Mitochondrial genome repositioning during the differentiation of the African trypanosome between life cycle forms is microtubule mediated

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SUMMARY

The cell cycle of the African trypanosome requires a precise orchestration of nuclear and mitochondrial genome (kinetoplast) positioning to ensure faithful segregation during division. The controls underlying these events must be subject to modulation, however, as the respective positioning of these organelles changes during the parasite’s complex life cycle. We have studied mitochondrial DNA repositioning during differentiation between the trypanosome’s bloodstream and procyclic form. We have found that repositioning occurs simultaneously with the DNA replication phase of the cell cycle of the differentiat- ing parasite. Furthermore, we demonstrate, at the cell and individual microtubule level, that this organelle repositioning is achieved via microtubule-dependent processes. Our results have implications for the control of cell differentiation and division in African trypanosomes.

Key words: trypanosome, Trypanosoma brucei, microtubule, mitochondrion, kinetoplast, differentiation, cell cycle, cytoskeleton

INTRODUCTION

The causative agents of African trypanosomiasis, Trypanosoma brucei spp., are protozoan parasites of mammals transmitted by the tsetse fly. The parasite life cycle involves a complex developmental programme with many biochemically and morphologically distinct forms being present within both the mammalian host and the insect vector (reviewed by Vickerman, 1985). The major morphological differences involve changes in overall cell shape and, in particular, the position in the cell of the mass of mitochondrial DNA comprising the kinetoplast. Repositioning of the kinetoplast from the extreme posterior of the cell to a location close to the nucleus was, for instance, discovered very early to be a fundamental event that occurred after transfer of the parasite from the host blood to the tsetse midgut (Robertson, 1913). Since the overall cell shape, staining of the kinetoplast and nucleus with Giemsa, and the single flagellum could all be ascertained by early cytologists, the position of these organelles became critical parameters for diagnosis of both the species of the trypanosome in an infection and their progression through the complex host/vector life cycle (Bruce et al., 1912; Robertson, 1912, 1913; Vickerman, 1965; Steiger, 1973; Brown et al., 1973). These changes in cellular architecture are also of fundamental significance to the organism. The molecular mechanisms that underlie them, however, have remained a mystery.

In the bloodstream, the trypanosome population undergoes fluctuating waves of parasitaemia, resulting from the cyclical outgrowth and immunological control of distinct antigenic variants of the parasite (reviewed by Cross, 1990). Within each parasitaemic wave the trypanosome population is heterogeneous, with the abundance of different morphological forms of the parasite varying as the parasitaemia progresses (Vickerman, 1965). Early on, the population is predominantly composed of cells which are elongate in morphology and are actively proliferative, the so-called ‘slender’ form. Subsequently, as the peak of parasitaemia approaches, the relative abundance of these forms decreases and they are replaced by the ‘stumpy’ form (Balber, 1972). This cell type shows a shorter and fatter cell shape, and is non-dividing. This stumpy form is apparently pre-adapted to differentiation through to the procyclic form; in particular they demonstrate the beginnings of mitochondrial reactivation at the structural and enzymatic levels (see Priest and Hajduk, 1994). In vitro analysis of the bloodstream trypanosome cell types has revealed that while both slender- and stumpy-enriched populations are capable of differentiation through to procyclic cells, the stumpy forms differentiate preferentially, efficiently and synchronously (Roditi et al., 1989; Ziegelbauer et al., 1990; Matthews and Gull, 1994a).

