ABSTRACT

Entamoeba histolytica, the causative agent of human amoebiasis has a biphasic life cycle comprised of motile trophozoite which converts to infectious cyst in the large intestine of host by the process known as encystation. With a view to understand the early signaling events of encystation the *Entamoeba* genome was analyzed to identify key signaling molecules. Chitin biosynthesis along with a distinct transcriptional up-regulation of yeast- like putative carbon catabolite derepressing kinases (CCDK) along with yeast CCDK regulatory gene homologs in *Entamoeba* during glucose deprivation indicates the presence of encystation-specific carbon catabolite derepression kinase-mediated signal transduction. The ortholog of a kinase involved in cellular differentiation of *Dictyostelium* was found to be highly expressed in encysting *Entamoeba* (EIN_049520) and was named as *Entamoeba invadens* cyst-specific protein kinase-EiCSpk3. A common origin of ancestry and considerable similarity in sequence and structure of EiCSpk3 with the carbon catabolite derepressing kinases of *Entamoeba* and other organisms explored in-detail by bioinformatics analysis suggests a functional homology. The early encystation- specific mRNA expression profile and metabolic stress response of EiCSpk3 was identified by further functional characterization and was found to be similar to CCDKs. A typical serine/threonine-specific in-vitro kinase activity of EiCSpk3 was further confirmed by straurosporine inhibition assay. Steady state kinetics of EiCSpk3 was found to be similar to CCDKs. Localization of the kinase to the cytoplasm during early encystation predicts a role in intracellular signal transduction. Transcriptional inhibition of glucosamine: fructose -6-phosphate (GFAT), the first and rate limiting enzyme of chitin biosynthesis and meiosis initiator candidates IME and SPO in response to EiCSpk3 down-regulation by RNA interference in encysting *Entamoeba* further supports functional similarity with CCDKs. A probable existence of cellular signal transduction mediated by EiCSpk3-like carbon catabolite derepressing kinases underlying glucose-starved *Entamoeba* encystation has been proposed.

Key words: Amoebiasis, *Entamoeba histolytica*, *Entamoeba invadens*, Encystation, RNA interference, Chitin biosynthesis, Kinase, Signal transduction, Meiosis, Carbon catabolite derepressing kinase, Glucosamine: fructose- 6 – phosphate aminotransferase.