Trends in Biochemical Sciences

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REVIEWS

Review

The Golgi as an Assembly Line to the Autophagosome

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Autophagy is traditionally depicted as a signaling cascade that culminates in the formation of an autophagosome that degrades cellular cargo. However, recent studies have identified myriad pathways and cellular organelles underlying the autophagy process, be it as signaling platforms or through the contribution of proteins and lipids. The Golgi complex is recognized as being a central transport hub in the cell, with a critical role in endocytic trafficking and endoplasmic reticulum (ER) to plasma membrane (PM) transport. However, the Golgi is also an important site of key autophagy regulators, including the protein autophagy-related (ATG)-9A and the lipid, phosphatidylinositol-4-phosphate [PI(4)P]. In this review, we highlight the central function of this organelle in autophagy as a transport hub supplying various components of autophagosome formation.

Highlights

The Golgi complex regulates production and delivery of proteins and lipids, and is a site of lipid metabolism needed for autophagy, in particular PI(4)P.

ATG9A is the sole transmembrane ATG protein and has a crucial role in the formation of the autophagosome, one new role being the delivery of the metabolizing enzymes of PI to the nascent autophagosome.

ATG9A trafficking from the Golgi and recycling endosome is controlled by the coat adaptor complexes AP1, AP2, and AP4, and several BAR-domain containing proteins BIF1, SNX18, and recently Arfaptin2.

The control of ATG9A delivery to the forming autophagosome allows *in situ* PI(4)P production for the initiation of phagophore formation.

Basic Mechanisms of Autophagy

Autophagy is a process whereby cellular material is degraded to procure nutrients or to remove organelles and proteins [1]. This highly conserved, essential process is mediated by a cohort of proteins called the ATG proteins, which are conserved from yeast to humans. There are many different cues that initiate autophagy, but perhaps the best known is amino acid starvation, which induces autophagy to offset the lack of nutrients. Autophagy can be seen as a complex pathway of membrane formation and reformation, centered on the *de novo* creation of a double-membraned autophagosome, which will fuse with the lysosome so as to degrade its cargo [1].

Many aspects of autophagosome formation are by now well understood and, in simplified form, can be viewed as a cascade starting from the mammalian target of rapamycin (mTOR), which activates the Unc-51-like kinase 1 (ULK1) complex, followed by phosphatidylinositol 3-phosphate [PI(3)P] generation at the ER by the phosphatidylinositol 3-kinase catalytic subunit type 3 [PI(3)KC3] complex I. The generation of PI(3)P at the ER leads to the recruitment of autophagy effectors to form the omegasome, the earliest autophagic structure, which grows into the phagophore. One of the effectors that bind PI(3)P is WIPI2B, which has an important role in the lipidation and membrane association of **LC3/GABARAPs** (e.g., LC3-II; see Glossary), which are essential for autophagy and are the most widely used markers of autophagosomes. Once the phagophore has grown and enclosed its cargo, it closes to form an autophagosome [1,2].

An important player in each step of this process is ATG9A, a transmembrane protein that cycles between the ***trans*-Golgi network** (TGN) and the **ATG9 compartment** [3]. Curiously, although essential at all stages for autophagosome formation, ATG9A does not have a defined function as far as we know [2]. Thus, many questions remain about autophagosomal membrane formation and the role of ATG9A. In particular, because it is the only transmembrane core autophagy protein, could ATG9A contribute lipids to help form the autophagosome? In addition, which proteins or lipid species could be trafficked by ATG9A to the expanding autophagosome?

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In this review, we address these questions from the viewpoint of the Golgi complex, focusing on the role of the Golgi in autophagy. In particular, we discuss the control of autophagosome formation through the cycling of ATG9A vesicles from the TGN, the role of ATG9A in the contribution of PI(4)P, a lipid gaining in importance in autophagy, and how this is regulated from a Golgi perspective.

Basic Biology of the Golgi Complex

The Golgi complex was first discovered in 1898 as an 'internal reticular apparatus', and comprises a series of interconnected, well-organized, sac-like structures [4]. Comprising three distinct subcompartments, the *cis*-Golgi, *medial*-Golgi, and *trans*-Golgi, and a fourth compartment, the TGN (Figure 1), it has been characterized as a trafficking hub of proteins on their way from the ER to the **endosomal system** or to the PM to be secreted. The correct trafficking of proteins

