

Lysosomal protein trafficking in *Giardia lamblia*: common and distinct features

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1. ABSTRACT

Giardia is a flagellated protozoan parasite that has to face different microenvironments during its life cycle in order to survive. All cells exchange materials with the extracellular medium through the reciprocal processes of endocytosis and secretion. Unlike more evolved cells, *Giardia* lacks a defined endosomal/lysosomal system, but instead possesses peripheral vacuoles that play roles in endocytosis, degradation, recycling, and secretion of proteins during growth and differentiation of the parasite. This review focuses on recent reports defining the role of different molecules involved in protein trafficking to the peripheral vacuoles, and discusses possible mechanisms of receptor recycling. Since *Giardia* is an early-branching protist, the study of this parasite may lead to a clearer understanding of the minimal machinery required for protein transport in eukaryotic cells.

2. INTRODUCTION

Giardia was initially described by van Leeuwenhoek in 1681 as he examined his own diarrheal stools under the microscope (1), but it was not until 1981 that the World Health Organization classified *Giardia* as a human pathogen. This unicellular parasite commonly causes diarrheal disease all over the world. Infections initiate with the ingestion of the encysted form of the parasite, which eventually ‘excysts’ in the upper small intestine of the host (Figure 1). The emerging trophozoites replicate and colonize the intestinal surface, and some of them encyst in the lower small intestine after sensing a specific stimulus (2,3) (Figure 1). In the course of encystation and excystation, trophozoites undergo biochemical and morphological modifications involving the secretory machinery of the cell. Recent studies on these changes have provided new insights into the mechanisms of

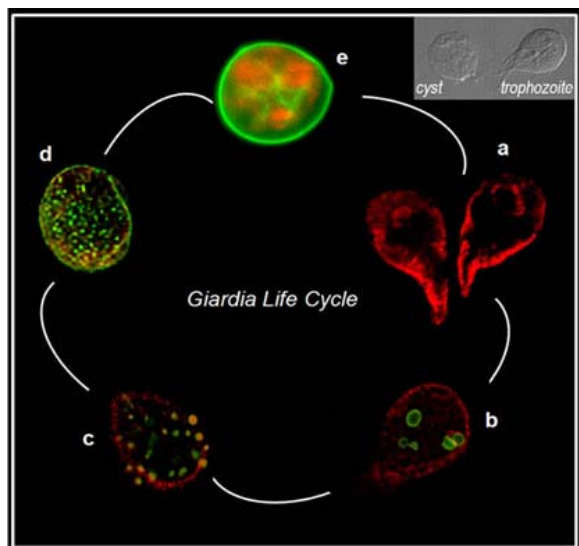


Figure 1. *Giardia* Life Cycle. The life cycle of this parasite involves only two stages: the cyst and the trophozoite (insert). *Giardia* infection occurs by the ingestion of infectious cysts from contaminated water or food. In the small intestine, excystation releases trophozoites that multiply by longitudinal binary fission. The trophozoites remain in the lumen of the proximal small bowel where they can be free or attached to the mucosa by a ventral sucking disk, causing the symptoms of the disease. Differentiation occurs when the parasites transit toward the colon and sense the stimulus for encystation. The earliest morphological change observed in encysting trophozoites is the appearance of large secretory granules, called encystation-specific secretory vesicles (ESVs), which transport cyst wall materials for subsequent release and extracellular assembly of the rigid cyst wall that protects the parasite outside its host. Direct immunofluorescence assays and confocal microscopy show adaptor protein 2 (AP-2, red) and cyst wall protein 1 (CWP1, green) at different time-points during stage differentiation. In growing trophozoites, AP-2 localizes in the lysosome-like peripheral vacuoles (PVs), while CWP1 is absent (a). At the beginning of encystation, AP-2 still localizes in the PVs and CWP1 in the ESVs. Later, AP-2 is seen surrounding ESVs, colocalizing near the plasma membrane (b). In the last step, ESVs containing CWP1 spread into small vesicles and partially colocalize with AP-2 (c). In the cyst forms (d), CWP1 is observed forming the cyst wall, while AP-2 remains inside.

secretion in this organism, but the molecular events that mediate intracellular protein trafficking and secretion in *Giardia* remain poorly understood or controversial (4).

Although once considered the 'missing link' between prokaryotes and eukaryotes, *Giardia* is an eukaryotic cell, since it possesses two nuclei with nuclear membranes, an endomembrane system consisting of the endoplasmic reticulum (ER) and endosomal/lysosomal vacuoles (named peripheral vacuoles or PVs), and a complex cytoskeleton (2). Strikingly, *Giardia* lacks other organelles that are characteristic of higher eukaryotes, such

as distinct early and late endosomes, lysosomes, and a Golgi apparatus, although protein sorting events associated with these organelles and lysosomal activity do occur in this parasite (reviewed by (4, 5)). Analysis of genes and proteins used for phylogenetic classification indicate that *Giardia* is indeed one of the earliest branching eukaryotes (6, 7), but some of the particular characteristics of this organism are probably a result of the secondary loss of complex cell structures due to its parasitic life style, rather than the primitive simplicity assumed for early diverging protists (4, 8). For these reasons, *Giardia* is a useful system to study not only the evolution of the endocytic and secretory pathways in eukaryotes but also how an organism may lose 'essential' subcellular organelles when becoming a parasite, while maintaining the minimal machinery necessary for fundamental cellular functions.

