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Flagellar Elongation Induced by Glucose Limitation Is Preadaptive for *Trypanosoma cruzi* Differentiation

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Trypanosomes must sense and respond to environmental change in order to progress through their life cycles. The American trypanosome, *Trypanosoma cruzi*, differentiates from the noninfective epimastigote form to the infective metacyclic form spontaneously in axenic culture. Here, we investigate the initial stimulus for that change and demonstrate that *T. cruzi* epimastigotes sense limitation of glucose in the medium and respond by undergoing significant morphological and biochemical change. As part of this change, the mean flagellar length of the population triples, which is correlated with an increased ability to maintain interactions with hydrophobic substrates, a requirement for differentiation to the next life cycle stage. Cell Motil. Cytoskeleton 46:269–278, 2000.

Key words: flagellum; differentiation; trypanosome; glucose

INTRODUCTION

Parasites with complex life cycles must interact with multiple environments. *Trypanosoma cruzi* and *Trypanosoma brucei* are pathogenic, flagellated protozoa of the Order Kinetoplastida, which have complex life cycles during which they parasitize multiple hosts [Vickerman, 1985]. Trypanosomes are model organisms for the study of the flagellar and somatic cytoskeletons [Bouck and Ngo, 1996; Gull, 1999] and are species in which the basic metabolic pathways—both conserved and divergent—have been well established for many years [Opperdoes, 1987; Fairlamb, 1989]. Trypanosome differentiation offers the opportunity to study the co-ordination of metabolic and cytologic change as a response to environmental stimuli in a simple and well-characterized system. Metacyclogenesis of the American trypanosome, *T. cruzi*, is a life-cycle differentiation process during which co-ordinated changes in metabolism and morphology occur to effect transformation from the non-infective, proliferative epimastigote form to the infective, non-proliferative trypomastigote form. Since this differentiation is marked by cell cycle arrest and acquisition of infectivity, its study has direct clinical significance for a disease that currently affects over 16 million people and has no immediate prospect of effective treatment [http://www.who.int/ctd/html/chagburtre.html]. *T. cruzi* metacyclogenesis occurs spontaneously at high density in culture and so is experimentally tractable; however, the exact nature of the stimulus has hitherto remained obscure.

In order to maximize their potential for transmission, parasites adapt to environmental stresses such as overpopulation by differentiating. For instance, in *T. brucei* slender bloodstream forms, quorum sensing of a trypanosome-derived "stumpy induction factor" has been described as a trigger for cAMP-mediated morphological change, mitochondrial activation, and cell cycle arrest [Vassella et al., 1997]. This response restricts parasitaemia to a level that will not kill the host immediately and so ensures a chronic infection. Another such differenti-

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ation occurs in the tsetse fly midgut, where procyclic forms of *T. brucei* give rise to invasive, non-dividing proventricular forms [Vickerman, 1985]. The trigger for this response remains cryptic, but may be mediated by interaction of procyclic surface glycoproteins with lectins of the tsetse midgut. Alternatively, lectin-carbohydrate interactions initiate a cell death pathway, akin to apoptosis, to limit the population density of procyclic forms in the tsetse midgut [Welburn et al., 1996]. In *Leishmania*, another genus of pathogens within the Order Kinetoplastida, the promastigote form multiplies in the phlebotomine sandfly gut, giving rise to metacyclic forms that are infectious. In vitro, this process occurs predominantly in the stationary phase of culture. In *Leishmania spp*., the cue for metacyclogenesis in vitro has been identified as the acidification of the medium, which accompanies growth to high density in culture [Bates and Tetley, 1993].

T. cruzi epimastigotes proliferate in the nutrient-rich environment found in the midgut of their vector, the triatomine bug, after the insect has taken a blood meal. As the meal is digested and the parasite density increases, the environment becomes nutrient poor and epimastigotes become more elongate [Kollien et al., 1998]. Eventually, epimastigotes reaching the insect hindgut attach by their flagella and undergo metacyclogenesis to human infective trypomastigote forms [Brener, 1973; Schmidt et al., 1998]. In *T. cruzi* epimastigote cultures, metacyclogenesis occurs spontaneously during stationary phase [Camargo, 1964], in a pH-independent manner [Ucros et al., 1983]. Metacyclogenesis is cAMP-mediated [Gonzales-Perdomo et al., 1988; Rangel-Aldao et al., 1988] and induction of metacyclogenesis has been ascribed to hydrophobic interaction of the parasite flagellar membrane with the plastic of the culture flask [Bonaldo et al., 1988; Kleffmann et al., 1998]. Although changes in epimastigote morphology during in vitro culture are documented [reviewed in Brener, 1973; Williams, 1985], a link between these changes and the restriction of metacyclogenesis to the stationary phase has not previously been explained. The studies presented here describe the conditions that induce requisite preadaptations of cultured epimastigotes for metacyclogenesis.

MATERIALS AND METHODS

Parasites

Since one potential source of morphological variation in *T. cruzi* could be genetic heterogeneity, a single clone—Br3c—derived from the Brazil strain of the parasite was used for all experiments. Br3c was selected for its ability to readily undergo all life-cycle transitions in vitro and its ability to cause myocarditis in mice (Leon and Engman, unpublished results). The observations recorded here are consistent with similar experiments performed on the Brazil and Y strains of *T. cruzi*, which are not shown.