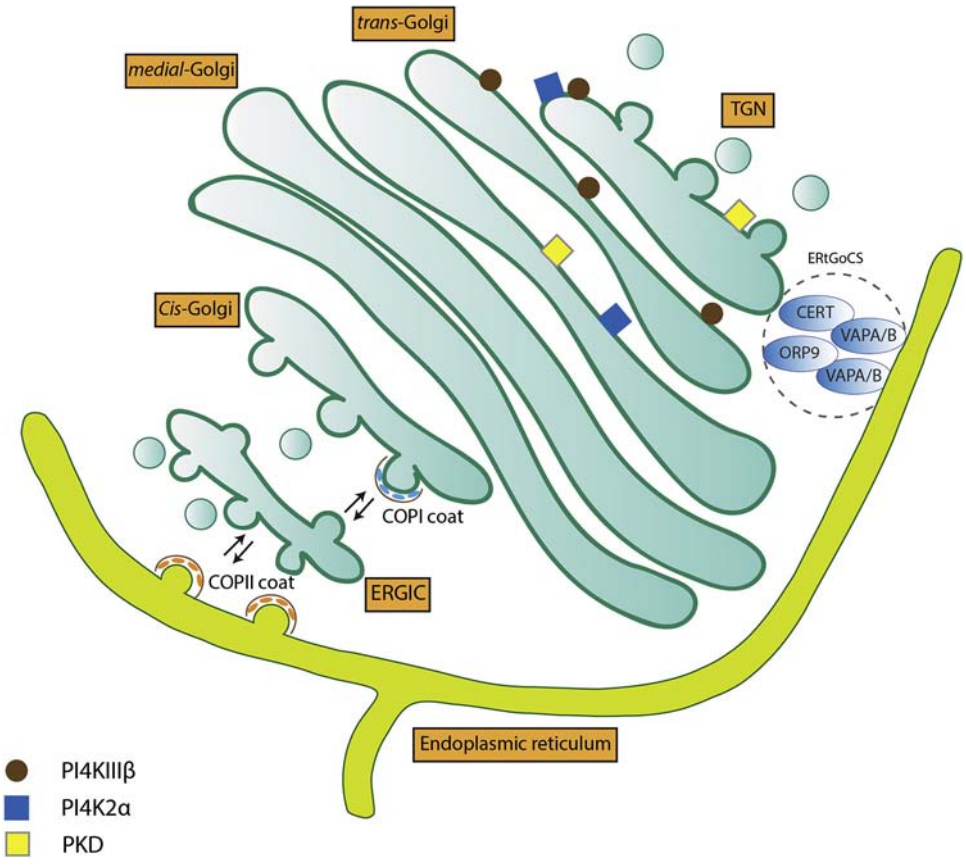


Figure 1. The Golgi Is a Central Trafficking Hub. Overview of the Golgi complex and the trafficking pathway. Endoplasmic reticulum (ER) cargo is sorted and loaded onto coat protein (COP)-II-coated vesicles at ER exit sites to be trafficked to the ER–Golgi intermediate compartment (ERGIC). If mislocalized, protein cargo will be sorted back to the ER through COPI-coated vesicles. Proteins can then be further trafficked along the *cis*-, *medial*-, and *trans*-Golgi, finally to be sorted to the *trans*-Golgi network (TGN) to be trafficked further into the cell, for example through the endosomal network. Lipid homeostasis is also important in the Golgi, and here we focus on phosphatidylinositol-4-phosphate [PI(4)P] regulation. The *medial*- and *trans*-Golgi in particular are enriched for PI(4)P through the action of PI(4)P kinases (PI4KIIIβ and PI4KIIα being the most prominent). ER–Golgi contact sites (ERTGoCS) are also important in regulating PI4P (see Box 1 in the main text for more information). Abbreviations: CERT, ceramide transfer protein; ORP9, oxysterol-binding protein-related protein 9; PKD, protein kinase D; VAPA/B, vesicle-associated membrane protein (VAMP)-associated protein A/B.

Glossary

Adaptor protein (AP) complexes: family of coat proteins involved in the assembly of cargos, both luminal and transmembrane proteins, into vesicles emerging from membranous compartments and delivered to different cellular destinations.
ATG9 compartment: clusters of tubules and vesicles adjacent to the nascent phagophores, or pre-autophagosomal structures (PAS in yeast), where ATG9 accumulates.
BAR-domain: a dimeric protein domain, named for the three proteins it was first discovered in (BIN, Amphiphysin, and Rvs), which is involved in the membrane curvature of lipid bilayers.
Clathrin-coated vesicles: clathrin is an important protein not only in vesicle formation during endocytosis, but also in generating vesicles that connect to the endosomal system.
COPI and COPII vesicles: transport vesicles coated in COPI or COPII complex proteins that traffic from the Golgi to the ER or from the ER towards the Golgi complex, to shuttle proteins between the ER and Golgi complex, respectively.
Endosomal system: collection of transport vesicles and compartments, including early endosomes, recycling endosomes, endosomes, and late endosomes. This network chiefly controls proteins cycling between the Golgi, PM, and lysosomes.
ER exit sites: regions of the ER coated with COPII, from where COPII vesicles traffic towards the Golgi.
ER–Golgi contact sites (ERTGoCS): membrane contact sites between the ER and Golgi apparatus, where the principal components are ER-localized tethering proteins that recruit proteins able to bind to Golgi membranes.
Golgi/TGN vesicular transport: the vesicular trafficking process of Golgi content, membrane, and/or luminal components. The specific content is recognized by different protein-bound vesicle carriers that participate in the cargo selection and formation of the vesicles.
LC3/GABARAPs: mammalian ATG8 homologs that are conjugated to the lipid phosphatidylethanolamine (PE) and bind the phagophore and autophagosome membrane. These proteins are commonly used markers for autophagosome formation and

