The role of the zinc finger proteins TcZC3H39 and ZFP29 in the RNA metabolism of *Trypanosoma cruzi*

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The regulation of gene expression is essential for the adaptation of *Trypanosoma cruzi* to different conditions imposed by its hosts during its life cycle. In trypanosomatids, this control is mainly mediated by post-transcriptional mechanisms which act on the metabolism of the mRNAs and proteins. In this context, the RNA binding proteins (RBPs) are key players since they interact with the mRNA by coordinating their destinations and fate in the cell. The RBPs repertoire in *T. cruzi* comprises different groups of proteins, including those with a C3H zinc finger domain. This project aims the study of the zinc finger proteins, TcZC3H39 and ZFP29 in *T. cruzi* by investigating their expression and localization throughout the parasite's life cycle, as well as the associated mRNAs targets in replicating and differentiating parasites. In order to investigate the biological role of specific genes we are using reverse genetics tools, such as gene knockout, to investigate the resulting phenotype changes. Recently, the CRISPR/Cas9 system has been described as an alternative method for gene knockout, which is being applied in several organisms, including *T. cruzi*. Therefore, to determine the role of TcZC3H39 and ZFP29 in the RNA metabolism of *T. cruzi*, we used the CRISPR/Cas9 system to knockout these genes and evaluate their respective associated phenotypes. Firstly, we proposed an improvement in the current strategies used to generate gene disruption by the CRISPR/Cas9 system in *T. cruzi*. The strategy consists in the combination of the advantages of the two earlier described methods and solution of their disadvantages. We validated the methodology by performing the knockout of the GP72 and α-Tubulin genes, used as control since their disruption phenotype is well described. After the establishment of the experimental protocol, we were able to attempt the knockout of TcZC3H39 and ZFP29. As a result, major changes were observed in the morphology and cell cycle of the parasites. Furthermore, the viability of the cells were compromised, suggesting that the TcZC3H39 and ZFP29 are essential for the parasite survival and may play important roles in its biology.