Quantitative proteomic analysis reveals constitutive histone PTM patterns and metabolic compartimentalizatio during the Trypanosoma cruzi cell cycle

Ana Paula Menezes[1], Maria Carolina Elias[1], Ariel Mariano Silber[2], Richard Burchmore[3], Julia Pinheiro Chagas da Cunha[1]

[1.] IB, Instituto Butantan, São Paulo;
[2.] USP, Universidade de São Paulo, São Paulo;
[3.] UofG, University of Glasgow, Glasgow;

Investigating the dynamics of the cell cycle in *Trypanosoma cruzi* is essential for comprehending how these organisms manage their proliferation. While chromatin- associated mechanisms are pivotal for cell cycle regulation in numerous eukaryotes, their influence on trypanosomes has been underappreciated, largely because of the extensive post-transcriptional regulation of protein-coding genes in these organisms. Previous studies have observed alterations in certain histone post-translational modifications (PTMs) throughout the *T. cruz*i cell cycle, but it is still uncertain whether these changes also affect the nuclear proteome. To explore these dynamics, our main goal was to investigate the nuclear proteins and histone PTMs throughout the cell cycle using quantitative proteomic approaches. We synchronized the cell cycle with hydroxyurea to isolate cells at G1/S, S, and G2/M phases. Following synchronization, we isolated nuclei and histones to perform a detailed quantitative proteomic analysis using LC-MS/MS with TMT labeling for nuclear proteins and DIA analysis for histone PTMs. Our results reveal that histone PTMs exhibit minimal variation across the cell cycle, indicating a constitutive process. This stability is supported by consistent profiles of H3K76 methylation and H4 N-terminal acetylation, which align with previously reported data. Furthermore, we observed that PTMs in variant histones were more pronounced compared to canonical histones, highlighting their potential significance in regulating nuclear processes. Expanding on this, our comprehensive nuclear analysis identified 2,937 proteins and revealed distinct proteomic signatures across the G1/S, S, and G2/M phases. Gene Ontology (GO) analysis in “cellular component” confirmed enrichment in terms related to chromatin and chromosome organization, validating the successful purification of nuclei samples. Additionally, we identified a significant number of metabolic enzymes, constituting approximately 30% of the total proteins, suggesting a critical role for metabolic compartmentalization during *T. cruzi* cell cycle. Moreover, the differentially expressed proteins in G1/S were enriched in GO terms (“biological process” and “molecular function”) related to energy generation and translation. Furthermore, our analysis of metabolic pathway enrichment revealed that these proteins are involved in pathways leading to acetyl-CoA production, though global histone acetylation was unaffected. These findings highlight the intricate interplay between metabolic processes and cell cycle progression, providing deeper insights into *T. cruzi* biology.

Agradecimentos: FAPESP