The synchronous differentiation of stumpy form cells through to the procyclic form can be induced in vitro (Ziegelbauer et al., 1990), permitting a defined biological dissection of the cytology and molecular biology of this process (Matthews and Gull, 1994b). This has revealed that coincident with the earliest differentiation events is a synchronous entry of the differentiating cell population into an active proliferative cell cycle from their division-arrested stumpy cell progenitors (Pays et al., 1993; Matthews and Gull, 1994a). The first detectable event is DNA synthesis, and this is followed by daughter flagellum outgrowth, segregation of the kinetoplasts, and subsequent mitosis and cytokinesis, events that reflect the...
well characterised procyclic trypanosome cell cycle (Sherwin and Gull, 1989a; Woodward and Gull, 1990). Analyses of the procyclic cell cycle reveal that the process must be very precisely governed. The organisation of the trypanosome cell nucleus, kinetoplast, flagellum, basal body and cytoskeleton is very tightly defined, and spatially co-ordinated (Robinson et al., 1995). This level of control is essential to ensure that the parasite faithfully segregates its single copy organelles during division, resulting in the production of two viable daughter cells. Similar stringent controls are likely to operate in bloodstream trypanosome cells, despite their body architecture being different from that of the procyclic form. There must be, therefore, a precise cellular remodelling during differentiation between these two forms. In this paper we have utilised the synchronous differentiation model to investigate one aspect of this remodelling-repositioning of the kinetoplast with respect to the cell nucleus and cell posterior. Our results, obtained at the cell and individual microtubule levels, implicate a role for microtubule assembly in kinetoplast repositioning. This represents the first molecular explanation for the classical cytological observations on the changes in the trypanosome organellar organisation as it progresses through its life cycle.

MATERIALS AND METHODS

Trypanosomes and differentiation conditions
For all differentiation analyses a pleomorphic line of T. b. rhodesiense EATRO 2340 was used; 1×10⁶ trypanosomes were inoculated into large, immunocompetent Balb/c mice. The morphology of the trypanosome infection was subsequently monitored and the parasites harvested when a level of >80% stumpy cells was achieved (typically 5 days post-infection). The cells were then placed directly into SDM-79 (Brun and Schonenburger, 1979) containing 6 mM cis-aconitate, 2 mM citrate, at 27°C, (for fluorescence analysis) or were purified over a sterile DEAE-cellulose column prior to culture, when protein extracts were to be prepared (Lanham and Godfrey, 1970). For microtubule inhibition experiments, rhizoxin solubilised in DMSO was included in the differentiation medium at a concentration of 20 nM or 100 nM (the final DMSO concentration was 10 µl/10,000 µl of culture medium). Control cultures were incubated in the absence of drug, but in the presence of DMSO. Rhizoxin was kindly provided by Shigeo Iwasaki (University of Tokyo, Japan).

Fluorescence and western blotting
Trypanosomes were prepared for fluorescence as described by Matthews and Gull (1994a). The resultant images were then captured either on Ilford XP-2 film, or using a Hamamatsu video camera linked to a Fluovision imaging system (Improvision; Coventry, UK). Cell-dimensional analyses were performed using Fluovision cytoanalysis software (Improvision, Coventry, UK). For such analysis, the shortest distance between respective cellular landmarks was plotted along the longitudinal axis of the cell when viewed at ×1000 final magnification. Composite 4,6-diamidino-2-phenylindole (DAPI)-FITC images were processed using Adobe Photoshop.

Trypanosome protein extracts were prepared by harvesting trypanosomes from culture, pelleting them at 1000 × g for 10 minutes, washing them with PSG (488.8 mg/l NaH2PO4, 2.55 g/l NaCl, 8.08 g/l NaHPO4, 15 g/l D-glucose), repelletting them, and lysing them in PGS/1% SDS containing protease inhibitors. Extracts were then separated by SDS-PAGE (Laemmli, 1970) and western blotted onto nitro-cellulose by standard procedures. Antibody binding was visualised by enhanced chemiluminescence (ECL; Amersham International, plc).

Electron microscopy
Detergent-extracted cytoskeletons were prepared and processed for immunogold labelling, positive staining and critical point drying as described by Sherwin and Gull (1989b).

