Expression analysis of cofilins in the excystation of *Entamoeba invadens*

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SUMMARY

Entamoeba hystolytica (Eh) cysts restore motility with the induction of excystation. The amoeba moves out, passing through a small hole made on the cyst wall. Reactivation of motility and its control by actin cytoskeletal reorganization are necessary processes in excystation. This study investigated important molecules in actin cytoskeletal reorganization: actin depolymerizing factor cofilin (Cfl). In addition, *E. invadens* (Ei) was used as the excystation and development model of Eh. For this, the cysts formed in an encystation medium were transferred into a trophozoite culture medium to induce excystation. A search of the Eh and Ei genome database revealed one type for Eh (EhCfl) and three types for Ei (EiCfl-1, Cfl-2, and Cfl-3). Phylogenetic analysis revealed that *Entamoeba* Cfl formed a clade that is separate from other organisms. Immunofluorescence staining using rabbit anti-EiCfl-2 antibody and mouse monoclonal anti-actin (Act) antibody revealed that both EiCfl and EiAct proteins of trophozoites were localized immediately beneath the cell membrane. In particular, staining in pseudopodia was intense for both EiCfl and EiAct, suggesting strong implications related to amoeba motility. Actually, EiCfl and EiAct were also localized around the area immediately below the cell membrane in cysts. Real-time RT-PCR revealed that Cfl mRNA expression in cysts was remarkably lower than that of trophozoites, and that the expression of EiCfl-1 and Cfl-3 was nearly absent even in trophozoites. Higher mRNA levels were observed in all EiCfl proteins 5 hr after excystation than those observed before excystation. Furthermore, remarkably increased mRNA levels of EiCfl-1 and Cfl-3 but not Cfl-2 were observed in the presence of cytochalasin D, by which enhanced excystation was previously reported by us. These findings demonstrate increased EiCfl expression by excystation induction, EiCfl colocalized with EiAct, and close correlation between EiCfl expression and amoeba motility. In addition, enhanced excystation by cytochalasin D was closely associated with highly increased mRNA levels of EiCfl-1 and Cfl-3.