ABSTRACT

Entamoeba histolytica is the protozoan parasite responsible for amoebiasis in human. The life-cycle of *Entamoeba* involves two stages, motile trophozoite, and cyst. In this study, *Entamoeba invadens*, the reptilian parasite, has been used as *in vitro* encystation model of *E. histolytica*. Encystation occurs due to low glucose conditions and hypo-osmotic shock that results in a variety of cellular changes to adapt to the adverse condition of the surrounding. In our quest to find out the mechanism of cellular homeostasis in *E. invadens*, we identified an adenosine monophosphate-activated protein kinase (AMPK) like protein in *E. invadens* (EiAMPK). AMPK is a well-known critical energy sensor in eukaryotic cells maintaining cellular metabolism. Although AMPK has been studied in different multicellular organisms and other protozoan parasites, it is not yet identified in *Entamoeba*. Bioinformatic analysis revealed that EiAMPK has 54% and 53% similarity with the human AMPK alpha subunit1 and AMPK alpha subunit2 protein sequence. Both the recombinant EiAMPK and the immunoprecipitated endogenous EiAMPK was able to phosphorylate SAMS peptide, an AMPK specific substrate *in vitro*. AMP kinase activity was found to be inhibited and activated by Compound C, the specific inhibitor and the activator AICAR respectively. Studies like expression profile and nuclear localization studies revealed that EiAMPK is a stress-responsive gene. There was an increase in expression of EiAMPK during various stress conditions. Localization studies revealed that the nuclear localization of EiAMPK also increased during stresses. EiH2B, a nuclear substrate of EiAMPK was identified by performing an *in vitro* phosphorylation assay. EiH2B was found to contain a potential AMPK target motif where Ser36 is highly conserved. The downregulation of EiAMPK has a negative impact on the encystation process. It regulates the encystation process by reducing the encystation efficiency and hinders the formation of a proper chitin wall. Downregulating EiAMPK also inhibits other cell physiological effects like cell aggregation, actin polymerization, which are the prerequisites for proper encystation. Our study identifies and characterizes an AMPK like protein in *E. invadens* as a stress-responsive kinase and deciphers its role during encystation in *E. invadens*. This study will help to have a better understanding of the stage differentiation process of *Entamoeba*.