Cell Culture and Media

For the purposes of these studies, initial populations of cells were cultured at 27°C in LDNT media (4 g/L NaCl, 0.4 g/L KCl, 8 g/L Na_2HPO_4, 2 g/L D-glucose, 5 g/L neutralized liver digest, 5 g/L tryptone, 10% (v/v) heat inactivated foetal calf serum, 100 mg/L haemin) essentially as previously described [Kirchhoff and Neva, 1985]: Briefly, cell density was allowed to reach approximately 5×10^7 cells ml^{-1}. Cells were then counted using a Neubauer haemocytometer and passaged to a density of 10^6 cells ml^{-1}. Thereafter, cells were passaged to a density of 10^6 cells ml^{-1} every third day to ensure that cultures remained in logarithmic growth at all times. Where stationary phase cells were required, cultures were allowed to grow for 12 days after passage or as specified. Conditioned and extinct media were prepared first by the centrifugation of the specified cultures at 500*g* for 15 min at 4°C and then by the filtration of the supernatants through a 0.22-μm filter. The pH of fresh LDNT medium is 7.2; consequently, the pH of conditioned or extinct medium was adjusted to a pH 7.2 by the addition of small amounts of 1 M NaOH as necessary.

Immunofluorescence Assay (IFA)

Samples for IFA were prepared as follows: epimastigote cells from culture were pelleted by microfuge centrifugation for 10 min at 500*g*, then washed once with PBS and pelleted again. The cells were then resuspended to approximately 10^9 cells ml^{-1} and settled onto poly-L-lysine coated slides for 10 min at room temperature in a humid chamber. Finally, cells were fixed for 5 min in 2% paraformaldehyde and permeabilized by immersion in methanol at −20°C overnight. IFA was performed essentially as previously described [Tyler et al., 1997]. Briefly, samples were rehydrated in PBS for 5 min, primary antibodies were applied for 1 h in a humid chamber at room temperature, samples were washed three times in PBS for 5 min per wash, secondary antibodies were then applied and the process was repeated. Finally, 1 μg/ml DAPI (4′,6-diamidino-2-phenylindole dichydrochloride: Boehringer Mannheim, Indianapolis, IN) was applied for 30 seconds and the sample washed in distilled water before mounting under a cover slip using Permafluor (Immunotech, Marseille, France) and sealing with nail varnish. Microscopy was performed using an Olympus IX70 inverted system microscope (Olympus America Inc., Melville, NY) and images were captured using the Delta Vision Image Restoration and Deconvolution System (Applied Precision Inc., Issaquah, WA).

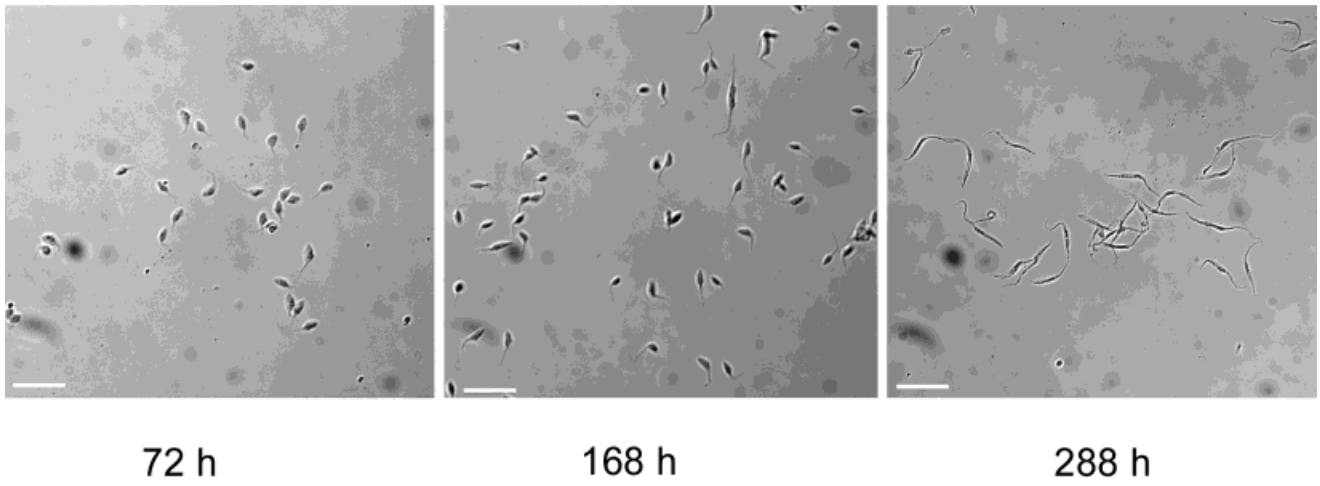

Fig. 1. Change in epimastigote morphology during culture growth in LDNT media visualized by DIC microscopy. After 72 h, cells were in exponential growth, after 168 h they were still growing, but no longer in exponential growth, and by 288 h cells they had reached the stationary phase of culture. Scale bars = 20 μm.

Measurement of the flagellar length of randomly captured cells was possible simply by tracing the line of the flagellum in captured images and employing the "measure distance" feature of the Delta-vision software.

Hydrophobic Adhesion Assay

The assay employed "silane-prep" microscope slides (Sigma Chemical Co., St Louis, MO), which are precoated with amino-alkyl silane to provide a hydrophobic surface. Trypanosomes tested were washed twice in sterile room temperature PBS and resuspended to a cell density of 10^7 cells ml^{-1}. One hundred microliters of this suspension was then spread evenly over a marked 1.5 cm^2 area of the slide. The parasites were allowed to adhere for 10 min in a humid chamber at room temperature. Fifty microliters of the suspension was then drawn off and recounted to determine the percentage of cells that had initially adhered. The slide was then washed three times in PBS for 5 min per wash, fixed in methanol at −20°C for 5 min, rehydrated in PBS for 5 min, and then mounted and examined by differential interference contrast (DIC) microscopy.

