Exploration of *Dictyostelium* as a Model System for the Development of Anti-amoebic Drugs

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Introduction

Entamoeba histolytica, a pathogenic human parasite, affects the gastrointestinal system and is responsible for millions of Amebiasis cases worldwide. It is passed through contaminated food and water in areas with poor sanitation such as developing countries. Its genome has been compared to other organisms, including animals (*H. sapiens*, *C. elegans*, *D. melanogaster*), plant (*A. thaliana*), yeast (*S. cerevisiae*), and the social amoeba *Dictyostelium discoideum*. 63 gene archetypes (73 genes in *Dictyostelium*) were found to be unique to both *Entamoeba* and *Dictyostelium* and consequently these represent potential targets for anti-amoebic drugs because these genes are not found in any other of the compared organisms, (Table 1). Historically, *Dictyostelium* has been used to study aspects of cell and developmental biology. Our goal is to utilize the experimental flexibility of *Dictyostelium* as a useful model to study the functions of these unique genes. As a first approach we are using RNAi to knock down the expression of these six genes (Table 1), looking for any changes in cell phenotype as a result.

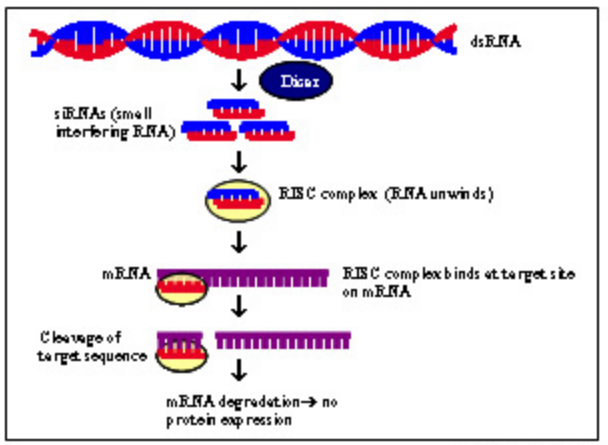

Figure 1: RNAi mechanism

Methods

DH1 wild type cells were transfected using six constructs, pC9A1, pC9B3, pC9C3, C9D2, pC9E2 and pC10B2. Blasticidin was used to select for cells that had integrated the construct, where all other cells died in the presence of this antibiotic. Colonies were identified, isolated, and maintained, while RNA was extracted for northern blots, to confirm gene knockdown. Additionally, preliminary phenotypic analysis was performed leading to the finding of one interesting cell line, pC90291 G7. Cells from this line were fixed and stained with the nuclear stain, DAPI (Fig 4). Many of these cells were multinucleated with varying numbers of nuclei per cell, indicating a possible defect in cell division.

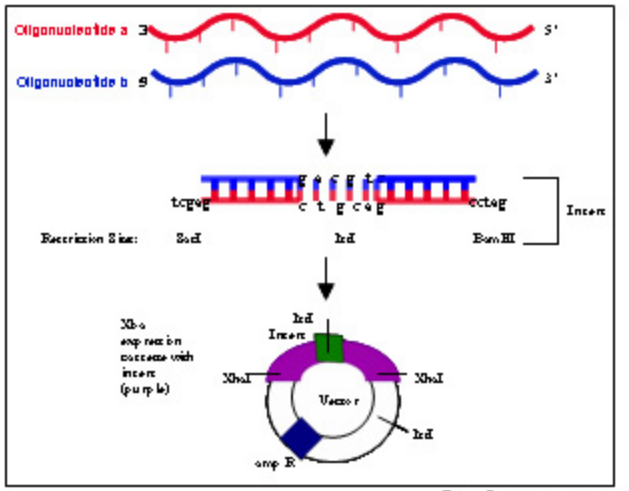

Figure 2: Design of constructs. Oligonucleotides were designed (Table 2), with specific restriction sites (SacI, XbaI and BamHI) to make the constructs for gene knockdowns.

Table 1: 73 genes unique to *Dictyostelium* or *Entamoeba* and their functions. Bolded genes were selected for knockdowns.

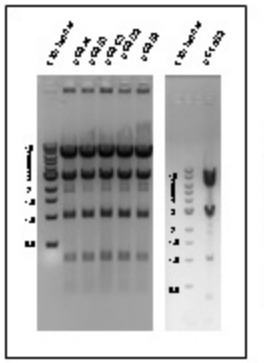

Figure 3: Two agarose gels confirming integration of insert into pTR4. DH1-wild type genomic DNA digested with XbaI and PstI, are based on the known positions of these restriction sites, but bands were expected at 1.6kb, 1.4kb, 1.1kb, and 400bp. It is apparent from these images that the expected bands were observed.

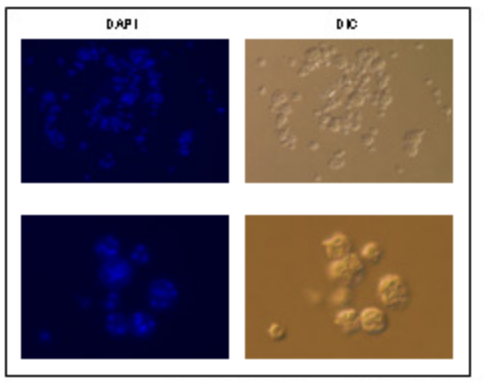

Figure 4: DAPI and DIC images of pC90291 G7 cells. These images are representative of the wide range of nuclei per cell observed in this cell line.

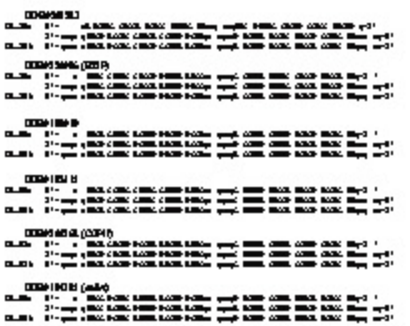

Table 2: Sequence of oligonucleotides used to make the knockdowns. The restriction sites (SacI, PstI and BamHI) are in lower case letters.

Future Directions

- Northern Blots to confirm gene knockdown
- Phenotypic analysis
  - -ability to go through a complete developmental cycle
  - -growth curves
  - -filling up, shaking cultures and DAPI staining for both
  - -repeat transfections
- Further analysis of the 6 selected genes, and the expansion of project to include the remaining 72 genes.
- Genes whose disruptions produce interesting phenotypes will be explored for the potential development of anti-amoebic drugs.