## Abstract

The life cycle of *Entamoeba* consists of highly motile trophozoites and non-motile, chitin walled dormant cysts. The conversion of trophozoites into cysts is known as encystation. The study of encystation is of public health importance since the cysts are responsible for transmission of the parasite. Here we studied the developmental events during encystation using the *in vitro* encystation model *Entamoeba invadens*. The encystation process starts with the formation of multicellular aggregates inside which cysts are formed. Time-lapse imaging of early encystation showed that *E. invadens* assumed a highly motile, stable bleb driven polarized morphology in the encystation media. The polarized morphology was found to be highly chemotactic and aggregation competent, that indicates the aggregate formation may be mediated by chemotaxis. Adenosine receptor antagonist, pentoxifylline was able to replicate the polarization, fast motility, and aggregation even in the growth medium which led to the identification of purinergic signaling that controls the morphology and motility of *Entamoeba.* Inside cell aggregates, chitin is deposited on the cell surface to make the cyst wall. During the early hours of encystation, a difference was observed in the number of the detergent resistance cells and chitin positive cells. This may to be due to the presence of cysts with incomplete chitin wall which could be intermediate stages of cyst wall deposition. Staining these cells for known cyst wall components showed that chitin wall formation started from a single point and eventually spread over the whole surface. In the encysting cells, the actomyosin cytoskeleton was reorganized into the cortical region, and it was found to be required for the proper deposition of chitin by anchoring the chitin synthase on the cell surface. Apart from the cyst, a rare multinucleate stage was found inside the cell aggregates, formed by continuous cell fusion and cytofission. Analysis of the changes in genomic content indicated cell fusion was followed by haploidization producing haploid nuclei. These nuclei later aggregated and fused to form a polyploid nucleus which could cause outcrossing in *E. invadens.* The presence of cell fusion, haploidisation, and nuclear fusion shows that MGC could be the sexual or parasexual stage of *E. invadens*. These results show that the encystation is not just the differentiation of trophozoite into the cyst, but a complex process involving intercellular communications, intracellular rearrangements, and genetic exchanges.