Although *Giardia* lacks a distinctive Golgi apparatus, it possesses perinuclear structures where the functions of the ER and Golgi co-localize spatially and temporally (i.e., ER-exist sites) (9). Similarly, the functions of early/late endosomes and lysosomes appear to be fulfilled by the PVs (10). Indeed, exogenous ferritin and Lucifer yellow are taken up into PVs (10, 11), and pulse-chase uptake experiments with horseradish peroxidase and fluorescent dextran show an early and persistent labeling of the PVs, indicating that these organelles play the role of both early and late endosomes in *Giardia* (10, 12). PVs have also been shown to be acidic, as evidenced by the uptake of acridine orange and the lysosomal markers LysoSensor and LysoTracker (10, 13, 14). Finally, PVs contain hydrolase activities, including acid phosphatase (AcPh), cysteine protease, and RNase activities, demonstrating their lysosomal character (13, 15-17).

A defining feature of eukaryotic cells is the compartmentalization of their cytoplasm into distinct membrane-bound organelles. As cellular components became more complex and abundant during evolution, subcellular compartmentalization developed as an essential means of preventing the inappropriate meeting of certain intracellular components, as well as facilitating efficient, ordered reactions (18). A key mechanism for the establishment and maintenance of subcellular compartmentalization is the formation of membrane-bound transport vesicles that assemble at the cytosolic surface of a donor compartment to deliver specific cargo molecules to an appropriate acceptor compartment. Clathrin-coated vesicles (CCVs) are the best-characterized type of transport vesicles that mediate cargo delivery to endosomal/lysosomal compartments in all eukaryotic cells. These vesicles are covered by a coat that is composed of the scaffolding protein, clathrin, and various oligomeric and monomeric adaptor proteins (APs). The APs recognize specific sorting signals in the cytosolic domains of transmembrane proteins, leading to the concentration of these proteins within CCVs. Many of these transmembrane proteins in turn act as receptors for the sorting of soluble, extracellular or luminal cargos that bind to them. Four structurally-related, heterotetrameric AP complexes named AP-1, AP-2, AP-3 and AP-4 have been shown to play roles in sorting transmembrane cargoes to endosomal/lysosomal

compartments in a variety of eukaryotes. AP-1, AP-2 and AP-3 function as clathrin adaptors, whereas AP-4 is likely a component of a non-clathrin coat. It is well-established that AP-2 mediates endocytosis from the plasma membrane, while AP-1, AP-3 and AP-4 participate in protein sorting at the *trans*-Golgi network (TGN) and/or endosomes. Orthologs of clathrin, AP-1 and AP-2 subunits are encoded in the *Giardia* genome (GDB, <http://www.Giardadb.org/Giardadb/>) (19). Moreover, AP-2 has been shown to participate in receptor-mediated endocytosis (RME) into PVs while AP-1 mediates delivery of hydrolytic enzymes to the PVs of *Giardia* (20). *Giardia* has also been found to encode orthologs of the subunits of the 'retromer' complex, another putative coat involved in endosomal-lysosomal sorting processes in yeast and mammals (21). These findings indicate that *Giardia* contains a conserved, yet simplified version of the coat-mediated sorting machinery present in other eukaryotes.

3. ENDOSOMAL-LYSOSOMAL PROTEIN TRAFFICKING

3.1. Clathrin and adaptor proteins

Clathrin is a heterohexameric complex composed of three clathrin heavy chains of ~190 kDa and three clathrin light chains of 23 or 25 kDa termed 'triskelion' that associates with the cytosolic side of transport vesicles emerging from the plasma membrane, TGN and endosomes (22). The unusual geometry of the triskelion allows it to assemble into regular polyhedral structures, the 'clathrin coats', which eventually give rise to CCVs. The dense protein coat of the CCV and its bristle-like morphology were first described by Roth & Porter (1964), who noted the involvement of these vesicles in RME of yolk proteins in mosquito oocytes (23). Clathrin was identified as one of the major coat proteins of CCVs by Pearse (1975), and the clathrate or lattice-like appearance of assembled clathrin was recognized in the naming of the protein (24). The formation of CCVs follows a sequence of coordinated steps, in which membrane invagination is coupled to growth of the clathrin lattice, leading to lattice closure and vesicle budding (25). More recently, a different type of clathrin-coated transport carriers (TCs), consisting of larger tubular/vesicular structures having one or more clathrin-coated buds, were identified (26). These TCs travel long distances from the juxtanuclear area of the cell until they fuse with peripheral endosomes. The function of the TCs might be to mediate long-range distribution of mannose 6-phosphate receptors (MPR) and their cargo hydrolases to the peripheral cytoplasm (27).

