Proteomics of intracellular and axenic amastigote lifeforms of Trypanosoma cruzi in replicative and non-replicative conditions

Trypanosoma cruzi differentiates between non-replicative and replicative forms during its life cycle. In this study, we performed a quantitative proteome analysis of T. cruzi amastigotes isolated from infected cells and amastigotes produced in axenic cultures. Intracellular amastigotes (AI) were isolated using DEAE-cellulose after mechanical disruption of T. cruzi-infected HeLa cells. Axenic amastigotes (AD5) were produced by differentiation of trypomastigotes in DMEM pH 5.0 for 9 h. AD5 cells were then incubated in pH 7.2, in LIT or DMEM medium. Cell counting showed that AD5 cells were not able to replicate while amastigotes grown in pH 7.2 in DMEM (AD7) or LIT (AL7) media were replicative. Proteins from AI, AD5, AD7, and AL7 were extracted and digested with trypsin. The resulting peptides were subjected to LC-MS/MS and protein identification and quantification using bioinformatics tools. Proteomics revealed that the AI isolation method was efficient since approximately 90% of identified protein groups were assigned to T. cruzi, when a concatenated T. cruzi and human protein databank was used for identification. Further, proteomic analyses of all amastigote conditions identified 3134 protein groups and showed differences between non-replicative and replicative conditions. Cluster analysis revealed up-regulated protein groups in non-replicative amastigotes related to the microtubule and dynein complex. On the other hand, a cluster of proteins with higher abundance in replicative conditions suggested the involvement of ribogenesis factors, ubiquitination, SUMOylation, SNARE complex, and nucleolar DNA-associated proteins in amastigote replication. Moreover, kinetoplast DNA-associated proteins and trans-sialidases presented increased abundance in replicative conditions. Further, differences in protein translation and energy metabolism were observed between replicative axenic amastigotes and AI. Overall, these results revealed biological processes involved in amastigote division, as well as interesting differences between axenic and intracellular amastigotes.

Agradecimentos: CNPq, FINEP