RESULTS

Kinetoplast migration during differentiation
In bloodstream stumpy cells, the kinetoplast lies close to the posterior limit of the cell body, often being displaced to one side by the large flagellar pocket at this life cycle stage. In contrast, in the procyclic cell the kinetoplast lies at a position approximately equi-distant from the cell posterior and the cell nucleus. The repositioning of this organelle can be examined in a synchronous differentiating cell system developed by Ziegelbauer et al. (1990). Synchronous differentiation is achieved by harvesting bloodstream form trypanosomes at a late stage in a wave of parasitaemia when there is a very high proportion of division-arrested stumpy form cells. When placed into in vitro culture media, at 27°C, containing cis-aconitate, these cells rapidly transform through to their procyclic form, with most events for which we have markers occurring in the first 12 hours. With this system we have used computer-enhanced microscopy to define a number of parameters for the structure of the differentiating cell and its organelles. Specifically, we have plotted the position of the cell nucleus and kinetoplast with respect to each other and to the posterior limit of the cell as the trypanosome undergoes differentiation. We have also plotted the position of the nucleus in relation to the anterior of the cell. Fig. 1 shows that the cytological restructuring of the trypanosome cell begins after approximately 6 hours under differentiation conditions, and that the process is complete 6 hours later. We have quantified several of the components responsible for the repositioning of the kinetoplast within the trypanosome cell body (Fig. 1). The first component is an outgrowth of the posterior end of the trypanosome cell, resulting in an increased nucleus to posterior distance of approximately 3 µm. The second component is a migration forward of the kinetoplast toward the nucleus, thereby decreasing this dimension by approximately 1.5 µm. A further contribution is potentially provided by movement of the nucleus forward toward the anterior end of the cell. This component is hard to define precisely using phase-contrast microscopy, because of the narrow anterior end of the trypanosome cell. However, it is apparent that it is complex, being made up of anterior directed movement of the nucleus and outgrowth of the anterior end of the cell. Since these processes are antagonistic and result in little net change in this dimension of the trypanosome cell, we have focused our subsequent analyses on events occurring posterior to the nucleus. Taking these individual changes into account, Fig. 1 shows the overall conclusion that the kinetoplast-posterior distance increases from around 1 µm to over 4 µm during the differentiation, so bringing the kinetoplast close to the nucleus of the procyclic cell. Significantly, the components of this change occur simultaneously, are exactly coincident with the DNA replication of the kinetoplast and nuclear genomes of the differentiating population (Matthews and Gull, 1994a; Pays et al., 1993), and are complete prior to segregation of the daughter kinetoplasts.
Trypanosome kinetoplast repositioning

Microtubule assembly and kinetoplast migration

We have previously used an antibody, YL1/2, to plot the progress of differentiating trypanosomes through their first cell cycle (Matthews and Gull, 1994a). This antibody is specific for the tyrosinated form of alpha tubulin in eukaryotic cells (Wehland et al., 1984; Kilmartin et al., 1982) and in trypanosome cells can be used diagnostically to define trypanosomes early in their cell cycle (Sherwin et al., 1987). The primary translation product of alpha tubulin in trypanosomes is tyrosinated, and can thereafter enter a detyrosination/tyrosination pathway mediated by the enzymes tubulin tyrosine carboxypeptidase (which detyrosinates) and tubulin tyrosine ligase (which tyrosinates). Dynamic (rapidly turned over or newly laid down) microtubules are tyrosinated, and then become detyrosinated as they age, events which have been scored on single microtubules in the trypanosome cell (Sherwin and Gull, 1989b). YL1/2 characteristically stains the basal body of the procyclic trypanosome cell and a posterior cone on the cell which fluctuates through the cell cycle. This posterior coning represents the extension of existing microtubules in the trypanosome and the interdigitation of new microtubules as the trypanosome lays down the cytoskeleton of the daughter cell.