The *Giardia* genome encodes an ortholog of the clathrin heavy chain (GiCLH) (28), with the open reading frame encoding an 1871-amino acid protein, with three C-terminal clathrin repeats and one N-terminal propeller, according to a protein family database (29). Perhaps because clathrin light chains share very low sequence homology between species, the identification of a clathrin light chain ortholog in the GDB has so far been unsuccessful. Analysis of GiCLH expression showed that clathrin is expressed almost equally in both stages of the parasite and is located in close association with the PVs in

trophozoites, and in the encystation-specific vesicles (ESVs) in immature cysts (5, 12, 30, 31). On the basis of these observations, it was suggested that recruitment of clathrin to late ESVs could serve to disperse large ESVs into smaller transport vesicles in response to the secretion signal (see below).

Several groups have presented evidence for a role of clathrin in endocytosis in *Giardia* (12, 32-34). However, neither typical membrane-associated clathrin lattices nor emerging clathrin-coated pits have been observed in this parasite. Instead, uncharacteristic coated pits were seen in close association with the PVs (10), suggesting that a distinct arrangement of clathrin might occur in this parasite. It is also possible that, as was observed *in vitro* (35), clathrin may be organized in a hexagonal array, forming tubes instead of vesicles. Similar to TCs in HeLa cells (26), the tubules might not break down into CCVs en route to PVs in *Giardia*. In mammalian cells, it has been shown that TCs contain MPRs, clathrin, Golgi-localizing, Gamma-ear containing, ARF-binding proteins (GGAs), and/or AP-1, and it was suggested that these might be uncoated during the TC-endosome fusion or could become integrated into the endosome membrane (26). In possible agreement with this hypothesis, *Giardia* clathrin and AP-1 were observed not only on ER-exit sites but also PVs (12, 30, 31, 33).

An unusual characteristic of the *Giardia* secretory pathway is that the ER tubular-vesicular network apparently extends to and contacts the PVs in the periphery of the cell (36). However, it was recently reported that no ER membranes invade the space occupied by PVs (37). An explanation that reconciles these observations might be that at least some of the clathrin-dependent trafficking in *Giardia* involves tubular carriers that extend from the ER-exit sites to the peripheral cytoplasm until they meet with distally located PVs.

Because clathrin has no affinity for biological membranes, its recruitment to membranes and capture of transmembrane cargo requires the action of clathrin-associated adaptor proteins (AP), which bind to clathrin through the amino-terminal domain of the clathrin heavy chain (38). Among these adaptors are AP-1, AP-2 and AP-3, which comprise two large chains (one each of $\gamma/\alpha/\delta$ and $\beta 1-3$, respectively), one medium-sized chain ($\mu 1-3$), and one small chain ($\sigma 1-3$) (39). These complexes are localized to different subcellular compartments, where they function in cargo selection (39). At least one of the large subunits in each AP complex ($\gamma/\alpha/\delta$) mediates binding to the target membrane. The other large subunit, $\beta 1-3$, recruits clathrin through a 'clathrin-box' motif (40, 41). The $\mu 1-3$ subunits are involved in the recognition of tyrosine-based, YXX \emptyset signals (where X represents any amino acid and \emptyset indicates a residue with a bulky hydrophobic side chain), and combinations of $\alpha\sigma 2$, $\gamma\sigma 1$ and $\delta\sigma 3$ recognize dileucine-based, (DE)XXXI (LI) signals (38, 42). A fourth AP complex, AP-4, is thought to be a component of a non-clathrin coat and to recognize a different type of signal (43). Besides the putative GiCLH, orthologs of two large, one medium, and one small subunit of each AP-1 and AP-2

are present in the *Giardia* genome. The colocalization of AP-1 with lysosomal proteins, its interaction with the GiCLH, together with the observation that lysosomal protein trafficking is altered in $\mu 1$ -depleted trophozoites, support the participation of this complex in the forward transport of proteins towards the PVs in *Giardia* (33). AP-1 also plays a central role during parasite differentiation, since $\mu 1$ depletion impairs encystation (33). On the other hand, AP-2 is localized to the PVs and plasma membrane in trophozoites and also neighboring the ESV in encysting cells (34). AP-2 participates in RME and is crucial in the internalization of lipoproteins (34). Although the $\beta 1$ -2 and $\mu 2$ mRNA transcripts change little during the completion of the cell cycle (31, 34), the role of the corresponding AP complexes appears essential for the adaptation of the parasite. AP-1 is not critical for *Giardia* trophozoite survival and multiplication, but it is necessary for cyst formation, acting indirectly in this process by transporting a transmembrane protein to the PVs (13, 14, 33). In contrast, AP-2 is essential for *Giardia* growth and survival, being involved in the endocytosis of essential molecules (e.g., exogenous lipids) (34) and in the fragmentation of ESVs into small transport vesicles containing cyst wall proteins during encystation (Figure 1).

The fast secretion and deposition of cyst wall material has been reported to involve in clathrin- and dynamin-dependent breakup of ESVs into small vesicles targeted for the plasma membrane (5, 12). It is possible that this parasite requires the concerted action of clathrin and adaptors as well as accessory proteins at the time of cyst wall formation.

