A lineage-specific mitosomal membrane protein possibly involved in vacuole-mitosome contact in *Entamoeba histolytica*

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The anaerobic protozoan parasite *Entamoeba histolytica* is the etiological agent of amoebiasis, a disease characterized by diarrhea and dysentery, which could lead to extra-intestinal abscess formation in the liver, lungs, and brain. This organism lacks mitochondria and instead possesses a highly degenerate and divergent mitochondrion-related organelle (MRO) called mitosome. Mitochondria and MROs are bound by a double-membrane barrier, lined with various proteins that allow for protein and metabolite transport, lipid transfer, signaling, dynamics and inter- and intra-organelle crosstalk. However, only a few protein homologs have been identified in the outer and inner membranes of the *E. histolytica* mitosome, suggesting that the mitosomal membranes are equipped with components that may have been modified by unique and possibly lineage-specific elements. Using a specialized prediction pipeline, we searched for proteins possessing α-helical transmembrane domains that are unique to *E. histolytica* mitosomes. A total of 25 candidate proteins emerged and following screening by localization analysis, we confirmed three novel ***E****ntamoeba*-specific **t**ransmembrane **m**itosomal **p**roteins (ETMPs). ETMP1 was predicted to have one transmembrane domain and two coiled-coil regions by *in silico* analyses. It was demonstrated to be integrated to the mitosomal membranes based on carbonate fractionation and immunogold analysis. ETMP1 forms a 180 kDa complex and immunoprecipitation analysis detected a candidate interacting partner that is localized to vacuolar and vesicular membranes. We expressed this ETMP1-binding partner and subsequent immunofluorescence and immunoelectron microscopy data demonstrated an unprecedented vacuole-mitosome contact.