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REVIEW

Sorting without a Golgi complex

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The endoplasmic reticulum-Golgi-target organelle route is one of the most studied events and has fascinated researchers for years. However, the conservative mechanism of protein sorting and delivery is now being challenged by the finding of unconventional pathways driving protein sorting and transport. Protozoa parasites are being rediscovered as good models for analyzing alternative targeting pathways, associated with their ability to adapt to diverse environments and hosts. Here, we have gathered all the available information about secretory protein trafficking in *Giardia lamblia*, with a focus on how this protozoan parasite is able to sort and direct proteins to different compartments in the absence of a Golgi complex.

KEYWORDS

encystation, ERES, *Giardia lamblia*, KDELR, secretory pathway

1 | INTRODUCTION

Cell differentiation mechanisms, which result in the formation of refractory or resistance stages, constitute a strategy commonly observed in both free-living and parasitic organisms in response to changing unfavorable environments. The life cycle of *Giardia lamblia* includes 2 stages: a mobile form, the trophozoite, capable of colonizing the host's digestive tract, and a resistant and infectious form, the cyst, able to survive under adverse conditions and colonize new hosts (Figure 1). In *Giardia*, the process of differentiation that converts the trophozoite into a cyst involves a precise disassembly of the cytoskeleton as well as coordinated deposition of the fluid material that will form the fibrillar cyst wall.

When the fine structure of a growing trophozoite is analyzed, just a few defined organelles can been observed: 2 nuclei surrounded by their nuclear envelope (NE), the endoplasmic reticulum (ER) and the lysosome-like peripheral vacuoles (PVs) with polarized distribution beneath the plasma membrane (PM) (Figure 2A). In addition, encystation-specific secretory vesicles (ESVs) can be observed only in encysting trophozoites (Figure 2B). From the morphological point of view, no Golgi complex, peroxisomes or mitochondria were ever observed by electron microscopy in the trophozoite or cyst. However, these absences are not correlated with the lack of Golgi or mitochondrial functions. In the last decade, the presence of organelles of mitochondrial origin has been described in *Giardia* in the form of mitosomes, which are located in the cytoplasm and between both nuclei,[1,2] and the expression of conserved mitochondrial proteins required for iron-sulfur protein maturation was proved.[3] Also, protein sorting to specific targets has been described during growth and encystation in this parasite,[4-6] showing that at least one of the many Golgi functions takes place in this unicellular parasite.

There is thus new evidence placing the ER at the center of the scene, playing a critical function as a core facility for protein (and lipid) sorting. This review aims to summarize and conciliate all the results obtained in this matter, showing that *Giardia*, like other parasites, contains a particular way of trafficking proteins, probably as a result of a reductive evolution process, involving loss of genes,

ABBREVIATIONS: BFA, brefeldin A; CWM, cyst wall material; ER, endoplasmic reticulum; ERES, ER exit sites; ESVs, encystation-specific vesicles; KDELR, KDEL receptor; NE, nuclear envelope; PNM, perinuclear ER membranes; PVs, peripheral vacuoles; TGN, trans-Golgi network; VSPs, variant-specific surface proteins

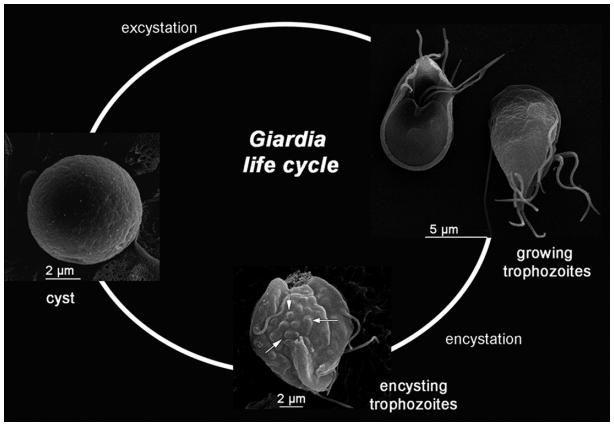

FIGURE 1 Life cycle of *Giardia lamblia*. After the ingestion of the infective cyst, commonly present in contaminated food or water, excystation takes place, releasing trophozoites that multiply by binary fission. The growing trophozoites are the vegetative form of the parasite that colonizes the intestinal epithelium of human and other vertebrates. When encystation is induced, the CWM is synthesized and transported in ESVs (arrows). The CWM is released forming the infective cyst that may survive in harsh environments until it finds a new host

organelles and functions.[7] The reduced giardial organellar components have been attracting scientists in the field for many years now. This characteristic had led to the idea that *Giardia* might be "the missing link" between prokaryote and eukaryote cell.[8] It has also been erroneously defined as "primitive" or "ancestral" due to its stripped-down subcellular complexity and the presence of genes with high sequence similarity to bacterial homologs.[9-11] *Giardia* was clustered at the base of the eukaryotic tree, suggesting its early divergence from other eukaryotes.[12] However, current opinion presumes that *Giardia* is not a vestigial cell, but rather displays a powerful example of adaptation to anaerobic conditions and to a parasitic lifestyle. In this context, the idea that *Giardia* keeps the basal mechanism for secretory trafficking of eukaryotic cells makes this parasite a very useful tool to understand its streamlining for efficient protein transport.