When we examined the extent of YL1/2 staining on stumpy bloodstream cells we noticed that there appeared to be only prominent staining of the cell’s basal body, with little staining of the posterior cone. As the cells underwent differentiation, however, the cellular staining increased progressively. Fig. 2 shows this induction of staining for tyrosinated alpha tubulin; on the left are shown phase images indicating the parasite cell shape, on the right is shown the YL1/2 labelling pattern. Cells, until approximately 6 hours through differentiation, show a staining pattern essentially the same as that seen for bloodstream stumpy cells. After approximately 6 hours, however, a brightly labelled posterior tip to the trypanosome cell is seen, which increases to a coned staining pattern between 8 and 10 hours through the differentiation. Finally, by 12 hours through the differentiation, the characteristic procyclic staining pattern is observed and there are the beginnings of basal body segregation, an event that occurs quite synchronously in the transforming population (Matthews and Gull, 1994a). Thus, it appears that the level of tyrosinated alpha tubulin increases during trypanosome differentiation.

To investigate whether increased YL1/2 labelling represented an increase in total cellular tubulin or an increase in the level of tyrosinated tubulin alone, we compared the respective levels of alpha tubulin and tyrosinated alpha tubulin in the differentiating population. Thus, we prepared total cellular protein at various time points and subjected these to western blotting with an antibody TAT-1, specific for all alpha tubulin isoforms (Sherwin and Gull, 1989b), and YL1/2, specific for tyrosinated alpha tubulin. Fig. 3A shows the Coomassie-stained gel used in this analysis, and demonstrates that with the exception of loss of the variable surface glycoprotein (VSG, arrowed), there is little detectable change in the protein profile of the differentiating population. Similarly, when the same samples were labelled for total alpha tubulin using the antibody TAT-1, there was little apparent change through the differentiation time course (Fig. 3B). In contrast, when the same blot was stripped and re-labelled with YL1/2, there was a clear induction in the staining through the time course, being at a low level in stumpy cells and increasing in level through the differentiation (Fig. 3C). Thus, at the cellular and protein levels trypanosome differentiation is accompanied by an increase in the level of tyrosinated alpha tubulin.
The YL1/2 staining pattern on differentiating trypanosomes prompted us to examine more closely these cells with respect to their cellular morphogenesis. When we did this we noticed that there was a very close association between the labelling pattern of the differentiating cells and the extent to which they had repositioned their kinetoplast. Fig. 4 shows in detail three cells at 10 hours through differentiation. These cells are all undergoing cellular morphogenesis but are not precisely synchronised, thereby allowing us to correlate kinetoplast position with the tyrosination status of the cells’ microtubules. Strikingly, in each case the degree to which the kinetoplast has been repositioned (shown in the phase/DAPI image, Fig. 4A) exactly correlates with the position and extent of tyrosinated microtubules (Fig. 4B). Furthermore, when we examined the relative position of the kinetoplast (revealed by DAPI staining) with respect to that of the basal body in the differentiating cell (revealed by YL1/2 staining), we noticed a consistent relationship. Fig. 5 shows a merged DAPI/FITC image of a representative cell 10 hours through differentiation. This illustrates the important point that the basal body always lies directly adjacent to, and anterior to, the kinetoplast during organelle migration.

