An ultrastructural study of *Trypanosoma cruzi* developmental stages during metacyclogenesis

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T*rypanosoma cruzi* is an excelent model to study cell differentiation, since it presents distinct developmental stages during its life cycle. At the insect midgut, epimastigotes undergo differentiation to metacyclic trypomastigotes, a process known as metacyclogenesis, that involves differential gene expression and ultrastructural alterations. The kinetoplast corresponds to an enlarged portion of the protozoan mitochondrion that contains DNA (kDNA) organized in a network of interlocked circular molecules. In epimastigotes the kinetoplast is in the anterior end of the cell, presents a disk-shaped morphology and contains condensed kDNA fibers, whereas in trypomastigotes the kinetoplast is situated into a globular structure in the posterior region of the cell and present a more relaxed kDNA arrangement. The different morphologies assumed by *T. cruzi* during the metacyclogenesis are still not fully characterized, thus causing controversies in nomenclature, mainly for intermediary forms. In this work we performed a detailed structural characterization of *T. cruzi* during *in vitro* metacyclogenesis using microscopy techniques, such as scanning (MEV), to reveal aspects of the parasite cell suface, and transmission electron microscopy (MET), to check organelle positioning. Focused-ion-beam scanning electron microscopy (FIB-SEM) and atomic force microscopy (AFM) were also employed in this study to furnished three-dimensional models and to study the topological characteristics of isolated kDNA networks, respectively. MEV analyses showed that at the beginning of differentiation a decrease of the protozoa total length was observed, however at the end this process metacyclic trypomastigotes increased its cell body size although the flagellum became shorter when compared to epimastigotes. By MET and FIB-SEM we observed that changes on the kinetoplast format and kDNA rearrangement occurred only after the complete migration of this structure to the posterior end of the cell body, whereas, the changes in the nuclear shape initiated when the metacyclogenesis was trigged and the kinetoplast started its migration. Changes in nuclear ultrastructure, as the loss of the nucleolus were observed in latter stages of differentiation process. AFM data showed that kDNA networks of distinct developmental stages of *T. cruzi* present different patterns of DNA distribution. In addition, the deep-etching technique confirmed that kDNA fibrils in trypomastigotes are more loosely packed than in epimastigotes and showed association with globular structures that probably correspond to proteins. Considering changes on cellular morphology, the location of the kinetoplast in relation to the nucleus, the flagellar position as well as the kinetoplast shape and kDNA topology three distinct intermediate forms were identify and we propose to name them as intermediates I, II and III. With

the proposal of this new nomenclature for the intermediate forms, we expect to organize and facilitate the classification of *T. cruzi* developmental stages during metacyclogenesis.

Supported by: FAPERJ e CNPq