2 | PLAYING ALONE: THE ER AS A CENTRAL SORTING WORKSPACE

The giardial ER is a membrane network distributed symmetrically from the NEs throughout the cytoplasm of the cell. Ultrastructural and biochemical analysis showed that it shares common aspects with the ER of most eukaryotic cells. For instance, the chaperone *G. lamblia* binding immunoglobulin protein (GlBiP) is located in the lumen of the ER. It possesses the classical Lys-Asp-Glu-Leu (KDEL) retention signal at its C-terminus and a signal peptide at its N-terminal end that determines its entry into the ER.[13-15] In addition, the ER proteins, *G. lamblia* disulfide isomerases (GlPDIs),[16] are also present at the lumen of the ER, contributing to the correct folding of newly synthesized proteins, and are retained in the ER by the presence of KDEL-like motifs at its C-terminus.[17] Recently, the KDEL receptor (KDELR), which keeps the ER-soluble proteins in the ER through retrieval mechanisms in many eukaryotic cells,[18] was found in *Giardia* (GlKDELR).[19] Finding that the KDELR is involved in the retrograde transport of ER proteins through the coat complex protein I (COPI) vesicles that bud from the cis-Golgi back to the ER in both yeast and mammalian systems, suggested that this might be a universal behavior. However, GlKDELR do not cycle between compartments but remain stacked at the ER in this parasite, playing retention rather than recycling functions (see below).[19]

Another conserved ER function involves the participation of the translocation machinery in the recognition of the hydrophobic signal sequences present in almost all secreted or membrane-inserted proteins.[20] Recently, the functional characterization of the coat protein complex II (COPII) components[21] enabled the identification of putative ER exit sites (ERES). The presence of the 5 core COPII components Sar1, Sec23/24 and Sec13/31 in the *Giardia* genome[12] led to the idea that this parasite might contain some structures with characteristics observed in other eukaryotes besides the clear lack of a Golgi complex. In this regard, Faso et al reported that GlSec23 and GlSec24 are located at the ERES, which were co-stained with a protein chimera that is transported to the PM.[22] GlSec23-HA also colocalized with one of the cyst wall proteins

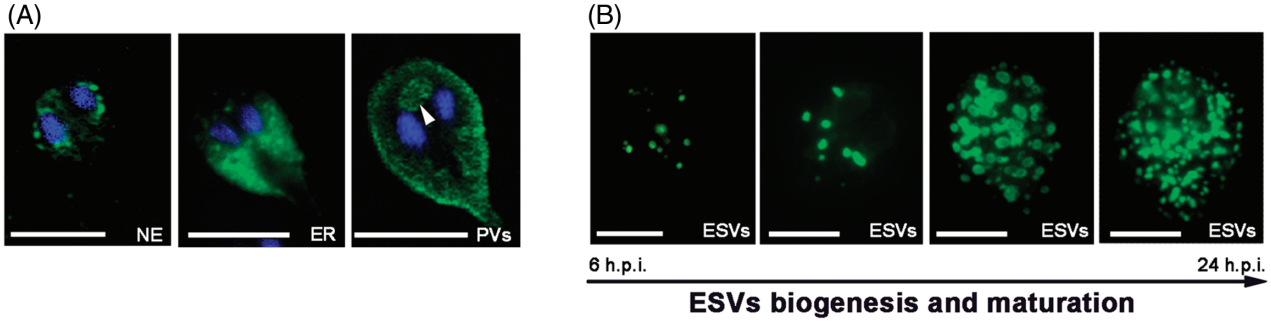

FIGURE 2 Membranous compartments of trophozoites. A, Each nucleus (blue) is surrounded by a NE. The ER is a membrane network distributed throughout the cytoplasm of the cell. Lysosome-like PVs possess polarized distribution underneath the PM and between the nuclei (arrowhead). Immunofluorescence assays and confocal microscopy show the NE, ER and PVs labeled with specific antibodies, that is, anti β-importin-HA (Mayol and Ropolo, unpublished), GlBiP and AP-2 mAb, respectively. 4′,6-Diamidino-2-phenylindole was used to stain the nuclei. (B) At 6 hours post-induction (h.p.i.) of encystation, ESVs are generated from the ER. Mature ESVs are denoted by the ring-like distribution of CWP1. Close to the cell periphery, the ESVs are reduced in size (~24 h.p.i.). Anti-CWP1 mAb was used for immunofluorescence assays and confocal microscopy. Bar, 5 µm