In order to investigate whether the correlation between dynamic microtubules and kinetoplast position was causative...
or coincidental we asked whether inhibiting the assembly of new microtubules would result in inhibition of kinetoplast repositioning. We did this by incubating the differentiating trypanosome population in 0, 20 nM or 100 nM of the drug rhizoxin. This compound is of the maytansinoid class of antitubulin agents and is a potent inhibitor of microtubule assembly in trypanosomes (Robinson et al., 1995) and other organisms (see Hamel, 1992, for review). Fig. 6 shows phase-contrast/DAPI images of cells 10 hours through differentiation in the presence of various concentrations of this drug. Fig. 6 (0 nM) shows control cells in the absence of rhizoxin: there has been clear repositioning of the kinetoplast in relation to the posterior end of the cell; the kinetoplast-posterior distance has increased from approximately 1 μm (as seen in stumpy cells) to approximately 4.8 μm (Fig. 7A). Where cells were incubated in 20 nM rhizoxin, kinetoplast migration has also occurred but to a lesser extent than in controls, the kinetoplast-posterior distance being approximately 2.5 μm (Fig. 6, Fig. 7A). Lastly, we show differentiating trypanosomes incubated in 100 nM rhizoxin. This cell population is still alive, being actively motile, but the distance between the cell posterior and the kinetoplast is akin to that seen in bloodstream trypanosomes, i.e. approximately 1 μm (Fig. 6, Fig. 7A). These cells also fail to demonstrate the characteristic polarised staining pattern with YL1/2, showing instead only a weak overall cellular fluorescence (data not shown). The second component of kinetoplast repositioning, decrease in the nuclear-kinetoplast dimension is also compromised in the presence of rhizoxin (Fig. 7B). At 10 hours through differentiation, 100 nM rhizoxin-treated cells have a kinetoplast-nuclear distance 0.9 μm greater than controls, representing an almost complete inhibition of this repositioning component. Indeed, the degree of this inhibition may even be greater than observed; the beginnings of cellular rounding that occurs in the presence of rhizoxin beyond 12
hours through differentiation may well mask to some extent the effect of the drug during earlier time points (K. Matthews, unpublished observations).

In the final analysis, we have related the contribution of the kinetoplast-nucleus distance to the position of the nucleus with respect to the posterior by a calculation of the kinetoplast index, KI (posterior-nuclear distance/nucleus-kinetoplast distance; Brown et al., 1973). Control cells have a KI of 1.9, representative of previous KI determinations for established procyclic cells, while 100 nM rhizoxin-treated cells have a KI of 1.1, equivalent to previously calculated values for a bloodstream stumpy cell population (Brown et al., 1973). Thus, by every criterion measured, the anti-microtubule drug rhizoxin inhibits kinetoplast repositioning in the differentiation of bloodstream cells through to their procyclic form, indicating that the process is microtubule dependent.

**Cellular morphogenesis is achieved by the extension of existing microtubules**

The induction of microtubule assembly at the posterior end of the trypanosome cell during trypanosome differentiation could take two forms. In the first, there might be extension of microtubules already present in the differentiating cell. Since the trypanosome has a highly defined microtubule polarity, with their plus ends all being oriented to the posterior of the cell (Robinson et al., 1995), an extension of pre-formed microtubules would be manifested as an increasing YL1/2 labelling pattern at this end of the cell. Alternatively, the observed pattern of tubulin tyrosination might be caused by the laying down of new, short, interdigitating microtubules at the posterior of the cell, as is believed to occur during cytokinesis (Sherwin and Gull, 1989b). In order to distinguish between these possibilities, we have examined the individual microtubule population within differentiating trypanosomes. This has been achieved by YL1/2 immunogold labelling of detergent-extracted trypanosome cytoskeletons subjected to positive staining and critical point drying. This procedure permits the observation of the preserved microtubule corset of trypanosomes and allows the tyrosination status of individual microtubules to be followed along their length (Sherwin and Gull, 1989b).

Fig. 8 shows the microtubule array of a representative trypanosome 8 hours through the differentiation, when kinetoplast repositioning is actively ongoing. YL1/2 labelling of these cytoskeletons demonstrates the presence of microtubules which are labelled at their posterior end and show less labelling toward the anterior of the cell. However, although individual microtubules are difficult to follow as they congress at the posterior end of the cell, in no case have we seen any evidence for large numbers of short interdigitating microtubules at the posterior end of the cell. Instead, it appears that modulation of the microtubule structure during trypanosome differentiation and consequent repositioning of the kinetoplast is caused primarily by the extension of existing microtubules (Fig. 8, inset panel).

