Expression of four chitinase genes of *Entamoeba invadens* differs in the encystation and excystation

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SUMMARY

Entamoeba histolytica (Eh) forms chitin-walled cysts in the encystation. The wall formation requires not only chitin synthase but also chitinase. In excystation, quadruplet metacytic amoebae emerge from the chitin-walled cysts by dissolving the wall. Consequently, chitinase function is necessary for both processes. In this study, we used *E. invadens* (Ei), a reptilian amoeba, as a model for encystation and excystation of Eh, and studied mRNA expression of chitinase (Chit) genes in those processes. Expression of three EiChits (EiChit1, EiChit2, and EiChit3) during encystation had been reported previously. We identified an additional enzyme designated as EiChit4 in the Ei genome database. We compared the primary structure and mRNA expression of these four Chits in excystation as well as the encystation using real-time RT-PCR. Like EiChit1, EiChit4 had an 8 x Cys chitin-binding domain (CBD) and Ser-rich spacer between CBD and catalytic domain, and was closer to EiChit1 than EiChit2 and EiChit3 in a phylogenetic tree. In trophozoites, all four Chits were little expressed. During encystation, the expression of all four EiChits increased remarkably in the early phase and then decreased in the later phase. In cysts, EiChit1 was expressed predominantly; EiChit4 was at small amounts, although EiChit2 and EiChit3 were expressed little. During excystation, as assessed 5 hr after the induction of excystation, the expression of EiChit1 and EiChit4 decreased drastically, while that of EiChit2 and EiChit3 increased. Furthermore, mRNA of only EiChit2 and EiChit3 increased remarkably when the excystation was induced in the presence of cytochalasin D. These data demonstrate different expressions of four Chit genes in the differentiation of Ei.