(CWPs) transported by a regulated mechanism (see below), suggesting that both continued and regulated protein secretion begin at the same ERES sites.[21] In addition, it was demonstrated that these 2 types of secretions depend on the small GTPase Sar1 (GlSar1),[22,23] which like its homologous in other eukaryotes is involved in the initial recruitment of COPII proteins at the ERES membrane.[23,24] GlSar1 cellular distribution from the ERES to the ER was affected by brefeldin A (BFA), an antibiotic produced by fungal organisms that completely redistribute the Golgi proteins into the ER in other cells.[25,26] Without a Golgi complex, this result suggests that the sites where the protein sorting takes place may be specialized ER membranes, sensitive to BFA.

Giardia does depend on exogenous lipids for energy production and membrane biogenesis. It was shown that ceramide is internalized through the endocytic pathways and targeted to the perinuclear/ER membranes (PNM) during growth.[19,27] Interestingly, it was found that the ERES are enriched in ceramide excimers, which in other cells are present exclusively at the region *trans* of the Golgi complex (TGN, trans-Golgi network).[19,28] This property was observed when the ceramide analogous BODIPY-C5 ceramide, which fluorescence emission shifted from green to red as the probe concentrates at the TGN in human skin fibroblasts,[28] colocalized with GlSec23 and the CWPs in this parasite.[19] Whether these specialized ERES in *Giardia* represent sites of synthesis of higher organized lipids is still unknown, but a recent report showed that the enzyme glucosylceramide transferase, which catalyzes the transfer of uridine diphosphate (UDP)-glucose and UDP-galactose to ceramide in the Golgi in other cells, is located at the PNM and some spots in the ER of *Giardia* trophozoites.[29]

Another particularity of this parasite is that the ER lacks a calnexin/calreticulin chaperone system that ensures the proper folding and quality control of newly synthesized glycoproteins.[30-32] Indeed, *Giardia* has a single nucleotide sugar transporter for UDP-GlcNAc, which appears to be involved in the synthesis of glycolipids rather than glycoproteins[32] suggesting that, in the absence of protein glycosylation, a compensatory glycan-independent quality control of protein-folding occurs, imparted by GlBiP and GlPDI family members, as was shown for *Caenorhabditis elegans*.[33]

Tremendous progress has been made trying to understand how *Giardia* is able to sort lipids and proteins to different target compartments. At this point, a critical role of the ER as a core center for protein and lipid selection has emerged in the absence of a Golgi complex. These findings place *Giardia* at the center of the scene, taking into account the importance of the endomembrane system in eukaryotic organization and the fundamental functions of this system for the establishment of parasitic lifestyles.

3 | (LOOKING FOR) THE GIARDIAL GOLGI COMPLEX

Subcellular compartmentalization probably originated by autogenesis, that is, from the elements present in the pre-eukaryotic cell.[34] The evolutionary mechanisms involved in the appearance of cellular compartmentalization are not known with accuracy, but it is quite clear that the presence of compartments made possible an increase in the abundance and the complexity of the cellular components. Probably the partition of the cellular volume facilitated the ordering of biochemical reactions within the cell, allowing the establishment of more complex regulatory pathways. In eukaryotes, the nerve center where the lipid and protein fluxes converge is the Golgi complex. As a general rule, the Golgi complex consists of flattened membrane-enclosed disks, called cisternae, joined together forming structures called cisternal stacks. Each stack is flanked on 2 sides by the cis-Golgi network (CGN) and the TGN.[35] The role of the CGN is the exchange of lipids and proteins between the ER and the Golgi, while the cargoes are classified at TGN in specialized vesicles for different cellular destinations.