**DISCUSSION**

When the bloodstream stumpy cell differentiates through to the procyclic form found in the tsetse fly midgut there is an extensive remodelling of the cell architecture and alterations in the position of the parasite’s organelles. These changes have been long recognised, are well described, and have been used diagnostically in the definition of distinct parasite life cycle
forms for over 90 years (Bruce et al., 1912; Robertson, 1912, 1913). Here we have investigated the mechanism responsible for one facet of this differentiation—kinetoplast repositioning, and have found it to be microtubule dependent. We have found this event to be completed early in the differentiation, and to be coincident with the DNA synthesis phase of the transforming cells as they re-enter their proliferative cell cycle. Thus, our results have implications for the control of both cell differentiation and division in African trypanosomes.

The kinetoplast repositioning was initiated 6 hours through differentiation, and was completed 6 hours thereafter. In previous descriptions of organelle migration during differentiation, the observed repositioning occurred over a far longer period, taking approximately 24-48 hours to complete (Brown et al., 1973). This kinetic difference was probably caused by differences in the differentiation media used; there have been intensive studies into the requirements for efficient differentiation of stumpy form cells since these early investigations (Czichos et al., 1986; Roditi et al., 1989; Ziegelbauer et al., 1990, 1993). Despite these temporal differences, our quantitative measurements of the kinetoplast migration are fully compatible with those of Brown et al. (1973). Our observations on the involvement of newly assembled microtubules (as assessed by tyrosination status) extend these descriptive studies and, for the first time, address the molecular mechanisms that underlie kinetoplast repositioning.

The pattern of tubulin tyrosination in the trypanosome cell has provided an invaluable marker in the analysis of the cell cycle of this organism. In the procyclic form, cells which are early in their cell cycle demonstrate labelling of their posterior cone and daughter flagellum with the antibody YL1/2 (Sherwin et al., 1987). Slender bloodstream trypanosomes which are actively proliferative show a similar labelling; their narrow posterior tip shows tubulin tyrosination as do daughter flagella (data not shown). In contrast, most stumpy cells demonstrate very weak posterior labelling. This is not surprising as stumpy cells are non-proliferative and the level of their non-tyrosinated alpha tubulin is likely to rise as the dynamics of the cytoskeleton declines and the sub-pellicular array ages. Once differentiation is initiated, there is an increase in the level of tubulin polymerisation and this apparently operates on pre-existing microtubules, rather than by the morphogenesis of new microtubules. The new assembly is visualised by the presence of tyrosinated alpha tubulin on the posterior end of the trypanosomes, which extends toward the anterior as differentiation continues. This distinctive tubulin tyrosination pattern is compatible with, and supportive of, very recent analyses on microtubule polarity in the procyclic trypanosome cell. Microtubules have an intrinsic polarity, bearing a ‘plus’ end and a ‘minus’ end, the plus end having the greater potential for seeding new tubulin assembly. Using a variety of cytological techniques, it has been shown that the trypanosome cell has a pronounced polarity with respect to its microtubules, and that the majority of microtubules are oriented with their plus end...
toward the cell posterior (Robinson et al., 1995). It is tubulin assembly on to these plus ends that is responsible for the staining pattern we have observed. Although there are few reports of the polarity of microtubules in the cells of eukaryotic microbes, there are numerous examples in the cells of metazoans in which the polarity of cytoplasmic microtubules is intimately associated with the morphogenesis of cells such as neurones and other cells with a defined shape and form in vivo (Baas and Joshi, 1992; Bre et al., 1987; Mogensen et al., 1989). In such cells, the intrinsic positioning of the microtubule organising centre and the polarity of the cytoplasmic microtubules influence organelle position and directed motility through the positioning of specific motors proteins (see Skoufias and Scholey, 1993, for review). Such proteins bind microtubules and generate motile forces on the organelles or proteins. Examples of such molecules motivating organelle transport in eukaryotic cells include the migration of Golgi vesicles (Cooper et al., 1990), endoplasmic reticulum dynamics (Dabora and Sheetz, 1988) and the cortical rotation of frog oocytes (Houliston and Elinson, 1991). The latter at least operates in an environment of unidirectionally oriented microtubules, perhaps analogous to the apparently unidirectional trypanosome microtubule corset.