RESULTS

Cellular Morphology and Mitochondrial Complexity of *T. cruzi* Epimastigotes Vary With Cell Density

During logarithmic growth, *T. cruzi* epimastigotes are tear shaped. They are generally between 12 and 14 μm in length, the posterior half of which is flagellar, and are 3–4 μm wide (Fig. 1, 72 h). As the culture density increases and cells approach stationary phase (168 h), cell lengths increase and by late stationary phase (288 h) cells of over 25 μm in length are common, although the cells have become narrower. A readily measurable parameter that increases with cellular elongation is the flagellar length. Flagellar lengths of individual epimastigotes increase up to fivefold between logarithmic and stationary phases. These cytologic differences are even more evident when single cells are analysed at high power for expression of mitochondrial and flagellar proteins by IFA (Fig. 2). What is immediately obvious is the increase in the overall length of the cell body and the flagellum. In addition, the simple twisting loops of the log-phase epimastigote mitochondrion become a more complex network, with a grid-like appearance and a long, somatic, anterior projection that runs parallel to the flagellum.

If the flagellar lengths of epimastigotes are carefully measured over time in culture, it becomes clear that the mean length of the population begins to increase as the trypanosomes exit logarithmic growth and enter stationary phase (Fig. 3). The flagellar lengths of 100 randomly sampled trypanosomes were measured and averaged for each of three time points: exponential or logarithmic growth phase, non-exponential growth phase, and stationary phase. The mean flagellar length of the population was found to increase two- to threefold during this period. This is surprising, since flagellar elongation represents a considerable metabolic investment by the parasite in production of proteins of the flagellar axoneme, such as tubulin, paraxial rod such as PAR and flagellar membrane such as FCaBP. It seems likely,

