Differential composition of the translational machinery of *Trypanosoma cruzi* during metacyclogenesis and cell cycle.

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Trypanosoma cruzi (T. cruzi), the causative agent of Chagas disease, primarily controls gene expression through post-transcriptional mechanisms. Our research group has identified translational regulation as a key process occurring during both metacyclogenesis and the G1/S transition of the cell cycle. Recent findings using various approaches suggest that ribosomes may exhibit compositional diversity at the protein level, potentially influencing regulatory outcomes. Based on this, we aimed to perform an in-depth characterization of the translational machinery in T. cruzi, focusing on the epimastigote and metacyclic trypomastigote stages of the life cycle, as well as on parasites synchronized in G1 and S phases. As a first step, we curated the current annotation of ribosomal proteins (RPs), assessing copy number, ribosomal location, N- and C-terminal extensions, expression levels, and possible extra-ribosomal roles. Experimentally, Ribo-Seq analysis revealed a global repression of RP mRNA translation in the metacyclic trypomastigote stage. However, some RP transcripts showed distinct behavior, suggesting selective resistance to this repression. Additionally, we applied a multi-omics approach—combining RNA-Seq, Ribo-Seq, and proteomics—on parasites synchronized in G1 and S phases. This analysis uncovered individual differences in translational efficiency of RP mRNAs, as well as in the steady-state protein levels of RPs. These findings support the idea that the composition of the translational machinery may vary during these biological transitions. To further explore this hypothesis, we conducted quantitative proteomic analysis of ribosome-enriched fractions obtained from epimastigotes, metacyclic trypomastigotes, and cell cycle–synchronized parasites in G1 and S phases. Through these experiments, we found that certain ribosomal proteins exhibit differential abundance in ribosome-enriched fractions across the different life cycle stages. We also observed variations in the levels of proteins associated with ribosomes. The differential abundance of components of the translational machinery could have regulatory implications, representing a potential new layer of gene expression regulation in T. cruzi.