When treated with the drug rhizoxin, microtubule assembly in the differentiating cells is inhibited and kinetoplast repositioning is blocked. It is important to note that this inhibition is observed whilst the overall microtubule structure of the differentiating cells remains intact. This is because the microtubule cytoskeleton of the non-proliferative stumpy cell (and early differentiating cells) is relatively non-dynamic and does not contain the large numbers of short interdigitating microtubules characteristic of proliferative cells establishing a daughter cytoskeleton prior to cytokinesis. As the differentiating cells complete S-phase and begin to construct their daughter cytoskeleton they become increasingly sensitive to rhizoxin and loose overall cell shape (K. Matthews, unpublished observations).

In the repositioning of the kinetoplast, the direct molecular motile force may act on the basal bodies, rather than on the kinetoplast itself. Robinson and Gull (1991) have shown previously that the mitochondrial genome of the trypanosome is attached to the basal body by some form of physical linkage. On the basis of experiments showing that kinetoplast segregation can be inhibited by microtubule inhibitors acting on the basal body segregation, it was suggested that there might be an archaic mechanism in which segregation of the mitochondrial genome is ensured by linkage to the cytoskeleton. This same linkage may be the mechanism through which kinetoplast repositioning during life cycle differentiation is achieved; we observed that the basal bodies ‘lead the way’ during the migration, always being anterior to the kinetoplast.

In the trypanosome life cycle, dividing forms alternate with non-dividing forms, the latter being adapted to transmission between hosts, or between distinct environments within the tsetse (Matthews and Gull, 1994b). Although these transitional stages appear in many ways pre-adapted to differentiation, we show here that this pre-adaptation does not extend to re-orienting the cell architecture in preparation for the cell cycle of the following proliferative stage. Instead, at least for stumpy-procyclic differentiation, it is clear that this reorganisation occurs during the cell cycle that is coincident with differentiation (although the two processes can be uncoupled; Matthews and Gull, 1994a). This is of significance because the relative positioning of the trypanosome’s organelles is apparently precisely controlled during a proliferative cell cycle; in particular the basal-body to posterior dimension and nucleus-basal body dimension remain strictly fixed during procyclic cell division. The differentiating cell, in contrast, greatly changes these dimensions within the DNA synthesis phase of the cell cycle that will result in two procyclic cells. This highlights the important conclusion that the differentiation cell division cycle must operate under distinct or relaxed structural controls different from those operative in a proliferative cell in logarithmic growth. Care should be exercised, therefore, in using cell cycle events of the differentiating cell as a paradigm for those of the procyclic cell (and vice versa).

Our results shed no light on why the trypanosome should be required to undergo the structural reorganisation accompanying cellular differentiation. It is possible that its function might be related to the increased mitochondrial activity in insect stages, the development of extensive RNA editing of mitochondrial transcripts in the procyclic cell or a requirement for physical proximity of the nucleus and kinetoplast to provide efficient passage of shared proteins and transcripts (Hancock and Hajduk; 1990; Mottram et al., 1991; Torri and Hajduk, 1988; Peterson et al., 1993). Alternatively, the requirement may not be kinetoplast based, but related to the basal body, which subtends the flagellum. The extreme terminal location of the basal body and elongate flagellum/undulating membrane in the bloodstream trypanosome may maximise the efficiency of trypanosome motility in the viscous mammalian bloodstream. Finally, the position of the kinetoplast may be dictated by the different requirements of the flagellar pocket, which is intimately associated with the organelle complex at this location in the cell. This might reflect the different nutritional requirements of the various trypanosome forms as they progress through their life cycle. Although finding the solution among these various possibilities will be the object of long-term future study, we have provided a first glimpse into how these events are controlled at the mechanistic level. Understanding these controls will allow us to study the consequences to the trypanosome of disrupting them.

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