacyclic trypomastigotes under identical conditions does not differentiate, but rather undergoes cell death over a 24 h period (Fig. 4B). This illustrates that, although the slender trypomastigotes can appear similar in morphology to the metacyclic forms, may invade cells by the same mechanism and have a similar metabolic state and some shared structural features, they are different functionally with respect to their commitment to the production of an amastigote form. In fact, it has been pointed out that bloodform trypomastigotes appear not to truly exit from the cell cycle, but to merely exist in a protracted G_1 phase (Gull, unpublished). In contrast, the metacyclic form shows characteristics of true cell cycle arrest and could be considered to be resident in G_0 until triggered to re-enter the cell cycle upon host cell invasion. This, coupled with documented differences in gene expression (Nogueira et al., 1982; Abuin et al., 1989; Araya et al., 1994), justifies the discrimination of bloodstream and metacyclic forms as physiologically distinct life cycle stages.

6. The significance of the intracellular epimastigote

Much of the research conducted on *T. cruzi* is conducted using a non-infectious extracellular epimastigote form, which is readily cultured in vitro, and hence, tractable to experimentation. The possible presence of an analogous, pathologically relevant, intracellular epimastigote intermediate would, therefore, be of tremendous interest. Morphologically speaking, the amastigote form itself is essentially an epimastigote, albeit with a short round cell body and a very short flagellum. Consequently, the presence of intracellular epimastigotes (Fig. 5) may simply indicate that, during an amastigote to trypomastigote transformation, elongation of the flagellum and the posterior cell body precedes translocation of the kinetoplast/basal body complex relative to the nucleus (Fig. 6). While this has some implication for the way in which the trypanosome cytoskeleton can be modified, it would have little implication on the way the life cycle is viewed. In kinetoplastids, however, there is a longstanding association between cellular morphology and the discrimination of life cycle stages (Wenyon, 1926). The intracellular epimastigote could be a true and obligate stage in the life cycle in the vertebrate host. An intracellular epimastigote might be taken to imply a three-stage life cycle (amastigote, epimastigote, trypomastigote) that would happen both in the vertebrate host and insect vector.

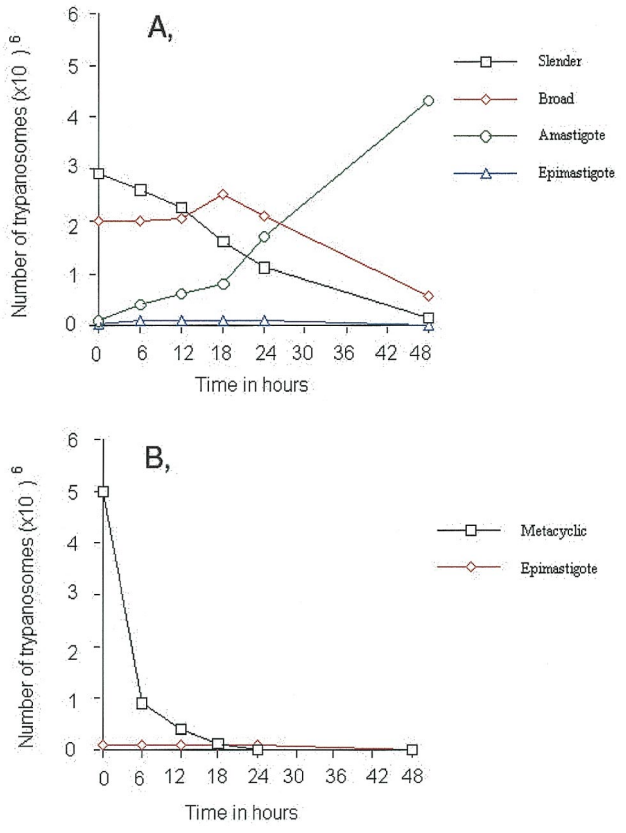

Fig. 4. Metacyclic trypomastigotes have different fates from blood form (tissue culture derived) trypomastigotes under similar conditions. In IMDM media at 37°C, blood form slender trypomastigotes were lost first from culture, the number of broad forms initially rose and then diminished, whilst amastigotes accumulated throughout the 48 h period. In contrast, metacyclic forms persisted for less than 24 h and did not differentiate.