Taken together, these results support the hypothesis that *Giardia* possesses molecular mechanisms for lysosomal protein trafficking involving adaptor proteins similar to those of other eukaryotes. AP-1 and AP-2 appear to be the only two adaptors involved in lysosomal protein trafficking in *Giardia*, since there is no evidence of the participation of other adaptor proteins such as AP-3, AP-4, and monomeric adaptors (i.e., the GGAs). It has been suggested that the two prototypic *Giardia* AP complexes predict the point of separation of *Giardia* after the first coordinated round of gene duplications, resulting in an AP-3 and an AP-1/2/4 ancestor (31). Phylogenetic reconstruction from comparative genomics has shown that all four AP complexes were present in the Last Common Eukaryotic Ancestor (LCEA), as was the F-COP subcomplex (41). However, the GGAs, which also exhibit homology to the ear region of the AP-1 γ protein are restricted to animal and fungal lineages (44). Therefore, individuality of the species lineage and secondary loss are common characteristics in the evolutionary history of the adaptins. Secondary losses of adaptors can be observed in *Drosophila melanogaster*, *Caenorhabditis elegans*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* which lack the AP-4 complex (44). In addition, comparative genomic and phylogenetic analyses of protozoan parasites have shown loss of the AP-3 complex in *Theileria* species, *Cryptosporidium parvum*, and *Babesia bovis*, while *Trypanosoma brucei* and *Leishmania major* lack AP-2 and AP-4, respectively (45). Thus, examination

of the role of the reduced set of AP complexes in protozoa provides insight deep into our cellular history and highlights the importance of essential cell biology adaptations of the ancestral cellular organization.

3.2. Lysosomal proteins

Soluble acid hydrolases destined for lysosomes are synthesized in the ER and transported to the Golgi complex, where their carbohydrate chains are modified by resident enzymes. In mammalian cells, the hydrolases are modified with mannose 6-phosphate residues that function as recognition signals for MPRs in late Golgi compartments. In yeast, the vacuolar hydrolases lack mannose 6-phosphate, and the sorting receptor is the product of the VPS10 (Vacuolar Protein Sorting 10) gene. In both cases, however, sorting signals present in the cytosolic tails of the receptors interact with clathrin adaptors and direct packaging of the hydrolase-receptor complexes within CCVs or clathrin-coated TCs (46, 47). Recently, multi-ligand type-1 receptors Sortilin, SorCS1, SorCS2, SorCS3, and SorLA, containing an N-terminal Vps10p domain, were discovered (48, 49). These are transmembrane proteins that convey Golgi-endosome transport and bind a number of unrelated ligands.

In *Giardia*, high hydrolase activity in the PVs has been implicated in protein degradation during growth (4), encystation (14, 50) and excystation (51). A family of three cysteine protease genes (CP1, CP2, and CP3) has been shown to encode members of the cathepsin B subgroup of the peptidase family C1 (17), and soluble CP2 has been found in PVs and ER of trophozoites (17, 52). Also, AcPh activity has been examined cytochemically, revealing communication of the PVs with the ER (10, 15, 53). Unlike AcPh in other eukaryotes, including protozoa, *Giardia* AcPh is a soluble protein that is transported from the ER-exit site to the PVs via AP-1 (33). It is thus possible that a specific receptor, possessing a function similar to the MPR or Vps10p, is involved in the trafficking of soluble hydrolases toward the PVs. Recent studies have identified a type-I membrane protein that interacts with AcPh and contains an YXX Φ -type motif in its cytosolic tail (Rivero & Touz, unpublished). *In silico* analysis revealed that this protein (GDB: GL50803_28954) might be orthologous to the Vps10p receptor. Further biochemical studies on this putative receptor in both vegetative and encysting trophozoites and its participation in hydrolase delivery are necessary to elucidate the exact function of this protein.

Comparative analysis of lysosomal proteins present in *Giardia* and other cells reveals some intriguing differences. For instance, AcPh is soluble in *Giardia* but exists as a type-I membrane protein containing a YXX Φ -type internalization sequence in cells as different as *Leishmania* and humans (54-56), with transport to the lysosome occurring through several cycles of plasma membrane internalization and recycling. In the lysosome of mammalian cells, the luminal domain of AcPh is processed and released in soluble form (57). Moreover, while the AcPh tail interacts with AP-2 in these cells, the lysosomal traffic of *Giardia* AcPh depends on AP-1 (33). Because

much of the machinery involved in lysosomal trafficking is derived from a few protein families (where the various family members perform the same basic mechanistic function), the analysis of the similarities and differences between organisms might provide further insight into eukaryotic cell evolution.

