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"PCR-cloning of a *Trypanosoma cruzi* scaffold protein-biotin ligase gene-fusion"

Trypanosoma cruzi (*T. cruzi*) is an arthropod-transmitted protozoan parasite that infects, approximately 6 million people and is the etiologic agent of Chagas' disease. Chagas' patients present with severe cardiac, gastrointestinal, or neurological pathologies that are life-threatening. Although primarily affecting Latin American countries, other regions, including North America have reported cases. There is no vaccine and current chemotherapies against this disease are inadequate due to their associated toxicity, ineffectiveness and the development of resistant strains.

A better understanding of parasite cellular and molecular pathways is expected to guide the development of better antiparasitic drugs by identifying new parasite drug targets. We are characterizing the molecular functions of a *T. cruzi* ribosome-associated scaffold protein, termed TcRACK1, to assess its potential as a potential new drug target.

Previous studies indicate that these proteins associate with ribosomes in an optimized manner, allowing for robust expression of parasite mitochondrial cytochrome c oxidase (COX) subunits. Expression of COX subunits is important for parasite mitochondrial function, survival and hence virulence in the mammalian host.

In order to understand how TcRACK1's precise association with ribosomes regulates COX subunit expression, the overall goal of this project is to identify TcRACK1 interacting proteins. This will be achieved using a biotin-ligase gene fusion approach, employing the biotin ligase TURBOID. We have optimized a PCR reaction to generate a TcRACK1-TURBO gene fusion that will then be inserted into the *T.cruzi* expression plasmid pBEX. When this fusion protein is ectopically expressed in *T. cruzi,* in the presence of added biotin, it will ligate biotin onto all proteins in its proximity.

These biotinylated proteins, representing TcRACK1-interacting and proximal proteins, will then be identified using streptavidin-affinity purification followed by mass spectrometric fingerprinting. The functional importance of these proteins in regulation of COX subunit expression will be tested in future studies.

Preliminary data showing our progress toward the expression of the TcRACK1-BioIDTurbo transgene in *T. cruzi* will be presented.