

Poster Presentation

Title: Characterizing Hypothetical Proteins in *Crithidia fasciculata*

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Introduction

Hypothetical mitochondrial proteins are often studied to elucidate functions in pathogenic trypanosomatids. Hypothetical proteins may be phylogenetically conserved and confirmation of their unknown functions would provide unique insights (1). The exact biological role of a hypothetical protein is typically determined through *in vivo* experimentation (2).

Crithidia fasciculata is a trypanosomatid model that can be studied to understand molecular processes in its pathogenic relatives. Open reading frames (ORFs) 7586 and 7685 from *C. fasciculata* were characterized *in silico* and subcloned to prepare for expression as GFP-fusion proteins for validation of mitochondrial localization.

Methods

Using various bioinformatics tools, mitochondrial localization probabilities were determined for ORFs 7586 and 7685. Different structural features such as O-GlcNAc sites, signal peptides, and transmembrane domains were searched for in each ORF. Each ORF was PCR amplified by adjusting parameters such as MgCl₂ or primer concentration. For ORF 7685, direct colony PCR and plasmid DNA isolation were performed after ligation into pJET 1.2 blunt cloning vector.

Results

The proteins encoded by each ORF have different predicted structural features. ORF 7586 had a putative signal peptide and transmembrane domains present in its amino acid sequence. ORF 7685 had predicted O-GlcNAc sites located at serine residues. Both amplicons were PCR amplified at their predicted lengths. Plasmid DNA from direct colony PCR of ORF 7685 was confirmed by sequencing.

Discussion

The proteins encoded by ORFs 7586 and 7685 likely localize to the mitochondrion and contain either a signal peptide and transmembrane domain (ORF 7586) or O-GlcNAc sites (ORF 7685). Input of O-GlcNAc sites to amino acid residues has been studied in *Trypanosoma cruzi*, a human pathogen, to understand how it maintains its structural integrity in the host (3). Transmembrane domains and signal peptides are necessary for proper function and cellular transport of mitochondrial proteins (4). Both proteins lack definite cellular function and require further investigation through mitochondrial localization in *C. fasciculata* to confirm these predictions.

Keywords: Bioinformatics, Mitochondria, Molecular parasitology, Trypanosomatids

References:

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