to their destination relies on the tight regulation of retention, selection, and transport via coat proteins, and the maintenance of signaling lipids in the Golgi [5]. Additionally, the Golgi is an important site for the post-translational modification of these proteins, especially glycosylation, which requires a host of specialized enzymes, such as glycan-modifying proteins [6,7].

Protein-Mediated Trafficking at the Golgi

As stated earlier, one of the prominent roles of the Golgi complex is the trafficking of proteins from the ER to the endocytic compartments and PM. Newly synthesized proteins destined for the Golgi are sorted into **COPII vesicles** at **ER exit sites** [8,9] and trafficked to the ER-to-Golgi intermediate compartment (ERGIC; Figure 1). From the ERGIC, which acts as a sorting station, two things can happen: (i) proteins are transported to the *cis*-Golgi in an anterograde pathway; or (ii) mislocalized proteins are recycled back to the ER in a retrograde pathway through **COPI** vesicles [8,10]. Proteins transported to the *cis*-Golgi pass through the *medial*- and *trans*-Golgi, arriving at the TGN to be sorted by different coat proteins (see later). These proteins are those destined for the endocytic compartment, the lysosome, or PM, where they are incorporated or released into the extracellular space.

Although this review does not focus on intra-Golgi trafficking (that which occurs between the *cis*-, *medial*-, and *trans*-Golgi), it does focus on trafficking out of the Golgi via the TGN and its impact on autophagosome formation. The architecture of the Golgi complex itself (ribbon-like versus Golgi stacks) is dependent upon tethers, including GRIP and coiled-coil domain containing 88 kDa (GCC88), and disruption of this ribbon-shaped architecture by loss of GCC88 leads to aberrant nutrient sensing and alteration of autophagy [11]. However, this aspect of Golgi biology will not be further covered in this review.

A variety of different cargo-containing vesicles form at the Golgi and are targeted to their destination through the binding of a protein coat. Despite the variety of the coats involved in TGN exit, only **adaptor protein (AP) complexes** (Figure 2) have been firmly connected with autophagy so far. These five AP proteins (AP1–AP5) [12,13] constitute a family of coat proteins that can be incorporated into either **clathrin-coated vesicles** (AP1 and AP2), or vesicles partially dependent (in the case of AP3) or independent (for AP4 and AP5) of clathrin. One of the first pieces of evidence for a role of **TGN vesicular transport** towards forming autophagosomes was illustrated by AP1 complex colocalization with LC3-positive structures upon rapamycin treatment, as well as autophagy inhibition in AP1 knockdown cells [14]. Here, we further discuss the role of vesicular exit from the Golgi in relation to autophagy, and focus on the heterotetrameric AP complexes.

PI(4)P Levels Are Tightly Regulated in the Golgi

Besides proteins, lipids, such as PI(4)P, also have a role in maintaining Golgi *cis*- versus *trans*-asymmetry and in protein trafficking; PI(4)P is also an important lipid for autophagy [15,16]. One of the main roles of PI(4)P in the Golgi, primarily at the TGN, is to recruit and target cytoplasmic PI(4)P-binding effector proteins. As an example of its role in Golgi protein sorting, Golgi phosphoprotein 3 (GOLPH3) is able to bind PI(4)P at the *trans*-Golgi and simultaneously interact with mislocalized Golgi membrane proteins and COPI to sort them for retrograde recycling [17]. Although important, multiple pathways beyond PI(4)P can lead to vesicular transport from the Golgi (**Golgi vesicular transport**; e.g., reviewed in [18]).

Besides its role in the formation of vesicles from the *trans*-Golgi, PI(4)P is also crucial for the formation of **ER–Golgi contact sites** (**ERTGoCS)**, which constitute an important lipid transfer site between the ER and the Golgi complex (Figure 1 and Box 1). These contact sites have a role in the regulation of lipid levels in the Golgi complex, necessary to maintain Golgi asymmetry and effector protein binding, as explained earlier, and can regulate other lipid-transporting enzymes (Box 1).

autophagy. They are also required for the sequestration of cargo to be degraded.
Selective autophagy: specific degradation of damaged organelles, protein aggregates, unwanted cytosolic components, or pathogens using the ATG protein machinery, and selective autophagy adaptors or receptors.
Sorting signals: generally short and linear amino acid sequences present within the cytosolic domains of transmembrane proteins that are recognized by protein coats and mediate the sorting of these proteins to specific cellular destinations.
***trans*-Golgi network:** most distal compartment of the Golgi complex.