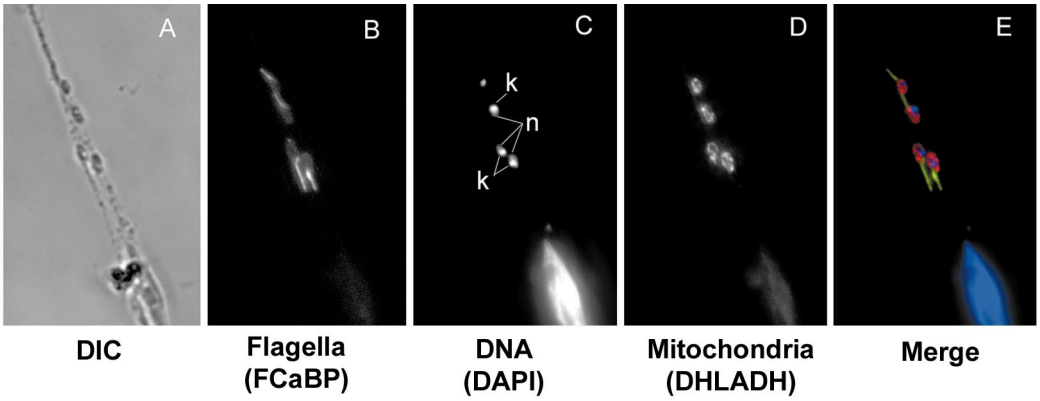

Fig. 5. Morphologically epimastigote forms can be visualised by fluorescence microscopy of infected cultured myocytes. (A) This shows differential interference contrast microscopy of an infected cell still containing some epimastigote forms after mechanical lysis of the cell. (B) The flagella are stained for flagellar calcium binding protein; (C), the DNA of the nuclei (n) and kinetoplasts (k) with the intercalating fluor DAPI; and (D), mitochondria cells are stained for dihydrolipoamide dehydrogenase. (E) The merged image; it is clear that the flagellum and kinetoplast positions relative to the nucleus make these morphologically epimastigote forms.

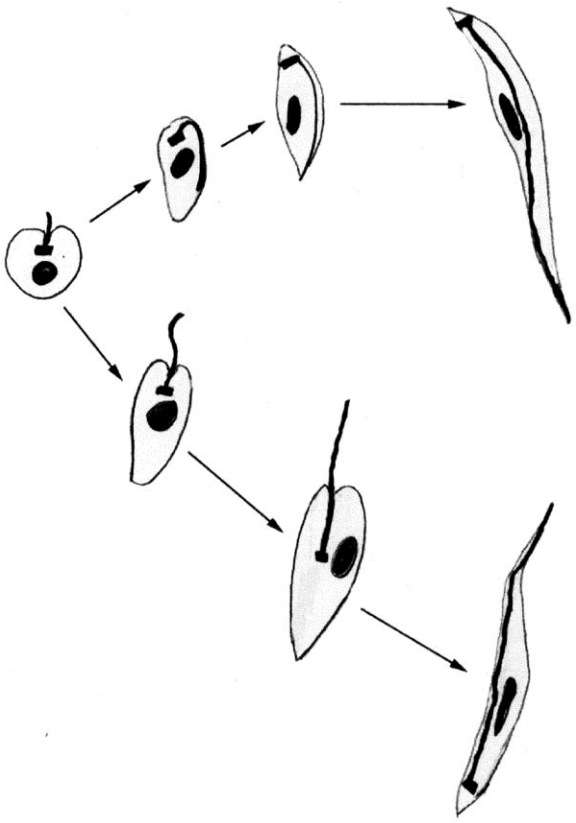

Fig. 6. Two possible cytological mechanisms by which an intracellular amastigote may transform to a slender trypomastigote. Above, simple elongation of the flagellum towards the anterior and co-ordinate extension of the flagellar attachment zone results in an intermediate form trypomastigote with a lozenge-like appearance. This form then simply elongates further to the slender trypomastigotes. Below, the amastigote elongates its flagellum and cell body to give an epimastigote intermediate with anterior free flagellum. The kinetoplast/basal body complex then migrates to a posterior nuclear position; the observation of intracellular epimastigotes (above) favours this mechanism.

Much more research is required to determine if this is indeed the case; however, recent reports of shared stage-specific epitopes and biochemical properties between extracellular and intracellular epimastigotes do lend support to this view (Faucher et al., 1995; Almeida-de-Faria et al., 1999).