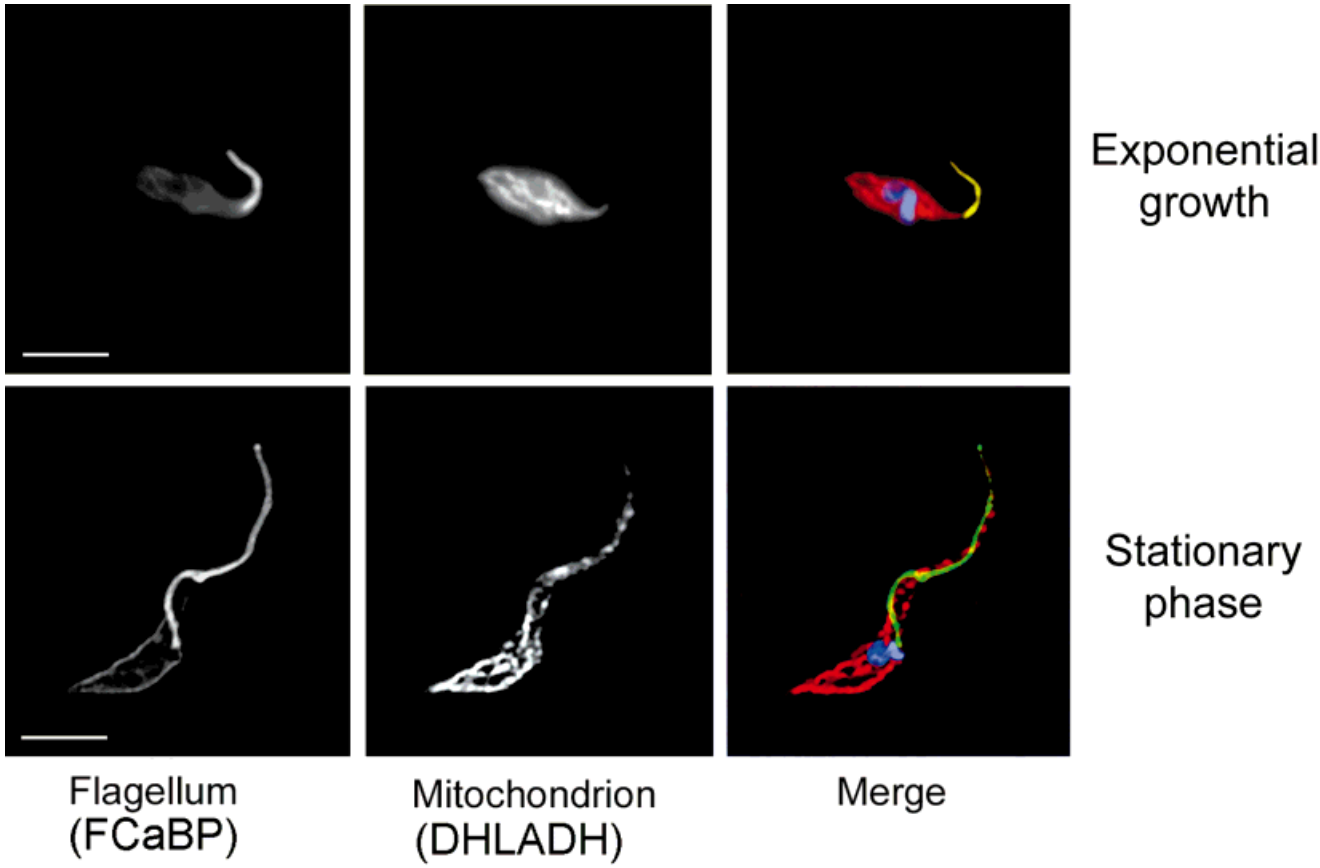

Fig. 2. Comparison of epimastigote cytologies during exponential growth and stationary phase. Samples from 72 h (exponential growth) and 168 h (stationary phase) cultures were stained and visualized by immunofluorescence microscopy for DHLADH (red) and FCaBP (yellow), which revealed the mitochondrion and flagellum, respectively. DAPI staining of the nucleus and kinetoplast is also shown (blue). The lengths of the cell body and flagellum length are much greater in the stationary phase cell (**left**). The mitochondrion of the stationary phase cell shows evidence of both increased size and complexity and its anterior aspect is particularly noticeable in these cells (**middle**). In the merged images, the flagellum (green) is seen to run alongside the anterior extension on the mitochondrion (red) for most of its length in the stationary phase cell, but not in cells from exponential growth. In spite of the differences in morphology, DAPI staining of the nucleus and kinetoplast (blue) shows that both cells are epimastigotes. Scale bar = 5 μm.

therefore, that such a significant cytologic change would have functional significance.

Outgrowth of the Flagellum Is Mediated by the Culture Medium

When cells change morphologically as a population during stationary phase, there are several possibilities as to how the change is mediated. These possibilities serve as potential explanations for the phenomenon of flagellar elongation we observe during epimastigote culture. Cells may communicate with each other through the secretion or excretion of soluble factors or by direct cell-to-cell contact. Alternatively, individual cells may simply react to the loss of a nutrient from the medium. If short range or contact interactions mediate the transition, then cell density may be critical in mediating transformation. If transition is due to depletion of a nutrient or accumulation of a stable factor in the medium, then the environment provided by the culture medium, and not cell density, will be important. To test these possibilities, stationary phase and log phase epimastigotes were passaged into fresh, conditioned, and extinct media (two flasks each) at a density of 10^6 cells ml^{-1} and incubated for 72 h. Conditioned and extinct media were prepared from cultures of epimastigotes 168 and 288 h after passage to a density of 10^6 cells ml^{-1} as described in Materials and Methods. Cells taken from log phase showed no significant change in flagellar length when placed in fresh medium, in contrast to cells taken from stationary phase, which decreased by approximately 50% over the 72-h period. Conversely, stationary phase cells placed in extinct medium showed no significant change

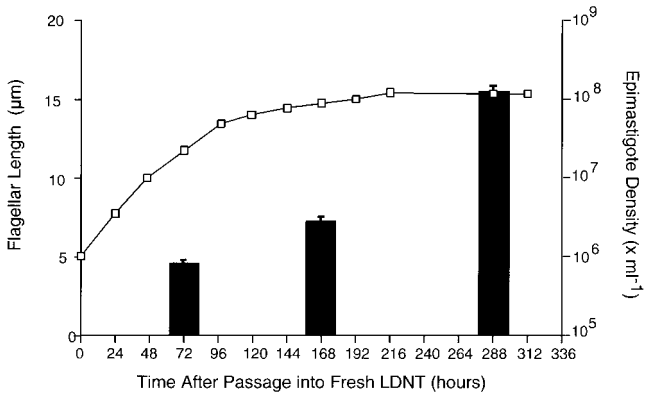

Fig. 3. Quantitation of the increase in flagellar length during epimastigote growth in culture. A subculture of *T. cruzi* epimastigotes initiated at a density of 1×10^6 epimastigotes ml^{-1} was sampled daily over a 2-week period and the cell densities (right axis) were plotted against time to give a growth curve. Cells grew exponentially for 96 h in culture, continued growing in a non-exponential manner for the next 120 h, and then reached a stationary phase where there was little further change in cell density over the period assessed. The flagellar lengths (left axis) were assayed during exponential growth (72 h), non-exponential growth (168 h), and stationary phase (288 h). This revealed significant increases in the mean flagellar lengths of the population at each time point tested (error bars reflect s.e.m., n = 100).