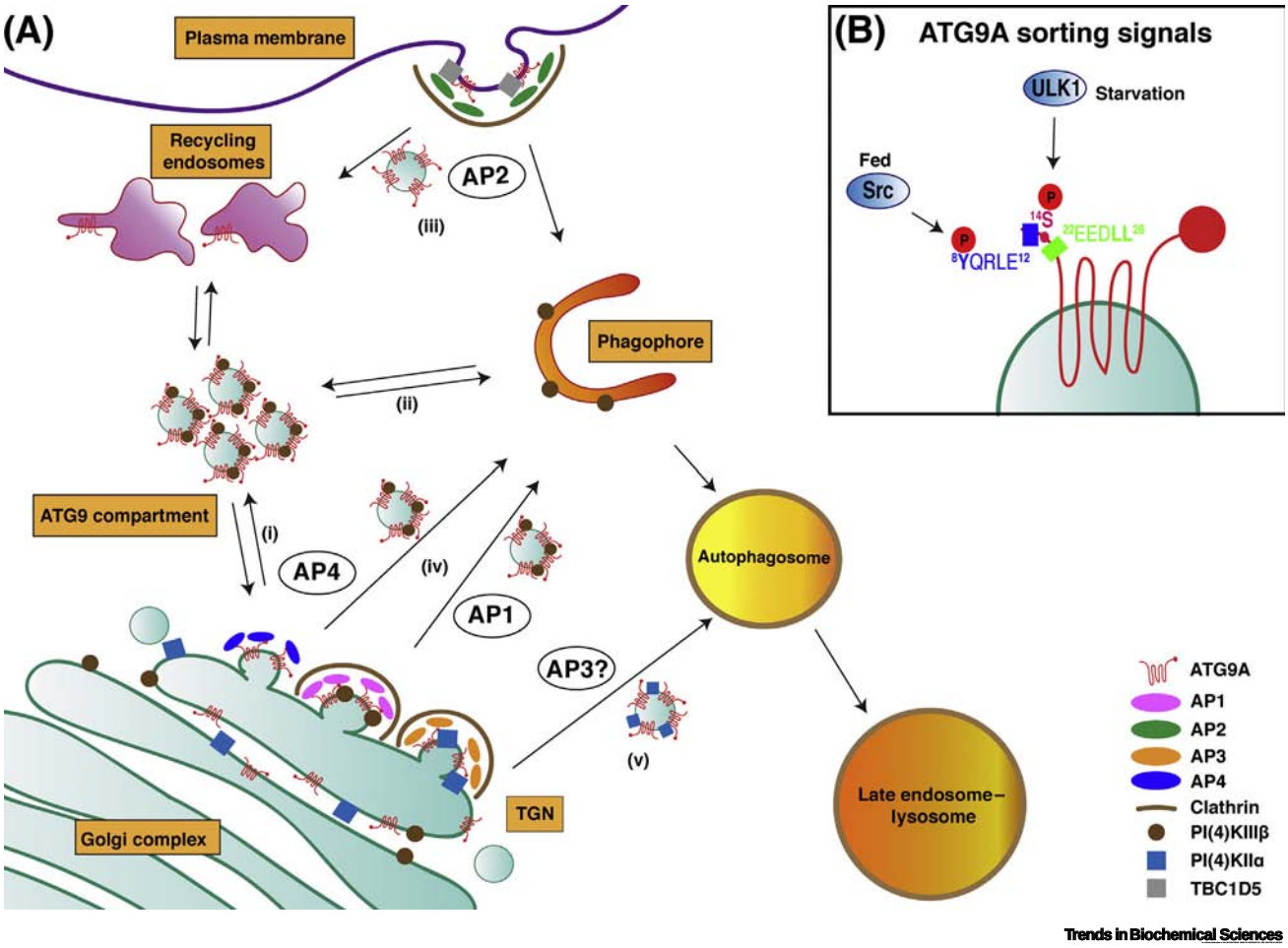


Figure 2. Adaptor Complexes Regulate Autophagy-Related (ATG)-9A Vesicular Trafficking. (A) Overview of ATG9A trafficking between different cellular membrane compartments coordinated by adaptor protein (AP) complexes. ATG9A vesicles traffic from the Golgi compartment to sites of autophagosome formation (phagophore) via AP1 and/or AP4 (iv) complexes, which deliver phosphatidylinositol-4-kinase (PI(4)K)-IIIβ. The AP4 complex can also direct ATG9A-positive vesicles to the ATG9 compartment (i,iv), from where ATG9A vesicles could traffic to the phagophore (ii). In addition, ATG9A interaction with TBC1 domain family member 5 (TBC1D5) can reroute the movement of ATG9A from the plasma membrane to recycling endosomes and autophagosome formation sites in AP2-derived vesicles (iii). PI(4)KIIα interacts with ATG9A and moves from the Golgi apparatus to autophagosomes via the AP3 complex (v), which might be another mechanism for ATG9A trafficking around the cell. (B) Schematic view of ATG9A N-terminal-sorting signals and its phosphorylation sites that facilitate binding of AP complexes. Src kinase and Unc-51-like kinase 1 (ULK1) phosphorylate ATG9 to regulate its trafficking in fed and starvation conditions, respectively.

