

Evolution of the endolysosomal ESCRT sub-complexes in the parasitic *Giardia intestinalis* and its fornicate relatives.

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Diplomonad protist parasites such as *Giardia intestinalis* and *Spironucleus salmonicida* are well known for causing significant healthcare and agricultural burden across the globe. Owing to both their parasitic lifestyles and phylogenetic position as Excavates, diplomonads in general have undergone tremendous divergence and reductions within their membrane trafficking components including in the endolysosomal organelles. In order to understand the evolution within this pathway, we identified and characterized the associated protein machinery, the ESCRT sub-complexes which are involved in the formation of multivesicular bodies (MVBs) containing ubiquitin-tagged cargo targeted for lysosomal mediated degradation, in both the basal free-living fornicate, *Carpodemonas membranifera*, and in parasitic diplomonads. Identification of the five sub-complexes was carried out by distant homology searching methods while characterized paralagous subunits were confirmed and sorted into specific protein subfamilies using phylogenetics. Overall we observed a trend of loss, specifically within the ubiquitin binding components such as TOM1, ESCRTI, and ESCRTII-VPS36 as a transition from free-living lifestyle to parasitism occurred. Because these components are necessary for the initiation of MVB formation and cargo recognition, we hypothesize that a loss in the canonical organelle must also have occurred within all diplomonads and that other mechanisms to carry out its role must be in place. A newly identified candidate protein in *Giardia*, a divergent CHMP7 homolog, provides one such potential example. Overall, these findings illuminate the dispensable nature of what are presumed to be important membrane trafficking organelles and therefore provide additional insight into protist parasite evolution within the metamonads.