The *Giardia* trophozoites do not appear to have structures similar to the membrane cisternae that characterize the Golgi complex.[36] Although clusters of parallel cisternae have been visualized in the parasite during encystation by electron microscopy, the absence of any Golgi markers makes it impossible to consider these structures as a true Golgi complex.[36,37] On the other hand, structures resembling the Golgi have been evidenced in vegetative and encysting trophozoites through the use of the Golgi marker NBD-C6 Ceramide.[27,38] However, it was not possible at that time to establish a correlation between the regions labeled with the fluorescent analog and the cisterna-like structures observed by electron microscopy. To address this point, a combination of BODIPY FL C5-ceramide staining, coupled to the diaminobenzidine photooxidation method[39-41] and transmission electron microscopy, was used to define ceramide-stained structures. In this way, it was possible to assure that ceramide labeled tubule-vesicular PNM cisterna-like structures of growing and encysting trophozoites.[19] The staining of ER membranes of early encysting cells was also detected at the site of ESV biogenesis.[19] This suggests that the ER possesses Golgi-structural properties from which secretory vesicles are formed.

When we focused on proteins that might help to find a Golgi-like complex in *Giardia*, several particularities appeared. For example, both the small ADP-ribosylation factor 1 (ARF1) GTPase and the giardial COPI-βCOP were detected in the cytoplasm, but also association with the PVs in growing trophozoites.[5,23] Still, the localization of these proteins is different from that of NBD-C6 ceramide, suggesting that these markers identify different compartments or regions of what was once considered a Golgi-like structure in the parasite.[38] In addition, the characterization of proteins involved in vesicular tethering and docking suggests the absence of a typical Golgi complex (see below).[10] Interestingly, the *Giardia* genome lacks glycosyltransferases and peripheral membrane proteins associated with the Golgi (termed "golgins") that function along the secretory pathway, but contains the Golgi-like proteins implicated in the endolysosomal pathway, like the homotypic fusion and vacuole protein sorting (HOPS) and the transport protein particle (TRAPP) complexes.[12,42] Although no characterization of these 2 proteins was performed, in silico analysis suggest that some features of the endolysosomal pathways are somehow conserved in this parasite. With the absence of Golgi matrix proteins and Golgi enzymes, the question that remains is whether the expression of these Golgi proteins is necessary to outline the structure and identity of the Golgi complex.[43-45]

4 | LESS IS MORE: CORE PROTEINS OF THE SECRETORY MACHINERY

Members of the subfamily of the Ras superfamily of low-molecular-mass GTPases (Rabs) and the N-ethylmaleimide-sensitive factor activating protein receptor (SNAREs) families play a central role in all steps of intracellular membrane trafficking. While 35 SNAREs and 60 Rabs were found in humans,[46] *Giardia* possesses only 7 Rab and 17 putative SNARE proteins on its genome.[10,12] The earliest step in vesicle trafficking and docking is achieved by the function of Rabs that cycle between the active GTP and the inactive GDP states. Rabs appear to function mostly as part of the vesicle-trafficking machinery, but there is evidence that some of them might function regulating the process.[44] In the context of a reduced set of organelles and key proteins involved in vesicle trafficking, it is not surprising that *Giardia* is able to successfully perform vesicle docking and fusion, although in its own way. For example, the giardial GlRab1 and GlRab2a/2b, similar to the human Rab 1A and 2, respectively, which participate in ER to Golgi vesicular transport, were localized in the ER, the PVs and at the membrane of the ESVs during encystation.[47] Later, by using a specific anti-GlRab1 pAb, GlRab1 was observing cycling between the cytoplasm and the sites of ESV biogenesis (ERES?), as the parasite life cycle goes from growing to encysting trophozoites.[23] Further analysis of Rab1 localization and function did not permit recognition of Golgi-like structures but reinforced the idea that the ER recruits Golgi-like Rabs. None of the Rab family members, F, D and 32, described in the *Giardia* genome were associated with biosynthetic-secretory trafficking, except for the Rab11 orthologs that have been localized in the PVs and linked with the transport of ESVs to the PM.[48]

SNARE proteins, on the other hand, participate in the last step of the fusion reaction, involving a complex formation by the interaction of SNAREs located on a transport vesicle with SNAREs from the target membrane. It was recently discovered that most vesicle SNAREs have an arginine residue (R-SNAREs), while a glutamine residue was found in syntaxins and SNAP-25-like proteins (Q-SNAREs). Concurrently, the Q-SNARE group was classified as Qa-SNAREs (syntaxins), Qb-SNAREs (25-kDa synaptosomal-associated protein-SNAP25-N-terminal SNARE motif), and Qc-SNAREs (SNAP-25 C-terminal SNARE motif).[46] The specificity in the SNARE complex function is fulfilled as long as the 3Q:1R ratio is preserved.[49,50] Expression and localization of 17 giardial HA-tagged SNAREs enabled speculation about the role of these proteins, specifically in secretory trafficking.[10] Interestingly, the closest orthologues to the SNAREs present in the Golgi and TGN were located in the PNM in this parasite[10] and at least 4 Q-SNAREs presented an ERES pattern, suggesting, once again, that the ER itself may perform at least the protein sorting and delivery function attributed to the Golgi complex in other cells.