Thus, its level is tightly regulated by the interplay between the PI(4) kinases (PI(4)K), most notably PI(4)KIIIβ, and phosphatases (i.e., SAC1), each of which is regulated by a complex feedback loop involving other Golgi-localized lipids and proteins (Box 1).

ATG9A Trafficking between the Golgi Complex, Recycling Endosomes, and Autophagosomes

ATG9A is the only conserved transmembrane ATG protein and, in mammalian cells, resides in the TGN, recycling endosome, and endosomal compartments (Figure 2Ai) [3,26,27]. During starvation, ATG9A accumulates in a vesicular compartment called the ATG9 compartment, from where it can be mobilized to interact with the forming and expanding phagophores (Figure 2Aii) [26].

Autophagy depends on the regulation of ATG9A trafficking, which is tightly modulated by different protein components. For example, the ULK complex promotes ATG9A trafficking between the TGN and the ATG9A compartment (Figure 2Ai) [26,28,29] and, similarly, p38α MAP kinase-interacting protein (p38IP) positively regulates ATG9A trafficking during amino-acid deprivation [27]. While not entirely clear why, the distribution of ATG9A in the absence of ULK1 appears to be primarily perinuclear, while perturbation of the p38IP interaction, which activates p38α MAPK, appears to cause the retention of ATG9A in the endosome [26,27]. Recently, AP complexes and **BAR-domain** containing proteins have also been implicated in the regulation of ATG9A trafficking from the TGN and the endosomal compartments to the site of the nascent autophagosome, as also discussed later and in Figure 2A and Box 2.

ATG9A Trafficking via AP Complexes

ATG9A contains two conserved N-terminal AP **sorting signals**, a canonical tyrosine-based motif (^{8}YXXØD/E^{12}) and a noncanonical dileucine motif (^{22}E/DxxLL^{26}), which confer binding to AP1, 2, and 4 complexes (Figure 2B) [30]. Phosphorylation of Tyr8 and Ser14 are essential for autophagy initiation and regulate AP binding [31]. Tyr8 is phosphorylated by Src kinase, which regulates ATG9A constitutive trafficking by enhancing its binding to AP1/2 complexes, as well as AP2 retrograde transport from the PM in response to human epidermal growth factor (hEGF) (Figure 2Aiii) [31]. ULK1 phosphorylation of Ser14 likewise promotes ATG9A binding to AP1/2 complexes, as well as its movement from the juxtanuclear compartment (TGN) to the ATG9 compartment under starvation stress. Additionally, both N-terminal sorting motifs are required for the transport of ATG9A back to the Golgi from recycling endosomes through an AP2 interaction [30]. Furthermore, ATG9A targeting to *Salmonella* in **selective autophagy** requires both N-terminal sorting motifs [30]. *Salmonella* growth arrest and clearance requires ATG9A for the recruitment of the PI(3)KC3 complex I and formation of a double membrane structure around the pathogen [32].

Box 1. Overview of PI(4)P Regulation at the Golgi

Enzymes residing in the Golgi have a role not only in maintaining its lipid composition, but also in producing and modifying lipids to be transported to the endocytic system. Here, we give an overview of the synthesis and regulation of PI(4)P and some of its effects (Figure I).

In mammalian cells, there are four different PI(4)Ks, of which PI(4)KIIα and PI(4)KIIIβ localize to the Golgi complex. These enzymes work by phosphorylating phosphatidylinositol on the 4 position hydroxyl group of the inositol ring. PI(4)KIIIβ is activated by protein kinase D (PKD) at the Golgi by phosphorylation at serine 294 [19], while, in turn, PKD is recruited to the Golgi by binding diacylglycerol (DAG) [20] (Figure IB). By providing a binding site for ceramide transfer protein (CERT), the level of PI(4)P itself indirectly regulates the supply of ceramide from the ER, which, together with phosphatidylcholine (PC), is converted into sphingomyelin and DAG by sphingomyelin synthase (SMS) (Figure IB). To complete the feedback loop, CERT is also a substrate for phosphorylation by PKD, which limits its activity [21]. Thus, the increase in ceramide through CERT leads to more DAG, recruiting PKD, which in turn activates PI(4)KIIIβ and inhibits CERT, leading to an equilibrium (Figure IB).

