Characterization of actin 4 of the human parasite *Trypanosoma cruzi*

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Actin is a highly conserved protein that performs numerous essential functions such as mobility, cytokinesis, vesicle trafficking, and chromatin remodeling. Actin can interact with other proteins such as actin related proteins (ARPs), which modulate actin polymerization, contribute to dynein-based motility and form chromatin-remodeling complexes. The primary sequence of ARPs suggests similarity, but not identity with actin. There are four loci annotated as actin (putative) in the genome of *Trypanosoma cruzi*. However, this annotation was based on primary *in silico* analyzes, without any functional characterization. An orthologous gene for actin 4 is found only in *Leishmania*, being, however, annotated as actin-like protein (AcL). Actin 4 primary sequence exhibits less than 50% of similarity and 35% of identity with other actin isoforms from *T. cruzi*. Nevertheless, when compared to other organisms, actin 4 is more closely related to ARPs and AcLs than to actins. By immunoblotting analysis, it was possible to determine that actin 4 is expressed in all developmental stages of *T. cruzi*. Moreover, actin 4 was found only in the soluble fraction of protein extracts obtained from epimastigote forms. Subcellular localization of actin 4 showed labeling throughout the cytoplasm with patches of staining, in both epimastigote, trypomastigote and metacyclic trypomastigote forms, shown by optical and electron microscopy. Parasites knockout for actin 4 gene, obtained with CRISPR-Cas9 methodology, showed alterations at the ultrastructural level, such as intense formation of vesicles with electron-dense content and appearance of multivesicular bodies. Despite these changes, neither the external morphology nor the growth of the knockout parasites were affected. In the future, parasites expressing actin 4-GFP fusion protein will be used for co-immunoprecipitation assays to determine protein interactions.