5 | TRAFFICKING TO THE PLASMA MEMBRANE

The major secretory cargo are surface proteins called variant-specific surface proteins (VSPs), which form a thick coat at the parasite surface and play a critical role in antigenic variation and cell survival. VSPs belongs to a family of proteins encoded by a repertoire of around 200 genes, only one of which is expressed on the cell surface at a given time.[51] These are type I membrane proteins containing a highly variable extracellular domain, a conserved transmembrane domain and an invariant 5-amino acid (CRGKA) cytoplasmic tail. Much data have been obtained about which domains drive these proteins to the PM. However, fewer results have been found in the study of how these proteins are selected for constant delivery to the PM and where this selection takes place. It was reported that export of VSPs is sensitive to BFA.[38] But, does a selected sorting to the PM exist in the absence of a bona fide Golgi complex? It was shown that, when the cytoplasmic tail of the VSPH7s was deleted, the protein still ended at the PM, and that, when its cytoplasmic tail is exchanged for another containing a lysosomal-delivery motif, the VSPH7-chimera ends up on the PV membranes instead of the PM of WB1267 transfected trophozoites.[4] These 2 results indicate that the lack of sorting signaling on the cytoplasmic tail of VSPs is actually the "signal" that eventually directs the protein to the PM. In favor of this claim is the fact that the VSPH7 gene (and protein) is not present in the strain WB1267, with a very low probability that VSPH7 without its cytoplasmic domain was driven to the PM by its association with native WB1267 VSPs. These results contradict those of Marti et al, but since their experiments were performed by the heterologous expression of a *Toxoplasma gondii* SAG1 surface antigen exodomain instead of a giardial VSP, this might behave differently.[52] Current analysis of VSP trafficking revealed that the post-translational modification of the conserved cytoplasmic tail of VSP by palmitoylation[53,54] and by citrullination[55] is not responsible for VSP trafficking but is essential for VSP segregation to raft-like domains of the PM (palmitoylation) and has a clear participation in the control of the antigenic variation in *Giardia*.[54,55] It was shown that the VSP constitutive pathway is maintained when the encystation process is triggered, showing a clear separation of these proteins from those that are transported in regulated vesicles.[21] Recent results suggest that the sorting site might be at the ERES, as a chimera containing VSP conserved domains showed colocalization with the ERES marker GlSec23 in early encysting trophozoites.[21] Analysis of the data as a whole coincides on specific and constant trafficking of the VSPs from the ER to the PM, which is clearly different to the routes for lysosomal or regulated secretion pathways.[4,22,38,56]

6 | CLASSICAL TRAFFICKING TO UNCOMMON LYSOSOMAL-LIKE ORGANELLES

The PVs are vacuoles of which the most striking feature is their high polarization beneath the PM of trophozoites, although they have been occasionally observed between the nuclei in the region termed the "bare zone."[56-58] Originally described as lysosome-like vacuoles,[59,60] recent studies showed that they belong to a new category of unique organelles that are involved in the sorting of internalized cargo, recycling and degradation of specific molecules "all-in-one".[56,57,61-63] Regarding secretory trafficking, the PVs are the final destination of membrane and soluble lysosomal proteins that are

delivered by the action of the heterotetrameric adaptor protein GlAP-1, associated with clathrin, at least to the clathrin heavy chain GlCHC. But where does the journey of lysosomal protein to PVs begin? It was observed that GlAP-1 and GlCHC are located at the ER beside the PVs,[29,64,65] and Δμ-AP1 knock-down experiments showed an accumulation of lysosomal-PV proteins at the ER, notably in the PNM and in a punctate pattern resembling the ERES spots.[65] Interestingly, the ER to PVs trafficking seems to comprise well-conserved characteristics that involve the Golgi complex in other eukaryotic cells. Such cases are the membrane protein encystation-specific cysteine protease (ESCP), which travels to the PVs using a tyrosine-based motif; and the soluble acid phosphatase (AcPh), which is transported by a Vps10p-like receptor (GlVps)[57] that also contains a similar lysosomal motif.[4] Thus, ESCP and the AcPh receptor ultimately bind to the μ1 subunit of GlAP-1, and GlAP-1 to clathrin, to specifically travel to the PVs. Not surprisingly, ESCP trafficking was impaired after BFA cell treatment.[4]