PI(4)P is also regulated through potential transfer and dephosphorylation at so-called 'ERTGoCS'. ERTGoCS are mediated by the ER docking proteins vesicle-associated membrane protein (VAMP)-associated protein A and B (VAPA and B), which can bind different proteins and tether them to the ER [22] (Figure IA). The aforementioned CERT is such a protein, targeting to the ER through a FFAT motif (two phenylalanines in an acidic tract), which binds VAPA/B, and by a pleckstrin homology (PH) domain, binding PI(4)P at the Golgi, where it transfers ceramide [23].

Besides CERT, oxysterol-binding protein 1 (OSBP1), and oxysterol-binding protein-related protein (ORP) 9 and 10 can also bridge the ER and Golgi through a FFAT motif and a PH domain [24,25], where they may mediate the exchange of Golgi-localized PI(4)P and ER-synthesized phosphatidylserine (Figure IA).

Besides the transfer of lipids, the ER is also able to modulate lipids at the Golgi through SAC1, an ER-resident protein phosphatase. SAC1 is able to dephosphorylate ER-localized PI(4)P in *cis* and, through an interaction with phosphatidylfour-phosphate-adaptor-protein-1 (FAPP1), is able to dephosphorylate Golgi-localized PI(4)P in *trans* [24,25] (Figure IA). Thus, ERTGoCS contact sites are important sites of lipid trafficking and modulation between the ER and the Golgi.

