

Studying translational machinery composition of *Trypanosoma cruzi* during metacyclogenesis and cell cycle by a multiomics approach.

Martín Rivara Espasandín^{1,2}, Santiago Radío³, Santiago Chávez^{1,2}, María Ana Duhagon^{2,4}, Pablo Smircich^{1,4}, José Sotelo-Silveira^{1,5}

¹ Departamento de Genómica, Instituto de Investigaciones Biológicas Clemente Estable, MEC

² Departamento de Genética, Facultad de Medicina, Universidad de la República

³ Institute of Evolutionary Biology (CSIC-Universitat Pompeu Fabra), 08003, Barcelona, Spain

⁴ Sección Genómica Funcional, Facultad de Ciencias, Universidad de la República

⁵ Departamento de Biología Celular y Molecular, Sección Biología Celular, Facultad de Ciencias, Universidad de la República

Trypanosoma cruzi (*T. cruzi*), the etiological agent of Chagas disease, regulates its gene expression mainly by post-transcriptional mechanisms. Our group observed that translational regulation is an important mechanism during metacyclogenesis and G1/S cell cycle transition. Recently, it has been shown by different approaches that the composition of the ribosomes may be variable at the protein level, leading to regulatory changes. Thus, we set out to perform a detailed characterization of the translational machinery of *T. cruzi* in the epimastigote and metacyclic trypomastigote life cycle stages, as well as in G1 and S cell cycle phases. First, we reviewed and polished the current annotation of ribosomal proteins (RP), analyzing copy number, location in ribosome, protein extensions at terminal ends, expression values and putative extra-ribosomal functions. Addressing this characterization in an experimental way, we observed through Ribo-Seq that there is a global repression of RP mRNAs translation in the metacyclic trypomastigote stage, but there is also individual variation, finding some RP mRNAs that resist that repression. We also performed a multiomics approach (RNAseq, Ribo-Seq and proteomics experiments) in parasites synchronized on G1 and S cell cycle phases. We observed individual variation in the translational efficiency changes of RP mRNAs and also in RP stationary level. These observations could be in line with the hypothesis that translational machinery composition may be variable during these transitions. To explore this hypothesis we performed quantitative proteomics on ribosomes enriched fractions, in parasites synchronized in G1 and S cell cycle phases. We observed differences in the protein abundance of some RP between ribosome enriched fractions of cell cycle phases, suggesting possible variations in translational machinery composition.