7. Impact of simple sugars on metacyclogenesis

We have previously shown that the depletion of glucose from the medium triggers elongation of the epimastigote flagellum (Tyler and Engman, 2000), which facilitates the hydrophobic interaction believed to trigger metacyclogenesis (Bonaldo et al., 1988). Other studies have also shown that rate of metacyclogenesis in culture is exquisitely sensitive to the availability of simple sugars (Adroher et al., 1988; Homsy et al., 1989; Krassner et al., 1990). Further, the mitochondrial metabolism of epimastigotes shifts, becoming cyanide insensitive when epimastigotes reach the stationary phase (Felix et al., 1978). We decided to determine whether the signal to undergo flagellar elongation was specific to glucose or whether other simple sugars could also inhibit flagellar elongation (Table 1, column 1) and, likewise, whether the concentration of other simple sugars affected metacyclogenesis in a similar manner to glucose (Table 1, column 2). It was observed that epimastigotes placed in phosphate-buffered saline (PBS) alone also underwent flagellar elongation, so we determined whether flagellar elongation under these conditions would also be reduced by the addition of other simple sugars (Table 1, column 3).

The glucose transporter of *T. cruzi* has been shown to transport glucose and fructose with similar efficiency; galactose, on the other hand, is not transported and neither are disaccharides like lactose (Tetaud et al., 1997, 1996). We assessed the specificity with which flagellar elongation was inhibited by glucose by testing a number of other simple sugars, and found that most were able to inhibit

Table 1
Effects of different saccharides on flagellar elongation and metacyclogenesis[a]

		Glucose$^-$ media: elongation (7 days)	Glucose$^-$ media: metacyclogenesis (21 days)	PBS: elongation (48 h)
Depleted		+++	−	+++
D-glucose	(20 μm)	++	+	++
D-glucose	(40 μm)	−	−	++
D-fructose	(40 μm)	−	−	++
D-galactose	(40 μm)	+++	+	−
D-mannose	(40 μm)	+	−	++
D-maltose	(40 μm)	+	−	++
D-lactose	(40 μm)	+++	−	+++
L-glucose	(40 μm)	+++	−	+++

[a] Both the abrogation of flagellar elongation in glucose-depleted medium and metacyclogenesis are critically dependent on the concentration of simple sugars. Neither glucose-depleted nor glucose enriched media will permit metacyclogenesis. Galactose which is not transported into the cell at a sufficient rate to abrogate flagellar elongation in depleted media does, however, support metacyclogenesis. Moreover, in PBS alone galactose is able to abrogate flagellar elongation, presumably since rate of uptake is increased in these conditions.

flagellar elongation in glucose-depleted medium. Galactose, lactose and L-glucose, however, which are not transported by the stereo-specific glucose transporter, did not inhibit flagellar elongation. There was an indication, however, that maltose and mannose were less effective, perhaps reflecting less efficient import into the cell or reduced metabolic potential.

What was surprising was that, in PBS, there was considerable flagellar elongation, even in the presence of glucose or fructose, and only in the case of galactose was inhibition of flagellar elongation apparent. This is in contrast to glucose-depleted medium, where galactose did not have a discernible effect. This was initially surprising since galactose does not enter the cell by the glucose transporter. Looking at metacyclogenesis, 20 μM glucose (or fructose) in the media supports up to 40% metacyclogenesis at 21 days after passage in the Br3c clone used for these experiments. Removing glucose or doubling the glucose concentration completely ablates metacyclogenesis, as does the use of any of the other saccharides tested, except, again surprisingly, 40 μM galactose, in which metacyclics were produced with, if anything, slightly higher efficiency than 20 μM glucose.

It is known that *T. cruzi* does take up galactose, albeit at a much lower rate than glucose since radiolabelled galactose is incorporated into some shed *T. cruzi* macromolecules (Couto et al., 1991). The inhibition of flagellar elongation in PBS by galactose can be explained if one hypothesises that the rate of saccharide uptake in PBS is dramatically increased compared with the uptake in media. If so, glucose and fructose would be used up so rapidly that elongation would have time to take place in the time frame measured. The increased efficiency of galactose uptake could be high enough to abrogate flagellar elongation, but low enough not to be exhausted over the time course measured. The other deduction from Table 1 is that a certain sustained low level of glucose/monosaccharide is required for metacyclogenesis. At lower glucose concentrations, in glucose-depleted media, cells cannot undergo metacyclogenesis, while at higher concentrations, cells grow to a higher density, then utilising the remaining glucose too fast for metacyclogenesis to occur. We would hypothesise then that serendipitously, the rate of galactose import turns out to be just right to sustain differentiation to the metacyclic form. From this interpretation of the data, we have developed a working hypothesis which is illustrated in Fig. 7. Obviously, validating such a model will require further study of the rates of saccharide uptake by *T. cruzi* under different conditions.