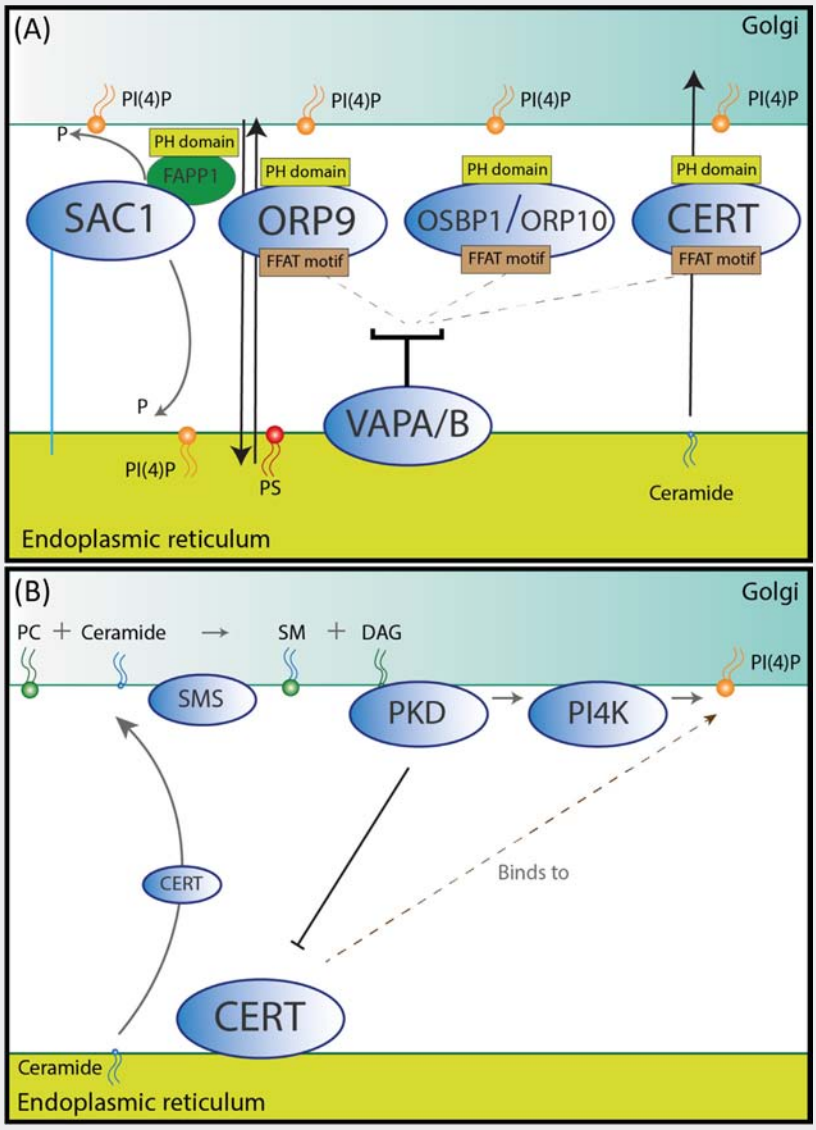


Figure I. Endoplasmic Reticulum (ER)–Golgi Contact Sites (ERTGoCS) as Hotspots for Lipid Transfer. (A) Shown here is a schematic of ERTGoCS between the ER and the *trans*-Golgi network (TGN). vesicle-associated membrane protein (VAMP)-associated protein A and B (VAPA and B) are ER transmembrane proteins that anchor the key lipid-metabolizing and transport proteins SAC1, oxysterol-binding protein-related protein 9/10 (ORP9/10), oxysterol-binding protein 1 (OSBP1), and ceramide transfer protein (CERT) to the ER. Each of these proteins is able to bind phosphatidylinositol-4-phosphate [PI(4)P] at the TGN through their pleckstrin homology (PH). SAC1 is a phosphatase that dephosphorylates PI(4)P, either in *trans* at the TGN by binding to phosphatidyl-four-phosphate-adaptor-protein-1 (FAPP1) or in *cis* at the ER membrane. ORP9 is proposed to transfer phosphatidylserine (PS) from the ER to the TGN membrane, supposedly in exchange for PI(4)P through countertransport, as has been reported for other ORPs (ORP5/8). ORP10 and OSBP1 are required for the structural integrity of contact sites, but not for lipid transfer. CERT is able to transfer ceramide to the TGN to be further processed. (B) Ceramide is transferred to the Golgi through ceramide transfer protein (CERT). There, sphingomyelin synthase (SMS) converts phosphatidylcholine and ceramide into sphingomyelin (SM) and diacylglycerol (DAG). DAG then acts as a binding site for protein kinase D (PKD), which in turn phosphorylates PI(4)-kinases [PI(4)K], which generate PI(4)P. CERT is able to bind PI(4)P and so transfer ceramide to the Golgi, where it acts as a substrate for the aforementioned SMS. As another feedback loop, PKD is also able to inhibit CERT, thus fine-tuning lipid levels at the Golgi.

Box 2. BAR-Domain Containing Proteins Involved in Autophagy

The BAR domain protein superfamily has been described as being crucial in several membrane-sculpting events [39]. By virtue of their ability to sense different membrane curvatures, BAR-domain containing proteins tightly orchestrate multiple intracellular trafficking pathways [10,40]. Three BAR-domain containing proteins have been implicated in autophagy.

Bif-1

Also known as SH3GLB1/endophilin B, Bif-1 belongs to the endophilin protein family characterized by the presence of an N-terminal N-BAR domain and a C-terminal SH3 domain. Initially discovered as a Bax-binding protein [41], Bif-1 has been shown to drive membrane curvature and liposome tubulation through the N-BAR domain [42]. *In vivo*, Bif-1 associates with the membrane compartment of different intracellular organelles, such as the Golgi complex or mitochondria, and regulates multiple membrane dynamics events and the formation of vesicles [42,43].

SNX18

Sorting nexin 18 (SNX18) is a member of the sorting nexin protein family characterized by a phox homolog (PX) domain, responsible for the binding of SNXs to PIs. SNX18 belongs to the PX-BAR protein subfamily and is involved in multiple membrane remodeling events of the endocytic system [44]. *In vitro*, SNX18 tubulates liposomes through the PX-BAR domain and shows the propensity to bind PI(4,5)P_2. *In vivo*, SNX18 binds dynamin 2 (DNM2) through its SH3 domain and mediates the formation of AP1-positive carriers, promoting DNM2 GTPase activity [44].

Arfaptins

Initially identified as ARF1-binding proteins [45], arfaptins localize at the TGN through the interaction with ARF-like 1 (Arl1) [46]; their binding to PI(4)P is driven by an amphipathic helix that precedes the BAR domain [47]. Arfaptins have been described as key regulators of several membrane remodeling events. *In vitro*, arfaptins can tubulate liposomes with different lipid composition [39]. *In vivo*, despite Arfaptin1 and Arfaptin2 sharing a homology of ~80%, they control distinct intracellular trafficking events. Specifically, Arfaptin1 regulates the formation of secretory granules [48], while Arfaptin2 has been described to regulate both the secretion of metalloproteinases-2 and -7, forming a complex with Arl1, PKD2, and GTP-bound ARF1 [49], and the function of ATG9A [16]. Furthermore, Arfaptin2 binds Rac1 and regulates the crosstalk between the Rac1 and Arl1 pathways [50].

Beyond N-terminal motif interacting proteins, the Rab-guanosine triphosphatase-activating protein (RabGAP) TBC1D5, the GAP for RAB7A, has been reported to interact with ATG9A and regulate its retrieval in AP2-clathrin-coated vesicles from the PM to the autophagosome formation sites (Figure 2Aiii) [33].

