

Abstract

Amoebae of the genus *Acanthamoeba* spp. are free-living unicellular organisms found in disparate ecosystems all over the world. Due to their ability to invade human body, evade its defensive mechanisms and cause extensive tissue damage, *Acanthamoeba* infection can lead to serious, if rare, diseases, affecting most commonly the eye and the central nervous system. Specific therapy for *Acanthamoeba* infections is not available.

A major reason for therapeutic failure in amoebiasis is the ability of the protist to differentiate into resistant stages. These are *cysts*, known to be formed under prolonged unfavorable conditions, both in the environment and the infected tissues, and the *pseudocysts*, less durable but rapidly formed under acute stress. The present thesis focuses on as yet unexplored mechanisms of resistance of cysts and pseudocysts. Moreover, further characteristics distinguishing cysts and pseudocysts as well as the processes involved in their formation are investigated.

One of the issues addressed is a presence of protective carbohydrate compounds mannitol and trehalose that participate in defensive reactions against abiotic stress in many organisms. Although putative genes for enzymes of the trehalose and mannitol synthetic pathways are present in the genome of *Acanthamoeba*, only one of the two compounds, disaccharide trehalose, was found. Trehalose was identified not only in cysts and pseudocysts but also in growing trophozoites. In contrast, none of the life stages were shown to contain mannitol. Detailed analysis of the sequences of the enzymes of the trehalose synthetic pathways, trehalose phosphate synthase (TPS), trehalose phosphate phosphatase (TPP) and trehalose synthase (TS), revealed that the genome contains enzymes belonging to two distinct enzymatic pathways, one being of a prokaryotic origin. Quantitative RT-PCR demonstrated a significant difference in expression profiles of the synthetic pathway genes in cyst and pseudocyst. Genes of both synthetic pathways are involved in trehalose synthesis during pseudocyst formation and at higher level than during encystation. Amounts of trehalose are nevertheless increased during both stress defense reactions. The presence of trehalose in mature cysts and pseudocysts was also demonstrated using mass spectrometry.

Furthermore, the relationship between encystation and pseudocyst formation and the progress of the cell cycle was also studied. Phylogenetic analysis and classification of the main cell cycle regulators in the *Acanthamoeba* genome revealed presence of 14 genes of 9 types of cyclins and 6 genes of 3 types of cyclin-dependent kinases.

By using flow cytometry analysis we clearly distinguished cell populations with distinct DNA content, G1 cell cycle population and population with newly synthesized DNA, G2 population. Our results strongly indicate that *A. castellanii* enters encystation from the G2 phase of the cell cycle. In contrast, initiation of differentiation into pseudocysts is independent of the progression of the cell cycle. Nevertheless, DNA content in mature cysts and pseudocysts is the same, both resistant stages survive harsh environmental conditions with G2 phase DNA content. We also described the effect of DNA synthesis inhibitors aphidicolin and hydroxyurea on *Acanthamoeba* growth and cell cycle. Our data revealed that neither of the studied compounds synchronized *Acanthamoeba* cell populations on the G1/S boundary, in contrast to what was described in other protists as well as mammalian cell lines. Along with this, we did observe concentration dependent impact of hydroxyurea on *Acanthamoeba* trophozoites growth rate.