In silico structural studies of Replication Proteins A1 and A2 from trypanossomatids (*Leishmania* and *Trypanosoma)*

Carlos A. H. Fernandes[1], Fábio F. Mattioli[1], Raphael S. Pavani[2], Marcos R. M. Fontes[1], Maria C. Elias[2], Maria I. N. Cano[3].

[1]Departamento de Física e Biofísica - Instituto de Biociências de Botucatu – SP, UNESP, Brasil, [2]Laboratório Especial de Toxinologia Aplicada – Instituto Butantan – SP, Brasil, [3]Departamento de Genética - Instituto de Biociências de Botucatu – SP, UNESP, Brasil.

Replication Protein A (RPA), the major single stranded DNA binding protein in eukaryotes, is composed of three subunits (RPA-1, RPA-2 and RPA-3) and is a fundamental player in DNA metabolism, participating in replication, transcription, repair, and the DNA damage response. However, these proteins were not yet characterized yet in trypanossomatids, among which are causative agents of some neglected tropical diseases, such as leishmaniasis (caused by species of *Leishmania* genus) and Chagas disease (caused by *Trypanosoma cruzi*). At the present work, we constructed *in silico* models of RPA1 and RPA2 from *Leishmania amazonensis* (LaRPA-1 and LaRPA-2) and *Trypanosoma cruzi* (TcRPA-1, TcRPA-2) by threading modelling and molecular dynamics simulations. Both LaRPA-1 and TcRPA-1 lack the N-terminal 70N domain, that is present in RPA-1 from higher eukaryotes; but present three subsequently OB-fold domains (OBF-1, OBF-2 and OBF-3) which are homologous to the DNA binding domains A, B and C (DBD-A, DBD-B and DBD-C) of RPA-1 from *Homo sapiens* (HsRPA-1) and *Ustilago maydis* (UmRPA-1). However, comparative structural analysis between LaRPA-1 and TcRPA-1 *in silico* models and HsRPA-1 and UmRPA-1 crystal structures revealed that trypanossomatids RPA-1 present different binding modes of single stranded DNA (ssDNA). Whereas in HsRPA-1 and UmRPA-1 structures all DBDs are able to bind ssDNA, in TcRPA-1 model only OBF1 and OBF2 are able to interact with ssDNA. Interestingly, in LaRPA-1 *in silico* model, OBF1 is the unique protein region that interacts with the nucleic acid. Regarding RPA-2, LaRPA-2 and TcRPA-2 present an OB-fold domain and a C-terminal winged helix-loop-helix domain (wHLH) homologous to RPA-2 from higher eukaryotes. *In silico* models of OB-fold domain from LaRPA-2 and TcRPA-2 adopt a similar tertiary structure conformation compared to the HsRPA-2 and UmRPA-2 crystal structures. However, the trypanossomatids RPA-2 *in silico* models present a ten residue insertion rich of flexible residues located in the neighborhood of the DNA binding channel. In the molecular dynamics (MD) simulation, this region presented a high root mean square fluctuation of the main chain, adopting multiple positions during 50 ns of MD simulation, even blocking the DNA binding channel. These data suggest that the DNA binding site of trypanosomatid RPA-2 is more structurally unstable than their homologues in higher eukaryotes, causing changes in the DNA binding affinity trypanossomatids RPA-2.

Funding support: FAPESP and NCC/GridUNESP.