Title: Development of a Tetracycline-Inducible Expression System for *Crithidia fasciculata*

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Abstract

Introduction

Diseases caused by eukaryotic trypanosomes (e.g. *Leishmania, Trypanosoma*) are a global health challenge, and cell biology of trypanosomes must be further investigated to effectively address treatment and control of these pathogens. We are developing a tetracycline-inducible system to control gene expression in *Crithidia fasciculata*, a trypanosomatid parasite of mosquitoes that we are using as a model for human pathogens. This system relies on constituent expression of the prokaryotic tetracycline repressor and T7 RNA polymerase. Ultimately, this system will be utilized to investigate the function of trypanosomatid targets through over-expression of GFP-fusion proteins or dsRNA for knockdown of specific transcripts (RNAi).

Method

To express the tetracycline repressor and T7 RNA polymerase in *C. fasciculata*, we are building two vectors (pCfInduce-TetR and pCfInduce-T7RNApol) that will be transfected into *C. fasciculata.* DNA sequences for each gene and flanking regulatory regions were amplified from *Crithidia fasciculata* DNA or existing vectors by PCR and transferred to the backbone plasmid pBSII. Insertion of each fragment into pBSII was verified by digestion with appropriate restriction enzymes.

Results

Currently, we have partially constructed each of the two vectors. We have ligated an 18S rDNA fragment into our pCfInduce-T7RNApol, and a 3' GSPS regulatory region has also been ligated into pCfInduce-TetR. Identity and insertion of each DNA fragment was confirmed by sequencing and restriction digestion. We are also working toward integrating DNA elements such as the phosphoglycerate kinase intergenic regions and selectable markers (hygromycin, G418) into these vectors in order to properly regulate expression of each protein and ensure that transfectants carry the desired expression cassettes.

Discussion

The remaining regulatory elements and selectable markers for each vector will be ligated to the vectors following refinement of PCR conditions and verification by sequencing. Following completion of pCfInduce-TetR and pCfInduce-T7RNApol, *C. fasciculata* will be transfected with both vectors, and expression will be confirmed by western blot. *Crithidia fasciculata* expressing both TetR and T7RNApol will be transfected with a third construct whose

transcription is under the control of the tetracycline repressor. Using this system, a variety of genes could be investigated in *C. fasciculata*, and manipulation of genes in this model organism may inform the development of new treatments to combat pathogenic trypanosomes.