Functional and structural characterization of RBP42 in *Trypanosoma cruzi*

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Trypanosoma cruzi, the etiological agent of Chagas disease, has unique characteristics in genome architecture and gene expression regulation. In this parasite, genes of unrelated functions are transcribed as long polycistronic pre-mRNAs and solved into monocistronic transcrips by the trans-splicing and polyadenylation processes. Due to these unique characteristics, gene expression regulation occurs mainly at the post-transcriptional level by RNA binding proteins (RBPs) that orchestrate transcripts processing, transportation, stabilization and degradation under normal and stress conditions. Moreover, there is a large variety of RBPs coding genes in the parasite's genome. The interaction between RBPs and mRNAs forms structures called ribonucleoprotein complexes (RNPs) that can aggregate into microscopically visible cytoplasmic structures known as RNA granules in response to stress. RNA granules could function as centers for degradation and storage of transcripts used in stress recover. To investigate the role of RBPs, epimastigotes of the CL Brener clone were transfected with the vector pRock_Neo to generate a cell line overexpressing TcRBP42. The phenotype of the transfected cells was characterized through growth curves in cells treated or non-treated with UV light, benznidazole and gamma radiation. The overexpression of the TcRBP42 in transfected cells was confirmed by detection of the histidine-tagged RBP42 in whole protein extracts by Western blot. The analyze of growth curves revealed a similar growth pattern between RBP42 overexpressing cells and control cells at normal conditions. In contrast, these cells were more sensitive to UV light (1000 J/m2 dose) and more resistant to gamma radiation. It was also observed that the RBP42 overexpressing cells are also more sensitive to benznidazole treatment. Given that RBP42 gene is annotated in the *T. cruzi* genome as a hypothetical protein, functional and structural analyses were performed to predict conserved domains and secondary structure by using the softwares InterProScan and PSSpred. In silico analyses revealed the presence of NTF-2 and RRM domains and a secondary structure similar to the one characterized for *T. brucei*. The RBP42 levels of gene expression were analyzed by RNAseq (package DESeq2 from R/Bioconductor) in non-transfected cells 4, 24 and 96 hours after exposure to 500 Gy of gamma radiation. The RBP42 gene is more expressed than the other genes in its genome vicinity. It was observed a two-fold change increase in the RBP42 gene expression levels after gamma irradiation, suggesting a role in the parasite stress response.