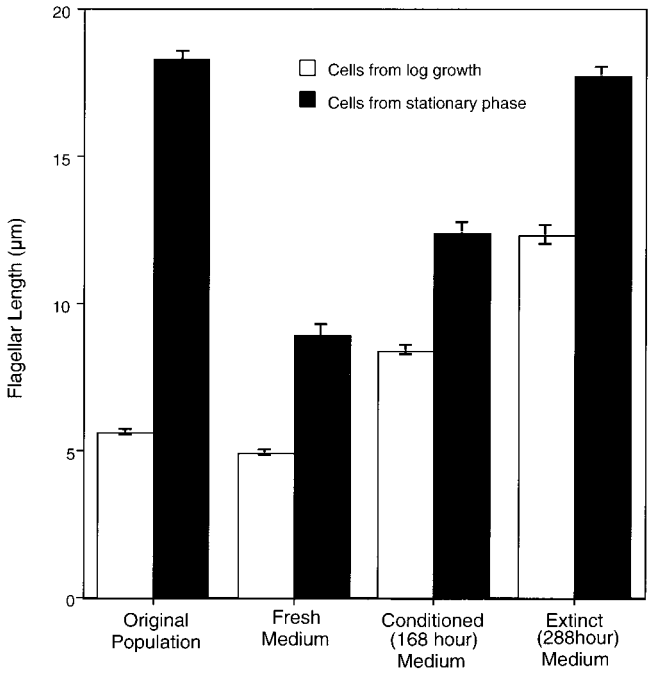

Fig. 4. The mean epimastigote flagellar length varies in response to the medium. Cells from 72- and 288-h cultures were passaged to a density of 10^6 trypanosomes ml^{-1} in three different media: in fresh medium, in conditioned medium (from 168-h cultures), and in extinct medium (from 288-h cultures). Samples were drawn from each of the six flasks after 72 h incubation. At this time-point, the flagella of cells that had been passaged into fresh medium did not lengthen significantly, whereas the flagella of cells passaged into conditioned or extinct media showed significant increases in mean length. Similarly, stationary phase populations passaged into extinct medium showed little difference in mean flagellar length after 72 h; however, when introduced into conditioned or fresh medium the mean flagellar length of the population shortened significantly (error bars reflect s.e.m., n = 100).

in flagellar length, while cells from the log phase of growth showed a twofold increase in flagellar length. In conditioned medium, cells from log phase showed significant lengthening, while cells from stationary phase showed significant shortening. These results together likely indicate that the mean flagellar length of the population is modulated in response to the variation in concentration of a substance in the culture medium.

Flagellar Length Increases in Response to Depletion of a Medium Constituent

The results in Figure 4 suggest that the morphological changes observed, marked by the increase in flagellar length, are due either to accumulation of a stable parasite-derived factor or depletion of a nutrient in the medium. If a parasite-derived factor were accumulating, then in extinct medium the level of factor might be high enough to exert its effect even at high dilution. Conversely, if the effect is a result of nutrient depletion, then the addition of isotonic buffer might be predicted to produce a similar or even greater effect than that afforded by extinct medium. We mixed fresh medium with either extinct medium or phosphate buffered saline (PBS) in different ratios (Fig. 5) and inoculated each mixture with log phase epimastigotes at a density of 10^6 cells ml^{-1}. Cells were sampled 120 h later and the flagellar lengths were measured. At low dilution neither extinct medium nor PBS produced a significant effect on mean flagellar length. Only with over 50% extinct medium was significant flagellar lengthening observed; however, at this and higher dilutions of fresh medium, the effects of PBS on the population's mean flagellar length was always greater than that of the extinct medium. We conclude from this experiment that flagellar length increases as a result of depletion of a medium constituent and not as a result of accumulation of a waste product or parasite-derived factor.

Glucose Depletion Leads to Flagellar Elongation

LDNT culture medium contains undefined constituents such as neutralized liver digest and bacto-tryptone, making systematic depletion of defined components difficult. To determine whether depletion of one of the supplements might be responsible for the flagellar lengthening phenomenon, we simply prepared media lacking each of them (glucose, haemin or FCS). Log phase epimastigotes were suspended to a density of 10^6 cells ml^{-1} in each and sampled 168 h afterwards for

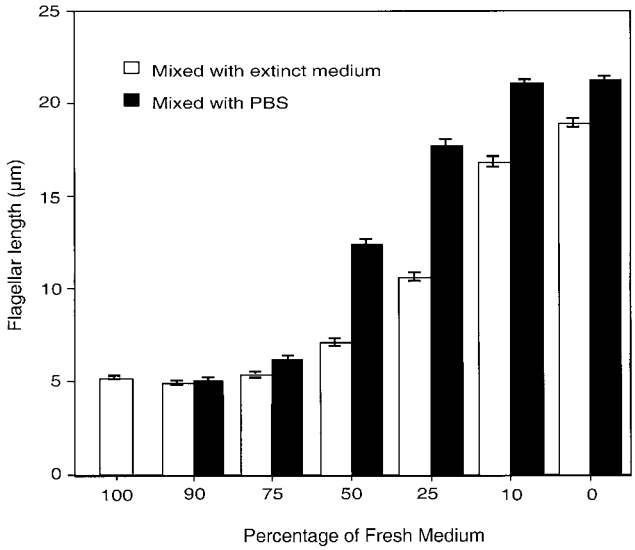

Fig. 5. Flagellar elongation is likely to be triggered by nutrient depletion. Epimastigotes from exponential growth were passaged to a density of 10^6 cells ml^{-1} in various mixtures of fresh medium and either extinct (288 h) medium or PBS. Cells were sampled after 120 h in culture and the flagellar lengths measured. Cells showed significant flagellar elongation only at high concentration of extinct medium or of PBS. This indicates that flagellar elongation probably occurs in response to depletion of a medium component (error bars reflect s.e.m., n = 100).

measurement of flagellar length. The flagellar lengths of cells cultured in media depleted of foetal calf serum or haemin were marginally greater than those of the control medium; however, when glucose was omitted, a clear and significant increase in the mean flagellar length of the population was observed (Fig. 6a). A similar, though lesser effect, was also seen when glucose concentration was merely reduced from 2 g/L to 1 g/L (Fig. 6a). Although this one experiment does not represent a comprehensive analysis of medium components, the result suggests that depletion of a simple sugar, rather than depletion of protein, lipid, or iron, is responsible, at least in part, for the flagellar lengthening observed in vitro.

When glucose was simply added back to conditioned culture medium, cells that were in the process of elongating their flagella responded by shortening their flagella instead, to a degree that was similar to that seen when passaged into fresh media (Fig. 6b). Together these results indicate that glucose depletion is most likely the trigger for flagellar elongation in vitro.

