PD-ABM-354

David A. Rakes, Research Coordinator
Center for Human Capacity Development
USAID/RND University Center
Room 900 SA-38
Washington DC 20523-3801

Final Report for grant period 9/93-9/95

Grant No. PCE 5053G003047-00
Title: Gene Expression during *Entamoeba* encystation.
PI:Vincent C. Bond, Ph.D.

1E.2.(a) Annual Workplan

Specific aims included identifying genes (specifically regulatory genes) that are uniquely specific to the process of encystation in *Entamoeba*, and placing those identified sequentially in the developmental pathway. By November 1993 we had begun processing *E. invadens* amoeba that had been induced to encyst and collected at various time points into the induction process. By the end of year one of this grant we identified two encystation specific transcripts using subtractive library techniques. These clones were sequenced to attempt to identify the gene. Sequence information identified one clone to be a late stage gene: that was already identified, chitin synthase.

In year two we moved to using differential screening of encystation-induced amoeba, very early after induction. Sanchez et al (1994), screening for encystation specific factors in *Entamoeba* using subtractive techniques, did not find early regulatory gene transcripts either. Their results combined with what we have obtained suggest: that one needs to look VERY early (the factors they found are made between 8 and 24 hr postinduction)in the encystation process (between 0 and 4 hours postinduction). Further, the evidence suggests that the early regulatory genes may be on during vegetative growth, but are either turned up or turned down on encystation induction.

We began a second series of screens only looking at very early time points after encystation induction (2 hr, and 4 hr). Additionally, this time instead of doing subtractive hybridization, which would only identify genes turned off or turned on during encystation, we used differential screening, a technique which would allow us to identify genes that are on in both vegetative stage and encystation induction stage, but are either upregulated or downregulated. Interestingly, we found three transcripts that appeared to be upregulated or downregulated following induction of encystation of *Entamoeba invadens* by assay using developmental Northern blots. As of the end of the grant period, we have sequenced one of those cDNAs. This cDNA clone appears to be a transcript with a fair amount of homology to Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH). This is interesting in light of a plethora of recent work showing that abundant metabolic enzymes (e.g., GAPDH, dihydrofolate reductase, thymidylate synthetase) appear to be involved in cellular homeostasis independent of the activity that characterized their initial description.

Some of our results were presented at an invited conference: Bi-National Symposium on Border Parasitic Diseases at The University of Texas at El Paso, March 1995 (See Appendix).

These results are also being prepared for journal publication in a paper that we are currently putting together for submission.

1E.2.(b)(1) Comparison of actual accomplishments with goals established for the period.

The overall goal of the project was to identify genes (specifically regulatory genes) that are uniquely specific to the process of encystation in *Entamoeba*.

The specific aims of the project were:

1. Enrich for encystation specific messages. We used differential screening of encystation-induced amoeba, very early after induction (1,2, and 4 hours postinduction). Several cDNA's believed to represent three transcripts either turned up or down following encystation induction were identified.

2. Examine the kinetics of expression of encystation specific clones. We examined the kinetics for one cDNA that was subsequently sequenced. Significant upregulation of the homologous transcript was observed at the earliest times examined postinduction (1 hour). Additionally, levels of this transcript dropped off after 8 hours postinduction.

3. Characterize encystation specific cDNAs. We have sequenced the above cDNA. This cDNA clone appears to be a transcript with a fair amount of homology to Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH).

1E.2.(b)(3) Other pertinent information including status of the finances.

Vincent C. Bond, Ph.D.
Principal Investigator

Walter W. Sullivan, Jr., Ph.D.
Vice President for Health Promotions

BIBLIOGRAPHY

Sanchez, L., Enea, V., and Eichinger, D. 1994. Identification of a developmentally regulated transcript expressed during encystation of *Entamoeba invadens*. Mol Biochem Parasitol 67(1):125-135

Differential Gene Expression during Entamoeba Encystation. Vincent C. Bond, and Yao-Qing Huang. Presented at the Bi-National Symposium on Border Parasitic Diseases at The University of Texas at El Paso, March 1995.

APPENDIX

Differential Gene Expression during Entamoeba Encystation. Vincent C. Bond, and Yao-Qing Huang. Presented at the Bi-National Symposium on Border Parasitic Diseases at The University of Texas at El Paso, March 1995.

We are investigating the regulatory mechanisms of encystation in *Entamoeba*, using *Entamoeba invadens* as a model system. Initially, we compared the protein profiles of newly formed cysts and trophozoites of *Entamoeba invadens* using high resolution two-dimensional PAGE and digitized video image analysis of silver stained gels. A total of 155 proteins unique to trophozoites and a total of 72 proteins unique to cysts were detected. Five of the most prominent cyst specific proteins were identified as candidates for further study. These results imply that extensive changes in gene expression accompany the progression of this parasite through its life cycle changes. These changes in gene expression can be taken advantage of to isolate those genes that are encystation specific. We are currently using a method called "differential display" to identify changes in mRNA expression patterns. Using this procedure we will identify and isolate genes either turned on (up), or off (down) early (2-8 hours) in the encystation process.