Like the MPRs, integral membrane proteins (e.g., LAMP/LIMP family proteins) are transported to lysosomes by binding of their cytosolic motifs to AP complexes (58). The carboxy-terminal β -sandwich domain of the μ subunits of AP-1, AP-2, and AP-3 binds directly to YXX Φ -type sequences while the $\gamma\sigma_1$, $\alpha\sigma_2$ and $\delta\sigma_3$ hemicomplexes bind to the (DE)XXXL (LI) sequences (59, 60). Although lysosomal integral membrane glycoproteins have not been identified in *Giardia*, it was reported that a cysteine protease termed ESCP (encystation-specific cysteine protease) is transported to the PVs through a tyrosine-based motif. This enzyme is homologous to cathepsin C enzymes of higher eukaryotes and possesses a transmembrane domain and a YRPI motif within the cytoplasmic tail. ESCP localizes to the PVs in growing trophozoites and also to the plasma membrane in encysting cells (14). Deletion of the YRPI motif or suppression of μ 1 mislocalizes this protein to the plasma membrane or to the ER-exit sites, respectively (14, 33).

More recent studies have shown that AP-2 participates in endocytosis of the *Giardia* Low-density lipoprotein Receptor-related Protein or LRP (34). *Giardia* LRP is a type-I membrane protein, which shares the substrate-N-terminal binding domain and a FXNPXY-type endocytic motif with human LRP1. This receptor localizes predominantly to the ER but is also found in the PVs and plasma membrane in *Giardia*, and internalizes both low density lipoproteins (LDL) and chylomicrons as shown by *in vitro* studies. The FXNPXY motif of LRP was shown to bind directly to the μ 2 subunit of AP-2, with this interaction being necessary for its proper localization, processing, and function.

One common characteristic of LDLR family members like the LRPs is that they have at least one copy of the FXNPXY-like sequence in their cytosolic tail, which serves as the signal for endocytosis or as a binding element for adaptor proteins involved in signal transduction (61). In other eukaryotes, FXNPXY signals are recognized by the adaptor proteins Disabled homolog 2 (Dab2) and Autosomal Recessive Hypercholesterolemia (ARH), which contain a phosphotyrosine-binding (PTB) domain (62). However, no PTB-containing proteins such as Dab2 or ARH are encoded in the *Giardia* genome, supporting the idea that AP-2 might be the key endocytic adaptor in this parasite. Indeed, it has been shown by surface plasmon resonance and photoaffinity labeling that the FXNPXY-like motif binds to μ 2 purified from bovine-brain-coated vesicles (63). Thus, the importance of the availability of intracellular adaptor proteins might determine the specific cellular function of lipoprotein receptors. Since *Giardia* trophozoites do not have the capacity of *de novo* synthesis of cholesterol, its acquisition may depend on the internalization of chylomicrons from the host intestine by LRP. Moreover, because the trophozoites normally thrive in an environment where they never come in contact with

LDL, it is possible that the binding of LDL to LRP represents an adaptation of the parasite to the culture medium.

3.2. Retromer complex and accessory proteins

3.2.1. Retromer complex

Another component of the endosomal-lysosomal transport machinery is the 'retromer', a peripheral membrane protein complex that has important roles in endosomal sorting of a variety of cargo molecules. Retromer was first shown to control the endosome-to-Golgi retrieval of vacuolar/lysosomal hydrolase receptors in yeast and humans, and over the past few years, it has been found to play a similar role in the transport of many other proteins in all eukaryotes from simple amoebas to plants and mammals (64, 65). In yeast, endosome-to-Golgi retrieval of the carboxypeptidase Y receptor, Vps10p, is mediated by a retromer that comprises five conserved proteins: Vps35p, Vps29p, Vps26p, Vps5p, and Vps17p. Cargo molecules such as Vps10p, are thought to be recognized by the Vps35p component, which together with Vps26p and Vps29p, forms the cargo-selective subcomplex. Vps5p forms another subcomplex with Vps17p, which regulates recruitment to membranes (66). Similarly, it was shown that a mammalian retromer complex participates in the retrieval of the cation-independent MPR (CI-MPR). Also in this case, retromer assembly and function involves the cooperation of a Vps35-Vps29-Vps26 subcomplex and a sorting nexin (SNX) dimer assembled from SNX1/SNX2 and SNX5/SNX6 subunit isoforms, which are homologous to Vps5p and Vps17p, respectively (65, 67, 68). Failure to retrieve the receptor results in its rapid degradation, leading to missorting of vacuolar/lysosomal hydrolases (65).

Searching the GDB reveals homologs of Vps35, Vps29, Vps26, and SNXs (GL50803_23833, GL50803_103855, GL50803_100864, and GL50803_16548/ GL50803_24488, respectively) (69) (Table 1). Consistent with the existence of retromer subunits in *Giardia*, protein interaction analyses showed that the Vps35 homolog directly interacts with the Vps10p-like receptor (gVps10p) (Rivero & Touz, unpublished). It is tempting to speculate that gVps10p transports AcPh (and other soluble hydrolases) to the PVs, and is then recycled back by directly attaching itself to Vps35 and the retromer complex in *Giardia*. Theoretical analyses of the Vps core of the retromer complex in *Giardia* have revealed unexpected similarities between this complex and the retromer core from human. For instances, the modeled structure of the *Giardia* Vps26 was found to share a striking similarity to molecules of the arrestin family with this fold consisting of two related β -sandwich subdomains. Also, the predicted structure of the *Giardia* small subunit Vps29 revealed a globular α/β topology with a central β -sandwich surrounded by α -helices, while the C-terminal fragment of Vps35 possesses a curved all α -helical structure (Figure 2). It remains to be elucidated how this subcomplex is formed and whether it is able to interact with other proteins. Although retromer is structurally conserved in *Giardia*, these investigations might shed light on retromer adaptation since this parasite lacks distinct endosomes and Golgi complex.