8. On commitment, cell cycle and morphogenesis

An important feature of the life cycle, which is apparently a fundamental difference from the *T. brucei* life cycle, is multipotency. Some *T. cruzi* life cycle stages may have multiple, viable potential fates depending on the environment in which they are placed. At the 37°C, 5% CO_2 environment of the host cell cytoplasm, amastigotes proliferate until the pseudocyst is full, and then differentiate to give trypomastigotes. At 27°C in fresh LDNT medium, amastigotes give rise to epimastigotes after a few rounds of cell division. What are the differences in the signals received which direct the cell whether or not to differentiate and into which cell type to transform? At what point is multipotency lost, when do cells commit to one lineage?

Another important observation is of amastigotes in newly passaged cultures of epimastigotes, even after years of continuous culture in LDNT (Wood and Pipkin, 1969). It is clear that the amastigote to epimastigote differentiation is reversible and that amastigote formation is favoured by high glucose concentration in the media. It is not clear whether glucose alone modulates this transition, and amastigotes comprise only a minority of cells even in sustained low density cultures. This leads to a question about what is responsible for the heterogeneity of cell types observed in low density cultures. It is also interesting that the transition

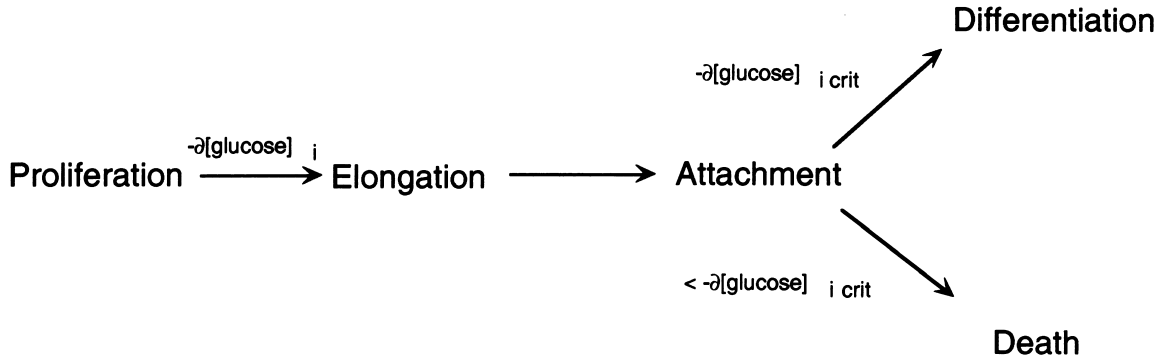

Fig. 7. Based on the above data and previous published work, we propose that the intracellular glucose concentration is they key factor both in elongation of the flagellum and in sustaining differentiation. Reduced levels of glucose signal flagellar elongation which is necessary for hydrophobic attachment and which generates the signal to differentiate; however, some glucose must remain to support the process of differentiation which will otherwise fail, resulting in cell death.

from amastigote to elongate epimastigote appears to be independent of the cell cycle position and that the size of the cells in differentiating cultures do not have an obvious relationship with preparedness for division. This, in turn, raises the question of whether size related cell cycle checkpoints are present during this phase of the life cycle.

Metacyclogenesis is the best characterised of the *T. cruzi* life cycle transitions. It involves an extreme morphogenetic event, changes in metabolism, expression of new surface markers, presumably a change in the hydrophobicity of the flagellar membrane to allow release from substrate, and exit from the proliferative cell cycle. The trigger for metacyclogenesis appears to be the hydrophobic interaction of the flagellum and an appropriate substrate. It is not clear though how this is transduced into a secondary (cAMP) signal, or what is the sequence of events that is triggered. Do attached cells arrest in the cell cycle and then differentiate or do they continue to replicate once attached? If they do replicate, do they do so only once, or multiple times? Does a terminal division give rise to one or two metacyclic forms, or to intermediate forms?

