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Abstract:

Trypanosomatids are parasitic members of the Euglenozoa, an early-branching protozoan phylum thought to share traits with the last eukaryotic common ancestor (LECA). Because the euglenozoan lineage branched early from LECA, studying trypanosomatid mitochondrial proteins may provide insight in the biochemical processes that may have occurred within a primitive mitochondrion and evolution of essential biochemical pathways found in higher eukaryotes.

A large number of proteins in the typranasomatid mitochondrion are uncharacterized, and we seek to clarify functional roles of putative, mitochondrial proteins and verify subcellular localization. Three novel proteins, ORF 508, 3806, and 9990, were selected from the genome (~ 11,000 genes, unpublished data) of *Crithidia fasciculata,* a non-pathogenic tyrpanasomatid, and localization to the mitochondrion was determined using multiple bioinformatics tools. Function of these proteins was performed by motif analysis, identification of conserved domains, and prediction of post-translational modifications. Motif analysis of ORF 508 shows multiple phosphorylation sites, which may be necessary for mitochondrial targeting as well as protection from degradation during apoptosis. Both, ORF 508 and 9990 contain N-myristoylation sites, which suggest the possibility that these proteins are dual-targeted to various subcellular locations and, membrane-associated. ORF 3806 and 9990 were proteins with conserved domains. ORF 3806 contains iron-sulfur cluster domain and therefore could play a role in cellular respiration. ORF 9990 contains a zinc binding domain which could play multiple roles in protein interaction. Thus far, ORFs 508/3806/9990 have been amplified using *C. fasciculata* genomic DNA, sequenced, and ligated into an GFP-fusion vector (pNUS-GFPcH), optimized for gene expression in *C. fasciculata.* Transfection of *C.* fasciculate with the GFP fusion constructs will permit validation of mitochondrial localization of each protein by microscopy.

Keywords: mitochondria; Trypanosoma; LECA; Euglenozoans