Table 1. Retromer complex subunits of yeast, mammalian cells, and *Giardia*

Yeast			Mammalian			<i>Giardia</i>		
Protein	MW kDa	Domains/folds	Protein	MW kDa	Domains/folds	Protein	MW kDa	Domains/folds
Vps35p	109		mVps35	92	α -solenoid	gVps35	87	α -solenoid
Vps29p	31		mVps29	20	metallo-phosphoesterase	gVps29	48	-
Vps26p	43		mVps26	38	arrestin	gVps26	57	arrestin
Vps5p	76	PX-BAR	SNX1/SNX2	59	PX-BAR	gSNX1?	27	PX
Vps17p	63	PX-BAR	SNX5/SNX6 (functional orthologs)	59	PX-BAR			

Comparative analysis of homologous subunits of the retromer complex in eukaryotic cells is detailed. ^a*Giardia* database version 2.2 (<http://giardiadb.org/giardia/>)

3.2.2. Dynamin

The GTPase dynamin plays an essential role in clathrin-mediated endocytosis (70). Additionally, dynamin and dynamin-related proteins have been implicated in such varied cellular processes as organelle biogenesis (e.g., mitochondria), membrane ruffling, actin regulation, nitric oxide production, and clathrin-independent endocytosis (71-74). Substantial evidence indicates that dynamin oligomerization around the necks of endocytosing vesicles and subsequent dynamin-catalyzed GTP hydrolysis are responsible for membrane fission (75).

Mammalian dynamins 1, 2 and 3 are the founder members of the dynamin family that, along with other large GTPases, possesses five identifiable domains: GTPase domain, middle domain, a lipid binding Pleckstrin-homology (PH) domain, GTPase Effector domain (GED) and C-terminal proline-arginine rich domain (PRD) (76). Recent studies indicate that *Giardia* possesses a single dynamin homolog (GIDRP, dynamin-related protein), with the predicted protein containing the N-terminal GTPase domain (33–219), the middle domain (230–523) and a C-terminal GED (628–719) (31). The *Giardia* dynamin (GIDRP, dynamin-related protein), has a PRD of 70 amino acids (538–608) (12). While the PRDs of dynamins are normally localized at the C-terminus, after the GED, in other eukaryotes, the PDR of the *Giardia* dynamin is inserted between the middle domain and the GED. Interestingly, a typical PH domain that could mediate direct interaction with membrane lipids is missing.

GIDRP partially colocalizes with clathrin at the PVs and is necessary for endocytosis of plasma membrane proteins but not for fluid-phase endocytosis in *Giardia* trophozoites. Moreover, the expression of a mutant GIDRP with reduced affinity for GTP and GDP impaired endocytosis and resulted in enlarged PVs, indicative of blocked vesicular fission in these organelles. Also in these cells, GIDRP is detected at the ER with only a minor proportion being present as a cytoplasmic pool. During encystation, however, both clathrin and GIDRP localize in part in the ESVs containing cyst wall material (5, 12, 31). Interestingly, matching the function observed in depleted- μ 2 encysting trophozoites (34), expression of a dominant-negative GIDRP affects the formation of small vesicles containing cyst wall proteins (CWPs) and blocks its exocytosis to form the cyst wall. Because close contact of the ESVs and PVs has been frequently reported (5, 12, 14, 31), exchange of material between these two structures may occur, with the PVs finally acting as sorting organelles, probably by delivering the CWPs to the plasma membrane and/or returning other proteins back to the ER-exit site.

3.2.3. Rabs and SNAREs

After formation, the transport vesicle must dock to and fuse with the appropriate target membrane. The best-characterized molecular process leading to membrane fusion involves the coordinated work of two types of protein called SNAREs (soluble N-ethylmaleimide-sensitive factor-attachment protein receptors) and Rab family GTPases. So far, over 50 members of the Rab family have been identified in mammalian cells, and each seems to have a characteristic intracellular localization and function. For instance, Rab5 plays roles in endocytosis, early endosome fusion, and caveolar vesicle targeting to early endosomes (77, 78), while Rab11 mediates slow endocytic recycling through recycling endosomes and Rab4 mediates fast endocytic recycling directly from early endosomes (79, 80). There is also a coordinated action of Rab5 and Rab7 in the sorting of cargo receptors by the retromer complex. First, Rab5 recruits PI3K to endosome membranes, promoting the formation of PtdIns (3)P, which in turn binds the retromer dimers, SNX1/2-SNX5/6. Then, these subunits associate with the cargo-interacting retromer subcomplex Vps26-Vps29-Vps35, which is an effector of Rab7 (81).