Flagellar Elongation Correlates With Increased Binding to a Hydrophobic Substrate

Metacyclogenesis is known to be dependent on the hydrophobic attachment of the flagellum to a substrate [Bonaldo et al., 1988] and existing studies suggest that the flagellum is the most hydrophobic exterior surface of the trypanosome [de Souza et al., 1978; Souto-Padron and de Souza, 1983]. We reasoned that by extending its flagellum, the parasite might increase the surface area available for hydrophobic interaction and, hence, preadapt for metacyclogenesis. If this were the case, we predicted, then elongate cells from stationary phase should bind a hydrophobic surface with greater avidity than the shorter, rounder, log phase cells. We used organosilane-coated slides as our hydrophobic substrate and allowed washed epimastigotes in suspension to settle upon them in a humid chamber. The suspension of epimastigotes was removed and the slides were washed, mounted, and viewed by DIC microscopy (Fig. 7). There were few log phase epimastigotes left adhered to the hydrophobic slides: mean cell density of 4.3 ± 1.4 (n = 50 fields) cells per 40 × objective field. In contrast, the surfaces of the hydrophobic slides remained covered with cells taken from stationary phase: mean cell density of 108.8 ± 5.6 (n = 50 fields) cells per 40 × objective field. We correlate this twenty-five-fold difference in binding to a hydrophobic substrate with the threefold difference in flagellar length between the log phase and stationary phase epimastigotes (Fig. 3) as evidence that flagellar elongation is a preadaptive response that can lead to increased hydrophobic binding and hence potentiate metacyclogenesis.

DISCUSSION

Functional stress responses to nutrient deprivation have been documented in most micro-organisms studied. From prokaryotes to yeast, deprivation of simple sugars leads to increased expression of subsets of genes that generally are concerned with either restricting the cell's nutritional requirements, for instance by cell cycle arrest, or with increasing carbohydrate metabolism. In *Escherichia coli*, several operons encoding carbohydrate uptake proteins are co-ordinately regulated in a cAMP-dependent fashion [Notley and Ferenci, 1995; Zambrano and Kolter, 1996]. In yeast, glucose limitation leads to de-repression of transcription of several genes, eventually causing G_1 cycle arrest and mitochondrial activation [Lesage et al., 1996]. In African trypanosomes, depletion of glucose has recently been implicated as a trigger for differentiation from the bloodstream form to the procyclic form [Milne et al., 1998]. In some cells, starvation can lead to functional, morphological changes. The intracellular bacterium *Legionella pneumophila* actually develops flagella in response to nutrient depletion, becoming motile and osmotically resistant in order to escape its spent host and disperse in the environment [Byrne and Swanson, 1998]. In this report, we likewise show