Another conserved lysosomal protein that participates in lysosomal trafficking is the *G. lamblia* ENTH protein (GlENTHp), an epsin-like monomeric adaptor the function of which is critical for AcPh trafficking.[66] Because *Giardia* utilizes some of the participants of the protein trafficking from the TGN to the endosomal-lysosomal system, it is tempting to conclude that the lysosomal protein delivery mechanism is one of the most conserved in this parasite. However, there are some points to highlight: (1) neither the function of the clathrin light chain was described nor clathrin-coated vesicles were observed in this parasite, (2) the GlENTHp acts as a dual epsin and epsinR monomeric adaptor, participating in ER to PVs trafficking and also in receptor-mediated endocytosis via GlAP-2,[66] (3) no other Golgi-related adaptor complexes or monomeric adaptors (eg, AP-3-4-5, GGAs, Hrs, etc.) are present in the *Giardia* genome, (4) no post-translational lysosomal protein modification was observed and (5) the giardial retromer complex that participates in the recycling of acid hydrolase receptors from endosomes to the Golgi in other cells, only contains the cargo-selection subcomplex, composed by GlVps35-29-26p, but lacks the structural subcomplex.[67] Still, there is no Golgi complex mediating all these mechanisms. Thus, in the light of the new discoveries, it seems unsurprising that the ER, particularly the PNM and the ERES, participates in the sorting of PV resident hydrolases. This is another point in favor of the hypothesis that *Giardia* evolved from a structurally complex ancestor by reductive evolution resulting from adaptation to a parasitic lifestyle.

7 | THE DE NOVO-REGULATED SECRETORY VESICLE FACTORY

Besides its pathogenic characteristics, *Giardia* has emerged as an interesting model to study regulated secretory vesicle genesis and trafficking, as it can be generated de novo just by exchanging the culture medium.[68] In brief, after sensing the stimuli that trigger encystation,[69,70] the synthesis and segregation of the CWPs to the ERES occurs.[22] CWPs are packaged and transported in the ESVs from the ER to the PM, where they are released in a still undefined process of exocytosis.[4,71-73] At the time of CWP secretion, the GalNAc homopolymer, which was synthesized and transported within vesicles distinct from ESVs, is covering the surface of encysting *Giardia* trophozoites and act as an anchor for these proteins, forming the extracellular matrix.[74,75] While most of the CWPs are attached to the developing cyst, it was demonstrated that CWP1 is also secreted to the media and incorporated into the cyst wall of distant forming cysts.[76] Encystation is tightly regulated and involves molecules that intervene at different time points, controlling the beginning and the ending of the process.[55,69,70,77]

How regulated secretory vesicles are formed and trafficked during *Giardia* differentiation is a central question that has been debated in the field for more than 2 decades. In most known specialized secretory cells (eg, neurons, endocrine cells, exocrine cells and

