Development of a yeast two-hybrid system to investigate protein interactions to the protozoan Trypanosoma cruzi, using bait proteins of the Rho signaling pathway.

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Introduction: The causative agent of Chagas disease, the Trypanosoma cruzi, undergoes profound morphological and metabolic changes along life cycle, indicating the presence of several transduction pathways. However, molecular tools to investigate protein interactions still require development. Objective: Considering this difficulty, we develop a two-hybrid system in Saccharomyces cerevisiae to investigate molecular partners of T. cruzi. Methods: For production of hybrids in yeast, were used the pGBK7 to clone our baits in fusion with the DNA Binding Domain of GAL4 (bait-Gal4BD), or the pGAD424 vector to subclone our cDNA library in fusion with the Activation Domain of Gal4 (cDNA-Gal4AD). The pGAD424 had its multiple cloning site modified with inclusion of adapters to allow the subcloning of fragments from normalized cDNA library for the three reading frames with Gal4AD. Occurring in vivo interaction, the two-hybrid formed will be able to activate transcription of genomic markers of S. cerevisiae MAV203 as HIS3, ADE1, and LacZ. After clone’s selection with chromogenic substrate, the clones will be sequenced. Results and Conclusions: After cloning Rho and actin coding regions in pGBK7 we carry out experiments to confirm the expression of baits in yeasts, discard both a possible toxicity and transcriptional auto-activation of genomic reporter HIS3. Subcloning efficiency of the original cDNA library with $10^6$ clones, estimated to the three reading frames of pGAD424 vector was approximately $10^5$ clones. The co-transformations of strain MAV203 with the constructions are in progress to dentify positive clones for these interactions.

Word Keys: two-hybrid, Trypanosoma cruzi, Chagas disease.
Supported by: PROCIÊNCIA-IFRJ, FAPERJ