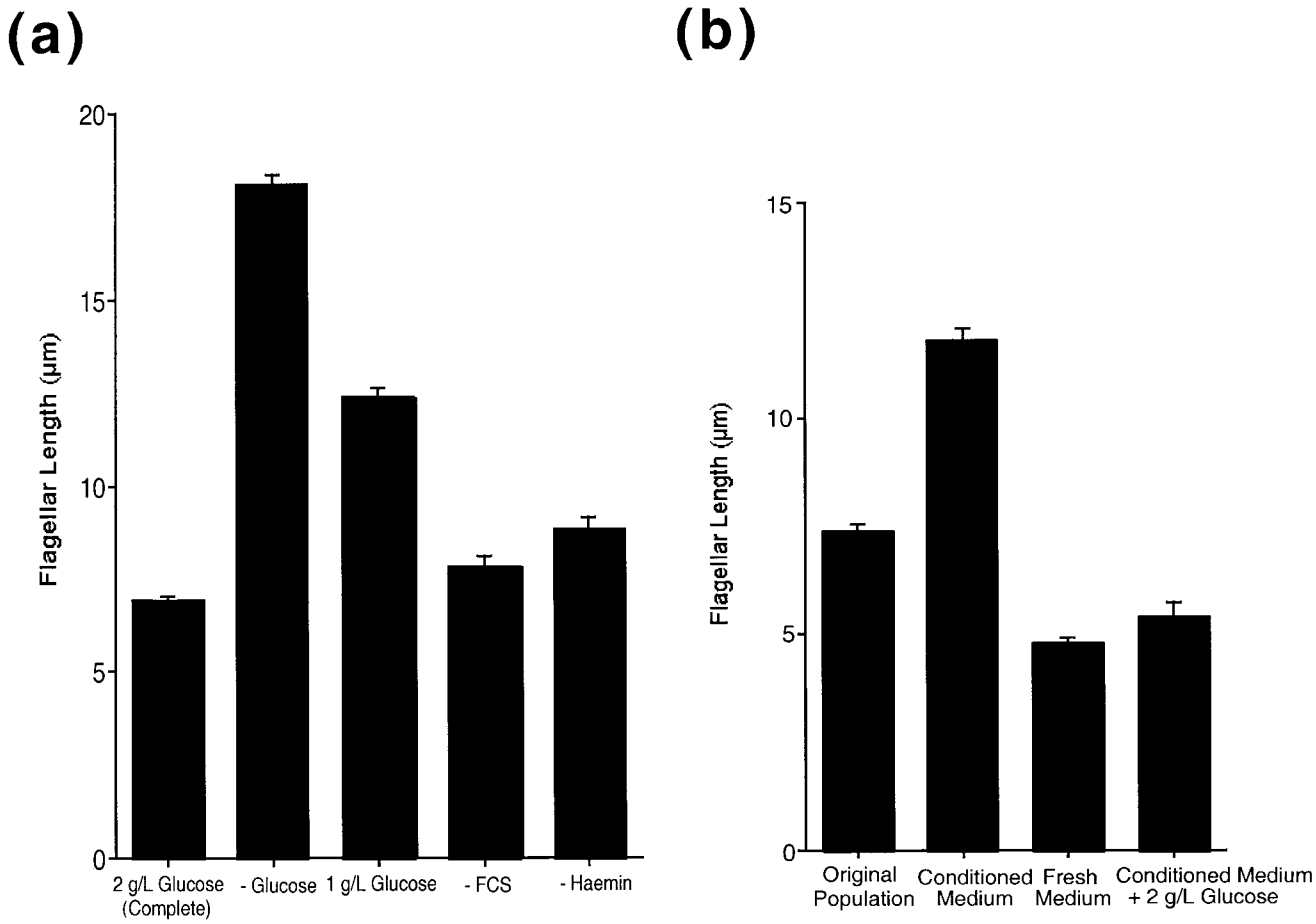

Fig. 6. Glucose limitation may be the trigger for flagellar elongation. **a:** Cells from exponential growth were passaged to a density of 1×10^6 cells ml^{-1} in complete medium or in media in which the major supplements had been omitted. Cells were sampled at 168 h after passage and flagellar lengths measured. The flagellar lengths of cells cultured in medium, which lacked added glucose or where glucose was added to half its usual concentration (1 g/L), were significantly greater than those of cells cultured in the other media (error bars reflect s.e.m., n = 100). **b:** Addition of glucose to conditioned medium (168 h) leads to a reduction in mean flagellar length similar to that seen by passage into fresh medium. Cells from 168-h cultures were passaged to a density of 10^6 trypanosomes ml^{-1} in fresh medium, conditioned medium (168 h), and conditioned medium supplemented with 2 g/L glucose. Forty-eight hours after passage into fresh media, or glucose supplemented medium, the mean flagellar length of each of these populations was significantly shorter than that of a similar population of cells introduced into unsupplemented, conditioned medium. This indicates that the morphological effects of nutrient depletion during culture can be at least partially complemented by addition of extra glucose (error bars reflect s.e.m., n = 100).

development of the *T. cruzi* flagellum in response to nutrient deprivation.

In *T. cruzi*, metacyclogenesis can be observed with good efficiency in vitro [Camargo, 1964]. Metacyclogenesis is observed in the stationary phase of culture, and metacyclic trypomastigotes are largely absent during logarithmic growth. The controversy over whether metacyclogenesis is induced by accumulation of a factor or depletion of a nutrient is long running [see Brener, 1973]. There is experimental support for the ability of a triatomine factor in haemolymph to induce metacyclogenesis [Isola et al., 1986] and for starvation medium to induce metacyclogenesis even in the absence of such a factor. Further, the possibility that accumulation of parasite factors or excretory products contributes to metacyclogenesis has not been ruled out. What seems certain is that a hydrophobic interaction between the flagellum and an appropriate substrate is necessary but not sufficient for differentiation [Bonaldo et al., 1988; Kleffmann et al., 1998] and that metacyclogenesis is mediated by a cAMP-signalling mechanism [Gonzales-Perdomo et al., 1988; Rangel-Aldao et al., 1988]. Our results provide support for the idea that nutrient deprivation has a role in potentiating metacyclogenesis.

Our initial observation was that, in stationary phase populations in which metacyclogenesis was occurring, the epimastigotes had much longer flagella than those undergoing logarithmic growth. We wondered, therefore,