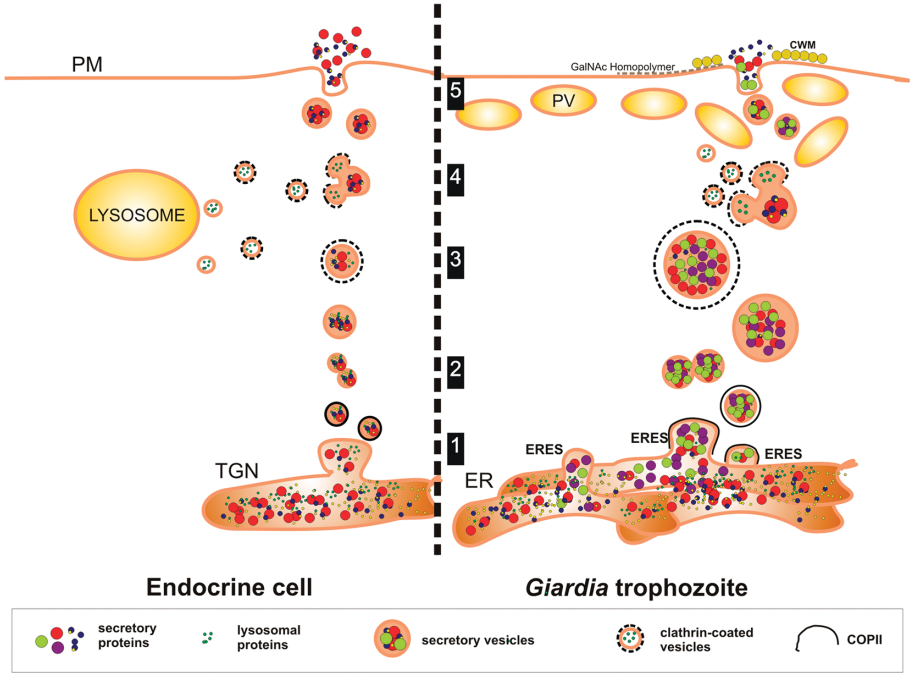

FIGURE 3 Comparison of the regulated secretory pathway of an endocrinal cell and a *Giardia* trophozoite. (1) Secretory vesicles bud from the TGN (or ERES for *Giardia*) as immature secretory vesicles. (2) Homotypic fusion is responsible for the increase in size of secretory vesicles during maturation. (3) Clathrin-coated immature vesicles also contain non-specific proteins. (4) Missorted proteins are removed by budding off clathrin coats small vesicles. (5) Acidification (unknown for *Giardia*) and condensation lead to the formation of mature vesicles. In endocrine cells, the mature vesicles are secreted after a secretagogue signal. In *Giardia*, no secretagogue is required and mature ESVs are sorted to the PM and sequentially secreted. The secreted proteins (CWM) are attached to the GalNAc homopolymer, which is differentially exported to the cyst surface. Modified from Reference [86]

hematopoietic cells), the secretory vesicles are permanently produced, stored in their cytoplasm and secrete their content only upon receiving a stimulation.[78] In contrast, the formation and release of ESVs in *Giardia* occurs following a different sequence of events, which involves reception of stimuli, synthesis of the cyst wall material (CWM), segregation of CWM into new-forming ESVs, trafficking to the PM and release to form the cyst wall.[79,80] One daring idea was that of the Hehl group in 2003, when they suggested that the ESVs are "transient Golgi-like compartments."[5] Since then, this statement was adopted by this and other groups on the field. This group proposed a cisternal maturation model for the ESVs as follows[5]: "(1) ESVs are generated from smaller pre-Golgi vesicles by homotypic fusion; (2) ESVs mature by retrograde transport via COPI-coated vesicles; (3) mature ESVs are analogous to single-cargo trans-Golgi cisterna and associated with clathrin; (4) ESVs disperse simultaneously into small secretory vesicles that fuse with the PM and release their contents." However, a rigorous analysis is necessary to support a definitive conclusion. It is true that, without a Golgi organelle, the ESVs are the only post-ER structures, which are visible only during encystation. It is a long way from there to the claim that they are Golgi cisternae.

Against this statement is the fact that none of the principal Golgi functions, such as O-glycosylation or lipid- and protein sorting to

TABLE 1 Main proteins of the mammalian and yeast Golgi apparatus and a comparison with the *Giardia* genome database

Category	Mammalian proteins	Yeast proteins	*Giardia* proteins
Glycosylation			
Glycosidases	α-Mannosidase I, II	–	None
Glycosyltransferases	GlcNAc phosphotransferase GlcNAc transferase I α1,6-fucosyltransferase β1,4-Galtransferase α1,2-sialyltransferase	Och1 Mnn9, Van1 Mnn9, 10, 11, Hoc1, Anp1 Mnn6 Mnn2, 5 Mnn1	– – –
Proprotein processing	Furin prohormone convertases Carboxypeptidase E	Kex2 Ste13 Kex1	GlFurin-like –
Protein transport			
Sorting receptors	Man-6-P receptor KDEL receptor p24 proteins p58/ERGIC53, VIP36 TGN38	Vps10 Erd2 Rer1 Emp24, Erv25, Erp1-6 Emp46/47 –	GlVps receptor GlKDELR – – –
Small GTPases	ARF1-5	ARF1, 2	GlARF-1
	Rab1, 1b, 2, 6, 6b, 10, 11, 12, 130, 33b	Ypt1, 6, 31, 32, Sec4	Rab1a, Rab2a, Rab 2b[a]
Membrane fusion	Syntaxin ERS24 Membrin rBet1 Syntaxin 5 mYkt6 GOS28 GS15 Syntaxin 6 Syntaxin 16 Vti1a VAMP3 or 4	Sed5 Sec22 Bos1 Bet1 Sed5 Ykt6 Gos1 Sft1 Tlg1 Tlg2 Vti1 Snc1 or 2	Qa-SNARE 2, Qb-SNARE 5, R-SNARE 1, R-SNARE 2[a]
Tethers/matrix	Giantin GRASP65	– Grh1	– –
	HOPS TRAPP	HOPS TRAPP	HOPS-like TRAPP-like
Coatomers			
COPI	ARF-1 α-COP β-COP β′-COP δ-COP γ-COP ζ-COP ε-COP	Arf1, Arf2, Arf3 Ret1 Sec26 – Sec27 Sec21 Ret3 Sec28	ARF-1 α-COP β-COP β′-COP δ-COP γ-COP ζ-COP –
COPII	Sar1 Sec13 Sec23 Sec24 Sec31	Sar1p Sec13p Sec23p Sec24p Sec31p	GlSar1p Sec13p GlSec23p GlSec24p (Sec24-like 1, 2 and 3) GlSec31p
Clathrin	AP-1 ab AP-3 AP-4 AP-5 EpsinR GGA Clathrin heavy chain Clathrin light chain	AP-1 AP-3 – – Ent3, Ent5 GGA1, GGA2 Clathrin heavy chain Clathrin light chain	GlAP-1 – – – GlENTHp (epsin-like) – Clathrin heavy chain Putative clathrin light chain

[a] The presence of these proteins in specific regions of the ER suggests a Golgi-like function.