Compared to other organisms, *Giardia* has a relatively small number of Rab and SNARE proteins (28, 82). The *Giardia* Rab11 has been localized to the PVs and cytoplasm but relocates to the ESVs during encystation (28, 36). A *Giardia* Rab1 that localizes to ER-exit sites and PVs is also associated with the ESVs during encystation (83).

Seventeen putative SNAREs have been identified and partially characterized, with five representing Qa-SNAREs, five Qb-SNAREs, four Qc-SNAREs and three R-SNAREs. Although some of these SNAREs localize to the PV area, their function has not been investigated and has rather been inferred from the participation of their orthologs in other cells (82). For example, different gSNAREs are present in the PVs/plasma membrane area, Qa1, gQa3, gQa5, gQb2, gQb4, gQb5, and gR3, with the presence of three different gQa SNAREs suggested to be involved in distinct pathways such as exocytosis, endocytosis, and PV-PV fusion (82).

The question of whether Rabs and SNAREs participate in delivery of lysosomal proteins to the PVs remains unanswered and could be addressed in greater detail by functional analysis. Since each member of the Rab and SNARE family retains analogous biological functions in almost all the species analyzed, it will be interesting to

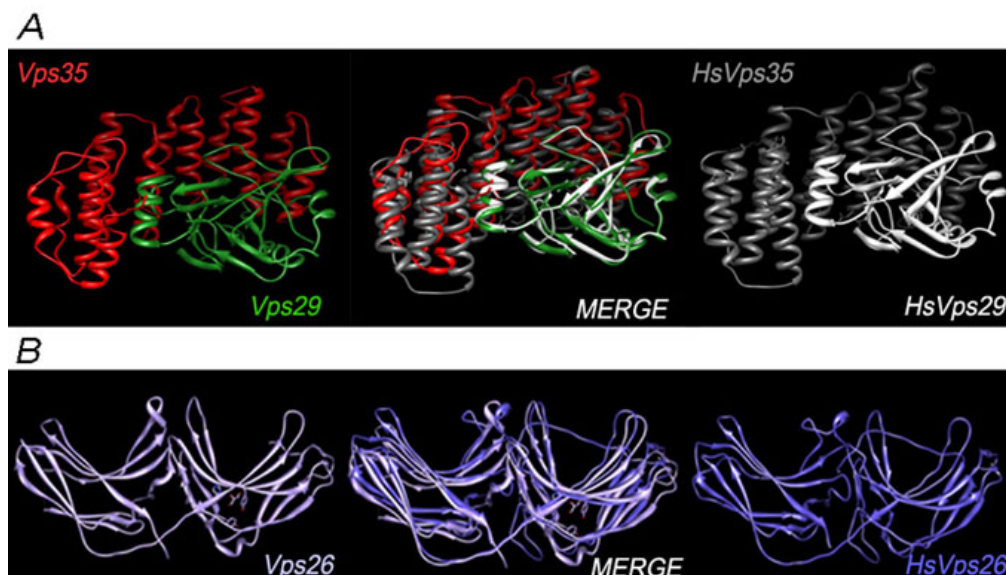


Figure 2. Three-dimensional Structure of the Giardial Vps subunits. A) Three-dimensional structures of Vps35 interacting with Vps29 are shown in ribbon diagram. The predicted 3D structures of the C-terminal domain of *Giardia* Vps35 (in red) and the *Giardia* Vps29 (in green) are highly homologous (MERGE) to the structure determined by x-ray crystallography of the human Vps35-Vps29 complex (HsVps35- HsVps29, in gray). B) 3D reconstruction of the medium subunit, Vps26. *Giardia* Vps26 (blue) and human HsVps26 (gray) show greatly related structures. The models were built using the MODELLER software (<http://toolkit.tuebingen.mpg.de/modeller>) and validated by means of VERIFY3D, SOLVX and ANOLEA.

determine whether in *Giardia* selective pressures might have been operating on distinguishing aspects of the lysosomal trafficking pathway, adapting the specificities of these proteins to accomplish their function.

4. SUMMARY AND PERSPECTIVE

Endosomal/lysosomal trafficking pathways exhibit significant complexity and diversity in terms of morphology, function, and mechanisms among different organisms and cell types. As shown by several studies, part of the *Giardia* transport machinery is fairly well conserved. The existence in this organism of the clathrin heavy chain and dynamin, a nearly complete repertoire of the retromer subunits and endosomal-lysosomal sorting motifs within cargo proteins, support an early acquisition of genes necessary for endosomal/lysosomal trafficking during eukaryotic evolution. Nevertheless, this parasite has experienced considerable diversification (Figure 3). The constraints of living under parasitic conditions have probably been the major driver for the reductive evolution of lysosome/endosome and Golgi compartments to maintain only those components that are essential for specific compartmentalization needs. For example, this parasite possesses only two of the four AP complexes, AP-1 and AP-2 that are involved in sorting signal recognition. No monomeric adaptor proteins have been identified so far. Also, despite the fact that the cargo-selection subcomplex of the retromer seems to be well conserved, we found substantial sequence divergence in the sorting nexin dimer. Whereas Giardial SNXs contain a PX domain important for targeting, they do not have a

recognizable BAR domain, suggesting that dimerization and membrane curvature detection might be somehow different in this parasite. Moreover, there are no clear orthologs of Rab5 or Rab7, which regulate retromer function in more complex cells. Moreover, although Rab11 has been associated with the PVs, further analysis will be necessary to assess Rab participation in membrane tethering and fusion to preserve the PVs identity. Similarly, investigation on the SNARE proteins closely associated with PVs will shed light on the mechanism of vesicle-vacuole fusion. Further studies of protein traffic to PVs promise to unravel not only the mechanisms by which this key organelle is assembled in *Giardia*, but also the fundamental organizational principles of endosome/lysosome biogenesis in all eukaryotes.

