# Characterization of the dynamin family in the human intestinal parasite *Entamoeba histolytica*

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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## Abstract

*Entamoeba histolytica* is an important human intestinal parasite that has a major impact on human health and is responsible for approximately 100,000 deaths each year. *Entamoeba histolytica* is one of several known eukaryotes that harbour strongly reduced mitochondria, called mitosomes, which have lost the vast majority of mitochondrial pathways as well as their organellar genome. While the occurrence and function of mitosomes have been well studied, little is known about their inheritance and division. Mitochondrial division in all studied eukaryotes relies on the participation of dynamin proteins for membrane scission. The central aim of this study was to characterize the dynamin protein family in *Entamoeba histolytica* and to analyze if they participate in mitosomal division. In relation to this work we studied the occurrence of mitosomes in the distantly related reptilian parasite *Entamoeba invadens* and revisited the phylogenetic relationships among mitosomal Hsp70, a protein we used for mitosomal localization experiments. Our studies revealed that *Entamoeba histolytica* contains two classical and two strongly derived members of the dynamin protein family, which we called Drp1, Drp2, Drp3 and Drp4. Drp1 and Drp2 exhibit the classical dynamin protein structure with a GTPase, middle and GTPase effector domain, while Drp3 and Drp4 only appear to contain the dynamin GTPase domain. Using phylogenetic reconstructions we could not identify closely, and thus functionally related, dynamins for Drp1 and Drp2 within the eukaryotic tree of life including the mitochondria-associated amoebozoan dynamins DymA and DymB. The structurally derived dynamins however, were closely related to amoebozoan and archaeplastidan proteins involved in cytokinesis and chloroplast division. All *Entamoeba* dynamins are differentially expressed in trophozoites with EhDrp2 appearing to be most abundant and Drp3 expressed the least. We conducted stage conversion experiments using *E. invadens* to understand the importance of dynamins during cyst formation. During encystation all dynamin expression levels increased. Interestingly, Drp3 expression is strongly upregulated in the mid cyst stages and Drp4 during the late phase of encystation. Thus, Drp3 and Drp4 appear not to be involved in cytokinesis and possibly evolved a novel function in the cyst formation process. We carried out Drp2 enzymatic characterization and localization experiments as well as

complementation studies using the related amoebozoan *Dictyostelium discoideum* in order to understand the role and function of *E. histolytica* Drp2 in the cell. We found that its kinetic characteristics are comparable to other members of the eukaryotic dynamin protein family by exhibiting low substrate specificity, the ability to oligomerize to higher structures and a substrate dependent cooperative enzyme activity. Drp2 localized to abundant punctate structures in the cytosol but did not co-localize with mitosomes. In addition, Drp2 was not able to complement *D. discoideum* DymA. Both findings suggest that Drp2 is not directly involved in mitosomal (or mitochondrial) division. We overexpressed Drp2 in *E. histolytica* and *D. discoideum* and found a significant effect on cytoskeletal organization. Both strains showed a strong impairment in amoeboid movement, cell-surface attachment and cell growth. Additionally, the number of nuclei was increased significantly. Our data imply that Drp2 plays an important role for cytoskeletal organization. Additionally in this study, we show that mitosomes are also abundantly present in *E. invadens* suggesting that mitosomes are characteristic for all *Entamoeba* spp.. Furthermore, we demonstrate that *E. invadens* cysts contain mitosomes in high abundance comparable to its vegetative life stage. Our studies verify that mitosomal Hsp70 is part of the amoebozoan protein family and of mitochondrial origin as shown by *in silico* characterization and localization experiments using the homologous Hsp70 antibody.

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