This table was verified and completed using The UniProt Consortium.[93] Data from the *Giardia* genome was obtained from the GGD.[12]

differential-targeted organelles, is accomplished by the ESVs. Also, all the steps described as part of ESV-cisternal maturation can be clearly associated with the process of regulated secretory vesicle maturation: (1) maturation of specialized secretory vesicles involves homotypic fusion of immature granules resulting in an increase in granule size[81]; (2) the recruitment of coatomer proteins during ESV trafficking can be associated with the vesicle maturation process, as was demonstrated for endocrine cells. This process involves recruitment of AP-1 and is regulated by ARF1[82,83]; (3) like endocrine cells, the incorporation of the GlCHC and the COPI complex to the ESV may be important for the removal of any membrane proteins inadvertently co-packaged into the ESVs[84]; and finally, (4) cargo molecules in maturing granules undergo condensation, acidification (not proved in *Giardia* ESVs), cargo-processing and removal of excess membrane and water, which yield a reduction in size[85] (Figure 3).

These 2 hypotheses are under continued testing and, while they seem incompatible, they have several consensus points, resolved over years of investigation. For instance, it is now known that the ESVs originate in specialized ER membranes, the PNM and the ERES, which present characteristics of a TGN but are also enriched in COPII coatomer complexes and in the small GTPase Rab1.[19,23] The budding of nascent ESVs requires CWM segregation and membrane selection.[19] The segregation of the CWM is determined by the expression of CWP1,[87] the common biophysical properties of CWP1, 2 and 3 involving their leucine-rich repeats,[5,88,89] and the 121 basic amino acids of the CWP2 C-terminal end.[90] During the maturation step, CWM proteolytic processing and partitioning occur.[71,73] Different steps have been claimed from this point, ranging from the sequential exocytosis and deposition of CWM to form the cyst wall,[73] to ESV protrusion,[72] to the interaction of ESVs with the PVs necessary for CWM discharge and release.[4,71] Much more work is required to conciliate these points of view, but we cannot exclude a co-participation of these pathways to accomplish cyst formation.

Regarding the role of the GlKDELR during encystation, new data have shown that it specifically retains GlBiP in the ER, at the zone of ESV biogenesis, rather than recovering it from the ESVs.[19] This finding was supported by analysis of the dynamic of the GlKDELR during encystation, showing a static behavior of the receptor.[19] Still, few GlBiPs were found inside the ESVs, as its expression is raised during encystation and the retention mechanism might be exceeded during this process.[90,91] Thanks to the advance in biochemical, genetic and cell biology approaches, we are confident that we will continue shedding light on these unresolved issues in the not too distant future.

8 | CONCLUSION

The reconstruction of the evolutionary histories that determined the origin of the diversity of life indicates that, contrary to intuition, reductive processes are more common than those of complexity. This finding has enabled models of evolutionary change to be proposed in which the loss of information, in the form of structures and/or functions, is the "price" for adaptation. In this context, processes of genomic and structural complexity play a key role, providing the material that is then shaped by the reductive forces during adaptive processes.[92] Table 1 presents the proteins associated with the Golgi complex in mammalian, yeast and *Giardia* cells. It is noticed the absence of key Golgi proteins in *Giardia*. When the dynamics and function of vesicular trafficking are analyzed, it becomes ever clearer that this parasite uses the ER as the central platform in the absence of a Golgi complex. It is also possible that an unstable Golgi structure has become too weak to stand and finally collapsed into the ER. However, the preservation of the ER nature of this membranous system and the lack of the central Golgi functions argue against this possibility. Moreover, it was possible to describe that these ER structures play Golgi functions during secretory transport. This result is significant because it shows how the temporal reorganization of pre-existing structures during the adaptive process can lead to novel functionalities and reduction of structures without loss of function. This represents an extreme example of function maintenance in the absence of characteristic structures. More studies are needed to determine the exact mechanism that mediates these changes in this particular group of organisms.

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Editorial Process File

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