Trypanosomes are organisms in which shape is dictated, at least in part, by the subpellicular array of microtubules. The lack of microfilaments and intermediate filaments make trypanosomes a clean system for studying the potential contribution of microtubules to morphology. It has been suggested that the composition of the subpellicular array with respect to tubulin isoforms may be stage-specific and may affect morphology. With the recent discovery in trypanosomes of new tubulin isoforms, posttranslational modifications of tubulin, plus and minus end microtubule associated proteins such as gamma-tubulin, in addition to microtubule-motors such as kinesin, these ideas will certainly begin to come under closer scrutiny. The clear polarity, asymmetry and motility of the trypanosome cell and its dependence on microtubule numbers, organisation and composition for defining morphology and flexibility make trypanosomes interesting model organisms for the study of morphology and motility. The extreme morphological and motility differences of the trypomastigote and amastigote stages of *T. cruzi* offer an excellent chance to highlight microtubule-based mechanisms of morphological control which would be difficult to discern in systems where such change is more subtle.

9. Defining the sphaeromastigote

Brack (1968) first described the sphaeromastigote as an early stage in the differentiation to the epimastigote form, but went on to say that it was the attached sphaeromastigote form that served as an intermediate in metacyclogenesis. In vitro, these observations seem difficult to reconcile since metacyclic trypanosomes arise only in stationary phase populations. What adds to the confusion is that the in vitro generated sphaeromastigotes described in vitro by

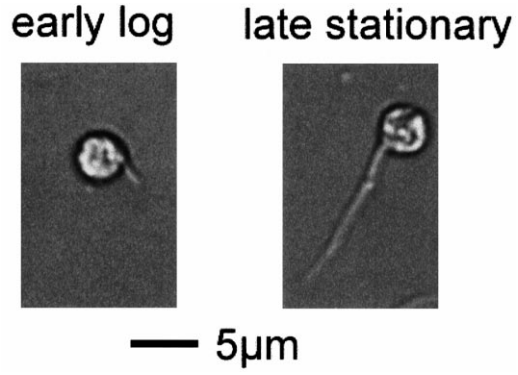

Fig. 8. Two different forms, both regarded as sphaeromastigotes. One is transitional from amastigote to epimastigote (left), the other from epimastigote to trypomastigote (right). In early log phase cultures, at a high glucose concentration, amastigote forms with slightly lengthened motile flagella are frequent (left). These cultures give rise to homogenous log growth. Epimastigote cultures with intermediate glucose concentrations have no sphaeromastigote forms and these cultures give rise to stationary phase cultures with low glucose levels, in which metacyclogenesis intermediates, with a round cell body and a long generally attached flagellum (right), are common. Although they look similar, these cells are quite different in terms of the fates to which they are committed.

some groups (for instance see Teixeira et al., 1999) are clearly a different cell type from that described by groups working in vivo (e.g. Kollien and Schaub, 1998). Fig. 8 illustrates the reason for the confusion. By DIC microscopy, and looking only at the visible cell body, very similar-looking forms are common in both early epimastigote populations and in stationary phase populations. During the middle of log growth, however, no such forms are observed and cultures have a uniform epimastigote morphology. This indicates that the forms seen are not the same form persisting through culture. Morphologically, these two forms are readily discriminated since the flagella of forms in the stationary phase culture are many times the length of the forms in early log growth cultures. Using immunofluorescence microscopy, it is possible to better visualise that the somatic pellicular membrane of the stationary phase form extends most of the way down the flagellum. This is in contrast to what is observed in the early log form. In this form, most of the much shorter flagellum is free once it emerges from the flagellar pocket. In all likelihood, these forms are functionally quite different. When glucose is reduced, the early log form elongates to give a true epimastigote, whereas the stationary phase form, which is generally attached to a substrate by its flagellum, is probably in the process of transformation from epimastigote to trypomastigote. In our depiction of the life cycle (Fig. 1), we have described the early log form as a sphaeromastigote, partly because it is more spherical, and partly because of our reading of what Christine Brack initially meant. It would be equally valid to refer to the stationary phase form as a sphaeromastigote; however, we recommend adopting the terminology used in this presentation since continuing to use the same term for both leads to confusion. In summary, it seems probable that both 'sphaeromastigote' forms are intermediates rather than life cycle stages per se.

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