Poster Presentation

**Title:** Exploration of putative mitochondrial gene expression in *Crithidia fasciculata* as a model for pathogenic trypanosomes causing human disease

**Authors:**
Baylye Boxall (bboxall1@student.gsu.edu)
Nadjean Sagesse (nsagesse1@student.gsu.edu)

**Faculty Sponsor:**
Dr. Paul Ulrich, Lecturer, Department of Biology (pulrich@gsu.edu)

**Abstract:**

**Introduction**

*Crithidia fasciculata* is a non-pathogenic relative of trypanosomatids that cause human disease. A closer look at the genome of *C. fasciculata* may yield potential drug targets in these drug resistant pathogens. Moreover, stimulation of cell death due to malfunctions in respiration pathways is a likely outcome to eradicate infection. Therefore, the likelihood of proteins localized in the mitochondria to be involved in cellular respiration will be explored.

**Methods**

Geneious was used to analyze the genome of *C. fasciculata* and extract the open reading frames (ORFs) (Kearse *et al.*, 2012). Out of 765 predicted mitochondrial sequences found using Predotar and WoLF PSORT, two ORFs with high probabilities were chosen for studying expression and related function within the mitochondria with NCBI Batch Conserved Domain (Horton *et al.*, 2007; Marchler-Bauer *et al.*, 2011; Small *et al.*, 2004). A structure of each protein was predicted using RaptorX (Kallberg *et al.*, 2014), based on existing structures containing similar domains and motifs. This structural data was used to visualize the active site of each protein in RasMol (Sayle & Milner-White, 1995).

**Results**

Open reading frames: ORF_4536 and ORF_10568 were each found to have mitochondrial targeting sequences as well as relevant conserved domains. Analogues in iron-sulfur cluster biogenesis were discovered for ORF_4536 and EF-hand calcium binding motifs for ORF_10568. These domains were verified using 3D structures.

**Discussion**

Based on individual activities between ORF_4536 and ORF_10568 in the mitochondria, a putative collaboration between the two with respect to respiration is proposed. Iron-sulfur clusters are involved in the transfer of electrons in the electron transport chain, and EF-hand calcium binding proteins are involved in the regulation of calcium with respect to the release of cytochrome c. We propose that calcium concentration may impact the affinity of iron-sulfur clusters to electrons in the electron transport chain. Understanding these mechanisms may offer potential suggestions for treatment of diseases caused by trypanosomes if genetic targeting can impair respiration.
**Current/Future Studies**

Genomic DNA isolations will be conducted to amplify both ORF_4536 and ORF_10568 in PCR for ligation into the pNUS-GFPcH vector for trafficking localization in *C. fasciculata* using microscopy (Tetaud *et al.*, 2002). Upon successful localization in mitochondria, both proteins can be monitored in their activities in relation to cellular processes like respiration.

**Keywords:** *Crithidia fasciculata*, Trypanosomes, Calcium-binding, Iron-sulfur cluster, Mitochondria, Respiration, Bioinformatics

**References:**


