ABSTRACT:

Entamoeba histolytica, the cause of amebic dysentery and liver abscess, infects approximately fifty million people each year in the developing world. It has been proven that the ability of *E. histolytica* to kill and phagocytose (the process of engulfing solid particles) host cells correlates with this parasite's virulence. It also has been reported that in many eukaryotic cells a superfamily of proteins called SNARE proteins play an imperative role in the phagocytosis process. But, despite the huge amount of information that is now known about SNARE proteins in many organisms, and the important role(s) that some SNARE proteins may have on *E. histolytica*'s virulence, nothing is known about these proteins in *E. histolytica*. As a precursor to studies of the role that SNARE proteins play in *E. histolytica* phagocytosis, we are characterizing four *E. histolytica* SNARE proteins to determine the functional groups to which they belong. We will then examine the role(s), if any, that each of these SNARE proteins have in *E. histolytica* phagocytosis. The ER-SNARE (Sec 22) protein is of particular interest because recent scientific studies suggest that during phagocytosis in mammalian cells the endoplasmic reticulum (ER) may fuse to the cellular plasma membrane, thus providing membrane for the forming phagosome, and that Sec22 plays an imperative role in mediating this fusion. To test if this occurs in *E. histolytica*, bioinformatics was first used to predict the functional group of each putative *E. histolytica* R-SNARE protein present in the genome database. This was done by using representative R-SNARE proteins from model organisms, and the computer program Phylip to build a phylogenetic tree. PCR was then used to amplify and clone the gene encoding an *E. histolytica* R-SNARE protein from each of the four functional R-SNARE groups while introducing a FLAG-epitope tag. We are now expressing these epitope-tagged SNARE proteins in *E. histolytica* parasites, and will be using immunofluorescent confocal microscopy to localize the expressed SNARE proteins while co-staining for cellular markers (e.g., the endoplasmic reticulum). Since the location of SNARE proteins correlates with function, this will enable us to test the functional classification deduced using bioinformatics. After characterizing these SNARE proteins, a dominant negative mutant of *E. histolytica* Sec22 (EhSec22) will be used to block the functions of the wild type protein. To verify the role of EhSec22 and the ER during phagocytosis, the *E. histolytica* cells will be fed beads of different sizes before assessing their phagocytic activity.