NUCLEAR STRUCTURE AND CELLULAR ORGANIZATION IN THE DIFFERENTIATION AND CELLULAR CYCLE OF *Trypanosoma cruzi*

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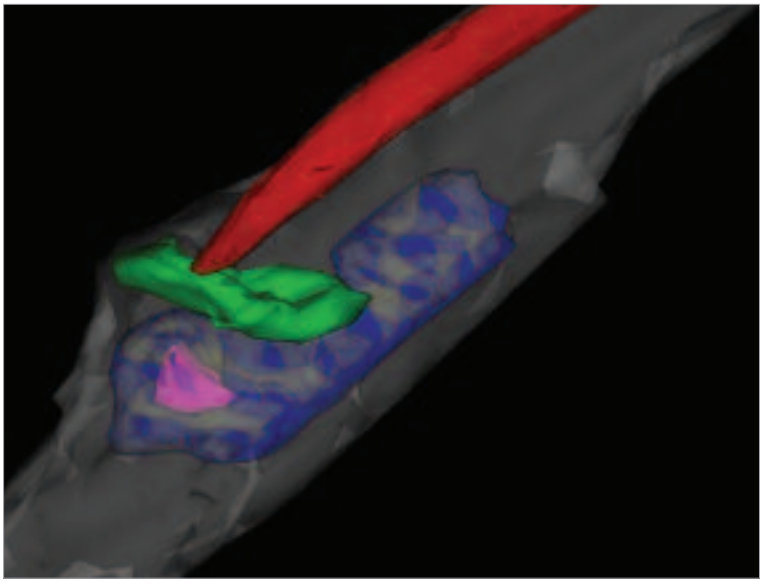
The establishment of microbial infections depends of parasite adaptation and survival in their hosts. To survive, the *Trypanosoma cruzi*, a protozoan parasite, causative agent of Chagas disease, alternates between forms that divide in the insect vector intestinal lumen (epimastigote forms), or in mammalian cell cytoplasm (amastigote forms), to non-proliferative forms but capable of entering mammalian cells after being exposed to extreme conditions in the blood stream or insect feces. Transformation from one form the other involves complex mechanism poorly understood, by which the parasite sense the environmental conditions, progressing through the cell cycle, or undergoing differentiation. Identification of the signals and mechanisms that led to one or other process is therefore relevant to understand and control infection. In a previous project, our laboratory started studies about the nuclear organization of *T. cruzi* with the goal to understand the process involved in the gene expression control during the different developmental stages of the parasite. We found large changes in the nuclear structure by comparing replicative and non-replicative forms of this protozoan. These structural changes correlated with a general transcription shutdown [1] and changes in the chromatin structure and organization [2]. To further understand the meaning of these changes we localized the replication and transcription sites in the nuclei of this parasite. We found that DNA precursors were incorporated close to the nuclear envelope, and later moved to the nuclear interior [3], in agreement with the findings that they are in continuous movement and become restrained to the nuclear periphery to replicate [4]. By using antibodies specific for the RNA polymerase II large subunit of *T. cruzi*, characterized in our laboratory [5], we observed that transcription is also located in restricted domains, close to the nucleolus and that these domains disappeared when transcription was inhibited (in preparation). To understand these differential localization of replication and transcription, we started to investigate the structure and modifications in the chromatin, recognized to have a central role in the organization and coupling of nuclear events [6]. We found differential phosphorylation and acetylation of histones during the cell cycle and after differentiation of the parasite into non-replicative forms. Therefore, our main goal in this project is to further characterize these structural and functional changes in the T. cruzi nuclei during the cell cycle and differentiation. These studies may help us to understand how the parasite controls his life cycle.



T. cruzi *epimastigotes undergoing differentiation. The figure is a 3D reconstruction of serial sections obtained by transmission electron microscopy of a parasite attached to a slide. Grey areas represent the cell body, red the flagellum, green the kinetoplast, blue the nucleus containing the nucleolus in pink and the electron dense chromatin in grey. Magnification: 50000X.*

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The major goal of our research has been the identification of novel mechanisms that would be useful as targets to combat parasitic diseases caused by trypanosomes. Gene transcription was found concentrated in a few nuclear regions, located in the nuclear interior of replicating forms of *Trypanosoma cruzi*, the agent of Chagas´ disease. These transcriptional regions disappear abruptly when infective and non-dividing trypomastigotes are formed. Chromosomes were found moving in the nuclear interior but during replication the chromatin is immobilized near the nuclear envelope. This nuclear organization was found dependent on histone modifications. Histone H1 was found phosphorylated in a cyclin-dependent kinase in *T. cruzi*. However, the *T. cruzi* histone H1 does not localize with the bulk of chromatin. It is rather concentrated in the nucleolus. Upon phosphorylation, occurring with the cell cycle progression, H1 dissociates from the nucleolus, suggesting that H1 is implicated in ribosomal RNA synthesis and/or processing regulation. Histones H4 and H2B were found mainly acetylated, while histones H3 and H2B methylated in *T. cruzi*. The acetylation sites were identified in the N-terminal portion of histone H4. Synthetic peptides with the identified modifications were used to obtain specific antibodies, which labeled nuclear domains involved specifically in the transcription, replication, or DNA repair. Insights about the mechanisms involved in the control of cell organization that could sense signals required for differentiation were also obtained. As trypanosomes decrease protein synthesis during differentiation, protein kinases involved in the regulation of protein translational initiation were studied. In our work a protein kinase involved in the phosphorylation of the eukaryotic initiation factor 2 (eiF2·) was characterized. This new kinase was detected in the flagellar pocket, suggesting that the cellular organization must be linked to the protein synthesis.

In a new program project we continued to work with the histone modifications and we started by looking the localization of several other kinases involved in the control of cellular morphogenesis. Kinases with characteristics of "Target of Rapamycin" (TOR) kinases, probably involved in the control of cellular growth were identified in trypanosomes. One of the kinases was found in the unique mitochondria of trypanosomes, while another, was found localized in vesicles similar to acidocalcisomes of these organisms. We are currently investigating their role in the control of cell cycle and differentiation.

In conclusion, our program projects provided some key features to start understanding the molecular mechanism controlling adaptative responses in protozoan parasites.

MAIN PUBLICATIONS

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