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Morphological Events during the *Trypanosoma cruzi* Cell Cycle

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The replication and segregation of organelles producing two identical daughter cells must be precisely controlled during the cell cycle progression of eukaryotes. In kinetoplastid flagellated protozoa, this includes the duplication of the single mitochondrion containing a network of DNA, known as the kinetoplast, and a flagellum that grows from a cytoplasmic basal body through the flagellar pocket compartment before emerging from the cell. Here, we show the morphological events and the timing of these events during the cell cycle of the epimastigote form of *Trypanosoma cruzi*, the protozoan parasite that causes Chagas' disease. DNA staining, flagellum labeling, bromodeoxyuridine incorporation, and ultra-thin serial sections show that nuclear replication takes 10% of the whole cell cycle time. In the middle of the G2 stage, the new flagellum emerges from the flagellar pocket and grows unattached to the cell body. While the new flagellum is still short, the kinetoplast segregates and mitosis occurs. The new flagellum reaches its final size during cytokinesis when a new cell body is formed. These precisely coordinated cell cycle events conserve the epimastigote morphology with a single nucleus, a single kinetoplast, and a single flagellum status of the interphasic cell.

Key words: cell cycle; flagellum; kinetoplast; nucleus; mitosis; *Trypanosoma cruzi*.

Introduction

The eukaryotic cell cycle is characterized by the progression of linked events that allow segregation of identical genetic material into progeny (Murray 2004). The mechanisms that ensure correct segregation have been extensively studied with respect to the control of cell growth, DNA replication, and mitosis, including the establishment of networks of interacting molecules expressed at different times of the cell cycle (Tyers 2004). However, much less is known about the relationship between the duplication and segregation of organelles and cell cycle progression.

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One reason for this is that most eukaryotes contain multi-copy organelles in each cell. Some protists, therefore, are very useful systems in this regard because they have single-copy and specialized organelles. Trypanosomes are flagellated protists that contain a single Golgi complex (He et al. 2004) as well as a large mitochondrion that hosts a single kinetoplast formed by a network of minicircle and maxicircle DNA molecules. A single flagellum connected to the kinetoplast at its basal body emerges from a flagellar pocket, an invagination of the plasma membrane (Gull 2003; Robinson and Gull 1991).

In *Trypanosoma brucei*, the agent of African trypanosomiasis, the morphological events occurring during the cell cycle are precisely determined (Woodward and Gull 1990). However, despite that *T. brucei* is considered an archetypal organism among trypanosomes, particularly when descriptions of structural organization are provided, morphological alterations that occur during the cell cycle of other species of trypanosomes must be different as they have distinct cell shapes, kinetoplast position, and flagellum insertion. *Crithidia* and *Leishmania* species, for instance, do not replicate their nuclear and kinetoplast DNA in the same order as *T. brucei* (Bhattacharya and Ghosh 1985; Cosgrove and Skeen 1970). In most cases, there is no migration of the basal body and kinetoplast segregation occurs close to the nuclear mitosis. In addition, although no detailed description has been provided, the new flagellum does not attach to the old one (Briggs et al. 2004).

Much less is known about the morphological alterations and the timing of the cell division cycle of *Trypanosoma cruzi*, the agent of Chagas' disease. *T. cruzi* cell division has been characterized previously (de Souza and Meyer 1974; O'Daly and Bretana 1976) and reviewed in (de Souza 1999, 2002; Tyler and Engman 2001), but a clear relationship between the generation of organelles and the cell cycle has not yet been established. Here, we describe the morphological events that occur during *T. cruzi* epimastigote cell division cycle, in particular, the replication of DNA, mitosis as well as kinetoplast and flagellum pocket division, and growth of the new flagellum.

Results

Morphological Patterns of the *Trypanosoma cruzi* Cell Cycle

To understand the morphological alterations that occur during the *Trypanosoma cruzi* cell cycle, exponentially growing epimastigote cells (Fig. 1 A) were searched for the presence of cells with differing numbers of organelles. After propidium iodide staining, these cells were analyzed by FACS (fluorescence-activated cell sorting) and no unusual ploidy was found (data not shown; Elias et al. 2002). A monoclonal antibody (mAb 25) that recognizes a 24 kDa flagellar calcium-binding protein found predominantly in the flagellum of the parasite (Engman et al. 1989) was used to label the flagellum, followed by 4′,6-diamidino-2-phenylindole (DAPI) staining to enable observation of the nucleus and kinetoplast. The culture presented cells with different morphological patterns and a quantitative analysis of these patterns is presented in Figure 1 B. Most of the cells contained just one nucleus, one kinetoplast, and one flagellum (1N1K1F) as illustrated in Figure 1 C (line a). A smaller number of cells contained one nucleus, one kinetoplast, and two flagella (1N1K2F) (line b). Cells with one nucleus, two kinetoplasts, and two flagella (1N2K2F) (line c), or two nuclei, two kinetoplasts, and two flagella (2N2K2F) (line d) were also found.

Immunofluorescence assays using an anti-tubulin monoclonal antibody showed that nuclear mitosis occurs in cells with 1N2K2F. The antibody reacts with *T. cruzi* subpellicular microtubules, abundant in these protozoa, and since it also recognizes microtubules that form the mitotic spindle, it allowed the visualization of mitotic cells. Figure 2 a—c shows examples of mitotic cells with

Figure 1. Morphological patterns of exponentially growing epimastigotes. Panel (**A**) shows a typical growth curve of *Trypanosoma cruzi* epimastigotes. Epimastigotes ($5-7 \times 10^6$ per ml) were harvested by centrifugation, immobilized on glass slides, fixed in 4% formaldehyde, permeabilized, and stained with mAb 25 and DAPI. Graph (**B**) shows average $\pm$standard deviation of three independent experiments ($n = 200$) indicating the proportion of cells presenting each morphological pattern concerning nucleus (N), kinetoplast (k), and flagellum (F). Panel (**C**) shows typical images of each morphological pattern (see Results) using phase contrast, or DAPI and mAb 25 staining (flagellum). N = nucleus, K = kinetoplast. Bars = 5 μm. Inserted box represents 4X zoom of the DAPI-staining image.