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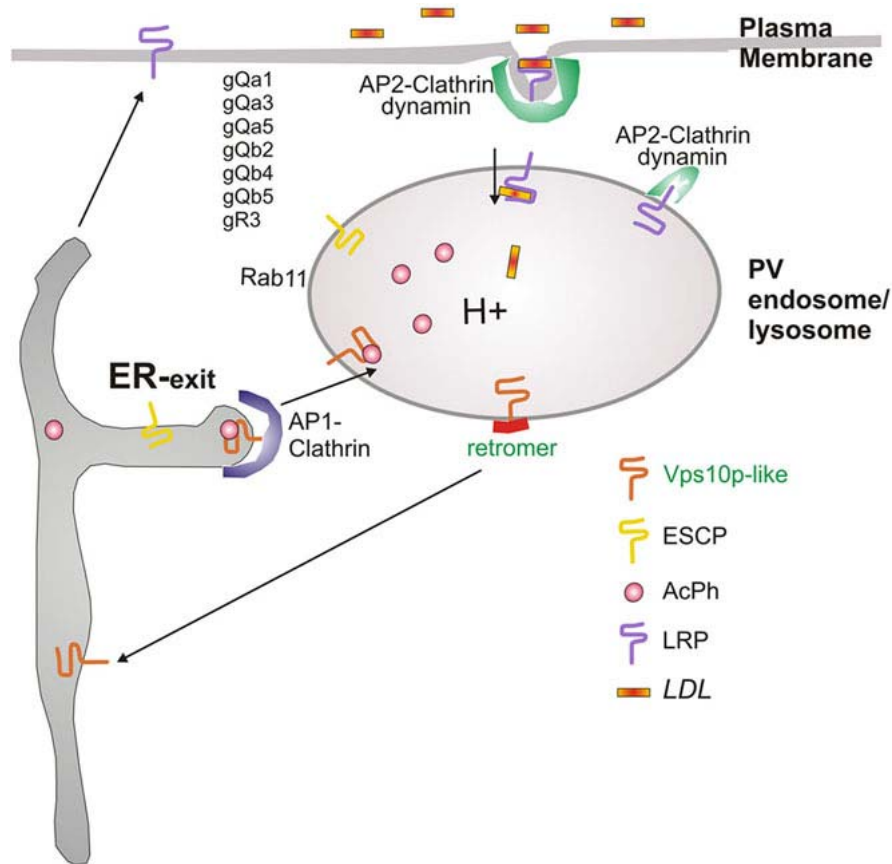


Figure 3. Schematic representation of the lysosomal protein trafficking in growing *Giardia* trophozoites. From the ER-exit sorting site, the membrane protease ESCP is directed to the lysosome-like PVs in AP-1 and clathrin-coated vesicles. For the same pathway, the hydrolase AcPh is probably associated with the membrane receptor Vps10p and AP-1. AP-2 is involved in LDL/LRP endocytosis and PV delivery. The cytosolic proteins, clathrin and dynamin, are localized in the PVs. Rab11, and the SNAREs Qa1, gQa3, gQa5, gQb2, gQb4, gQb5, and gR3 may participate in vesicle trafficking to and/or from the PVs. The retromer complex may participate in receptor recycling from the PVs to the ER. H⁺ represents the acidic pH of the PV lumen. Unconfirmed protein participation is depicted in green. Modified from (21).

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Abbreviations: CCV: clathrin-coated vesicles. TGN: *trans*-Golgi network. MPRs: mannose-6-phosphate receptors. APs: adaptor proteins. ER: endoplasmic reticulum. PVs: peripheral vacuoles. AcPh: acid phosphatase. RME: receptor-mediated endocytosis. TCs: transport carriers. CLH: clathrin heavy chain. ESVs: encystation-specific vesicles. GGAs: Golgi-localizing, Gamma-ear containing, ARF-binding proteins. Vps: vacuolar protein sorting. gHR: *Giardia* Hydrolase Receptor. ESCP: encystation-specific cysteine protease. PTB: phosphoTyrbinding. CP: cysteine protease. LRP: Low-density lipoprotein Receptor-related Protein. SNX: sorting nexins. CWPs: cyst wall proteins. SNARE: soluble Nethylmaleimide-sensitive factor-attachment protein receptors. LDL: low density lipoprotein.

Key Words: Adaptor protein, Lysosome, *Giardia*, Clathrin, Protein trafficking, Review

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