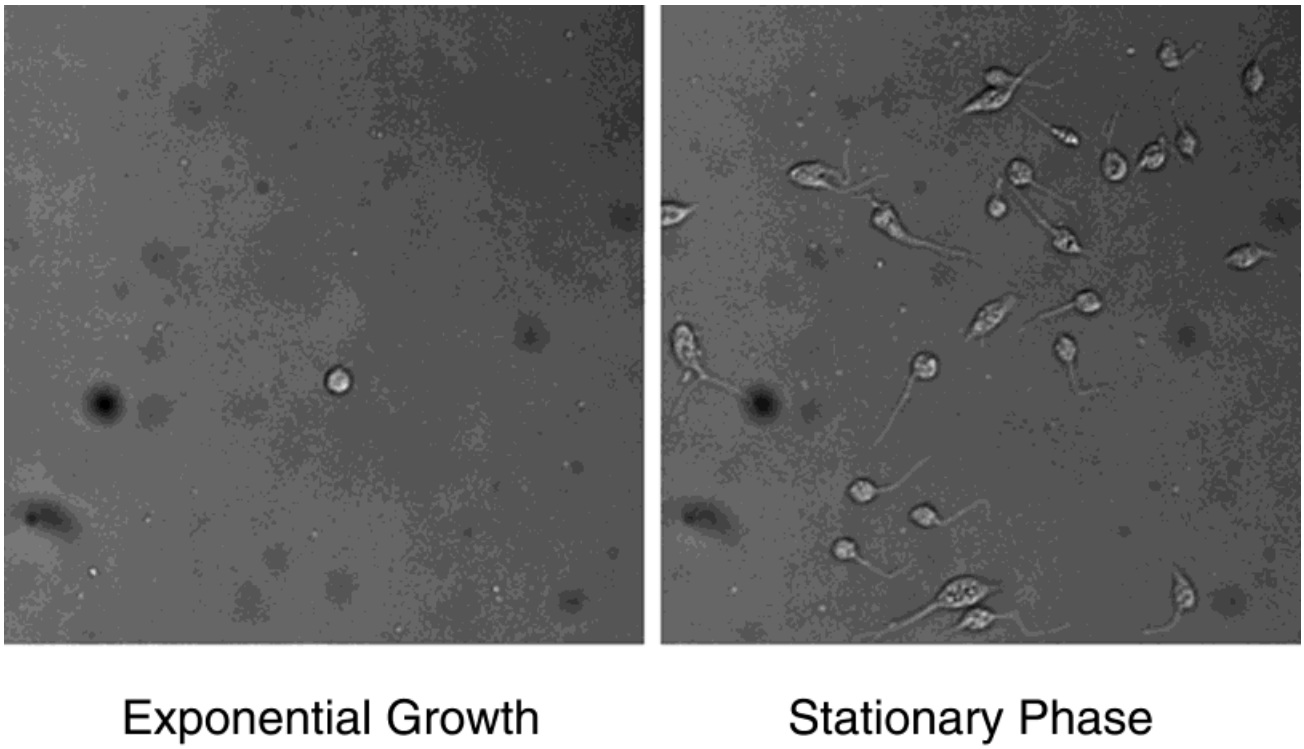

Fig. 7. Flagellar length correlates with binding to a hydrophobic substrate. One hundred microliters of cells drawn from an exponentially growing population (**left**) and a stationary phase population (**right**) were washed, suspended to 1×10^6 cells ml^{-1} in PBS and settled onto "silane -prep" coated microscope slides for 10 min, then washed three times, mounted and examined by DIC microscopy. Under these conditions, few cells from the exponential population and that had flagella adhered to the slide, in contrast to the many adherent cells of the stationary phase population that possessed long flagella.

whether the close contact evident at these high trypanosome densities was responsible or whether the increase in flagellar length occurred as a result of nutrient depletion or of waste product accumulation. We determined that the elongation occurred in response to changes in the medium and that these changes were not due to concentration of a parasite derived factor, but more likely to be due to loss of a nutrient. Fortunately, by omission of medium supplements we were able to identify the simple sugar glucose as one nutrient whose depletion leads to flagellar elongation. We confirmed its effect by adding extra glucose to cells, which had already partially elongated, finding that cells responded by shortening their flagella. Although we have not eliminated the possibility that the depletion of other nutrients (particularly other simple sugars) may have a similar effect, drastic reduction of soluble iron by the removal of haemin, or of lipid by the removal of FCS, did not have such an effect. Even when iron limitation led to cell death (after two passages in the absence of haemin), cells did not die with the elongate morphology and flagellum characteristic of stationary phase and glucose starved cells (not shown). We conclude, therefore, that flagellar elongation is not a general response to nutrient depletion.

Glucose depletion has previously been shown to lead to activation of mitochondrial enzymes in stationary phase *T. cruzi* epimastigotes [Carneiro and Caldas, 1983; Cazzulo et al., 1985]. Notably, cultures become sensitive to cyanide and refractory to the alternative oxidase inhibitor SHAM [Felix et al., 1978] indicating a shift from use of the trypanosome alternative oxidase to predominantly cytochrome mediated respiration. Mitochondrial chaperones also show evidence of increased expression in the stationary phase, consistent with the increased structural complexity observed here (Tyler and Engman, unpublished results) [Carreira et al., 1997]. It is not surprising, then, that in order to accommodate the extra mitochondrial complexity, the cellular morphology might be co-ordinately altered, leading to an increase in cell body length. What is not intuitively obvious is why an epimastigote should invest in its flagellum upon sensing an incipient scarcity of nutrient. A longer flagellum might be expected to confer increased motility allowing escape from a localized region of depleted nutrient; however, the triatomine midgut is an enclosed environment, so it seems unlikely that much is gained by simply moving faster. Instead, we favour the explanation that a lengthened flagellum might be a preadaption for differ-

entiation to the next life-cycle stage, the metacyclic trypomastigote.

Previous studies suggested that flagellar elongation may occur when epimastigotes attach to a substrate by their flagella [Kollien et al., 1998; see also Vickerman and Tetley, 1990]. In stationary phase cultures, sugar depletion may encourage hydrophobic attachment of the flagellum simply by reducing the level of glycosylation on epimastigote surface proteins, thus making the trypanosome surface more hydrophobic. This, in turn, could then cause the flagellum to attach and then to elongate. Alternatively, glucose depletion may signal more directly to increase the gene expression of the flagellar components required for the construction of a longer flagellum, perhaps using part of the same pathway used to increase mitochondrial gene expression.

Previous freeze-fracture studies have observed high concentrations of sterols and low numbers of intermembrane proteins present in the flagellar membrane relative to the plasma membrane in *T. cruzi* [de Souza et al., 1978; Souto-Padron and de Souza, 1983]. An indication of this work is that the flagellar membrane is likely to be more hydrophobic than the plasma membrane, a reason that hydrophobic attachment is mediated by the flagellum and not by the plasma membrane. This interpretation predicts that the elongation of the flagellar membrane in response to glucose depletion should effectively increase the ability of the parasite to adhere to a hydrophobic substrate. We tested this hypothesis and found that it was indeed the case. It has been clearly demonstrated that flagellar attachment is required, but not sufficient for metacyclogenesis. Since glucose depletion promotes hydrophobic attachment, our conclusion is that, in vivo, upon sensing depletion of simple sugars from the blood meal, the epimastigote reacts by extending its flagellum in order to adhere to the hindgut wall of its insect vector and undergo metacyclogenesis.

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