Interestingly, despite being expressed 40 times less in HeLa cells (HeLa) compared with AP1 or AP2 [34], the TGN-localized AP4 complex was recently described to have a key role not only in the movement of ATG9A vesicles during autophagy (Figure 2Aiv), but also in neuronal deficiencies [35–38]. AP4 deficiency leads to a form of hereditary spastic paraplegia (HSP) called AP4 deficiency syndrome, in which ATG9A has a central role. Retention of ATG9A within the TGN results in a decrease of axonal ATG9A, causing a reduction in the formation of autophagosomes along the axon and, consequently, in the neuronal soma. AP4 and ATG9A interact via the canonical tyrosine-based motif (^{8}YXXØD/E^{12}) [35]. Depletion of AP4 subunit epsilon (AP4ε KO) causes an accumulation of ATG9A at the TGN [35,36], which increases the levels of LC3B and the number and size of autophagosomes [35,36]. These data together suggest that AP4-dependent ATG9A sorting is a requirement for proper autophagy induction.

Considering the multiple interactions of ATG9A with different AP complexes described earlier, further studies are needed to understand the cellular stimuli and molecular determinants required for the specific sorting of ATG9A by each AP complex, as well as the differences between constitutive, steady-state ATG9A trafficking, and starvation or other induced ATG9A trafficking events mediated by the AP complexes.

ATG9A Trafficking via BAR-Domain Containing Proteins

In addition to AP complexes regulating ATG9A vesicle trafficking, it is also fine-tuned by different BAR-domain containing proteins (Box 2 and Figure 3). Bif-1, in conjunction with the UV radiation-resistance associated (UVRAG)/Beclin1 Vacuolar protein-sorting 34 (Vps34) complex II [51], was the first BAR-domain containing protein described to activate autophagy and was later reported to be a key player in the formation of ATG9A vesicles from the Golgi complex [52] and recycling endosomes [53]. Additionally, SNX18 has been reported as a PX-BAR containing protein involved in the formation of ATG16L1 and ATG9A-positive vesicles from recycling endosomes [54,55]. Recently, a mass spectrometry (MS) analysis of isolated ATG9A vesicles revealed the presence of Bif-1 and additional BAR-domain containing proteins Arfaptin 1 and 2 [16]. However, whether there is interplay between the different BAR-domain containing proteins participating in ATG9A vesicle trafficking remains an open question.

Bif-1 Regulates the Formation of ATG9A Vesicles from Recycling Endosomes in concert with Dynamin 2

Interaction of Bif-1 with Beclin1 through UVRAG enhances PI(3)KC3 complex II lipid kinase activity, and depletion of Bif-1 or PI(3)KC3 complex II prevents starvation-induced fission of Golgi membranes and blocks the redistribution of ATG9A from the Golgi to the ATG9 compartment, thus impairing autophagy [52]. Furthermore, it has been shown that Bif-1 interacts through its SH3-domain with dynamin 2 (DNM2) to regulate the formation of ATG9A vesicles that originate from the Rab11-positive recycling endosomes [53] (Figure 3). The N-BAR domain of Bif-1 is responsible for the formation of ATG9A-positive tubules, while the GTPase activity of DNM2 drives the membrane fission events that lead to the formation of ATG9A-positive vesicles. Given the close proximity of the Rab11-positive compartment to the TGN, it is reasonable to assume that a unique pool of ATG9A-positive membranes in the Rab11 compartment is sorted by Bif-1, while the effect of Bif-1 on the starvation-induced fission of Golgi membranes might be unrelated to ATG9A vesicle trafficking. However, this hypothesis has yet to be clarified.

SNX18 Promotes the Formation of ATG9A Vesicles from the Rab11-Positive Compartment via DNM2

SNX18 was initially linked to autophagy through regulation of ATG16L1 and LC3-II-positive membranes from the endosomal compartment [54]. On recycling endosomes, SNX18 directly binds LC3 and sorts it together with ATG16L1 to the perinuclear area. Further work showed that ATG9A and ATG16L1 trafficking from the tubular endosomal compartment to the autophagosome formation sites is dependent on SNX18 binding to DNM2 through its SH3 domain [55] (Figure 3). Loss of SNX18 restricts ATG9A to the juxtanuclear recycling endosomes, impairing autophagic flux. Intriguingly, SNX18 and Bif-1 share the capacity to bind DNM2 through the SH3 domain and induce membrane sculpting through the BAR domain. However, it is still unclear whether SNX18 and Bif-1 can cooperate or compete in the binding to DNM2 to regulate ATG9A vesicle trafficking from the Rab11-positive compartment.

Arfaptin2 Controls Initiation of Autophagy through ATG9A Vesicle Trafficking

A stable isotope labeling with amino acid in cell culture (SILAC) MS study of immunoisolated ATG9A vesicles revealed that Bif-1, Arfaptin1, and Arfaptin2 are specifically enriched on these vesicles during amino acid starvation [16]. However, Arfaptin2, and not Arfaptin1, regulates the initiation of autophagy through the trafficking of ATG9A vesicles between the Golgi complex and the ATG9 compartment (Figure 3). Depletion of Arfaptin2 or disruption of its ability to bind PI(4)P caused a pronounced dispersal of ATG9A from the juxtanuclear region, together with a

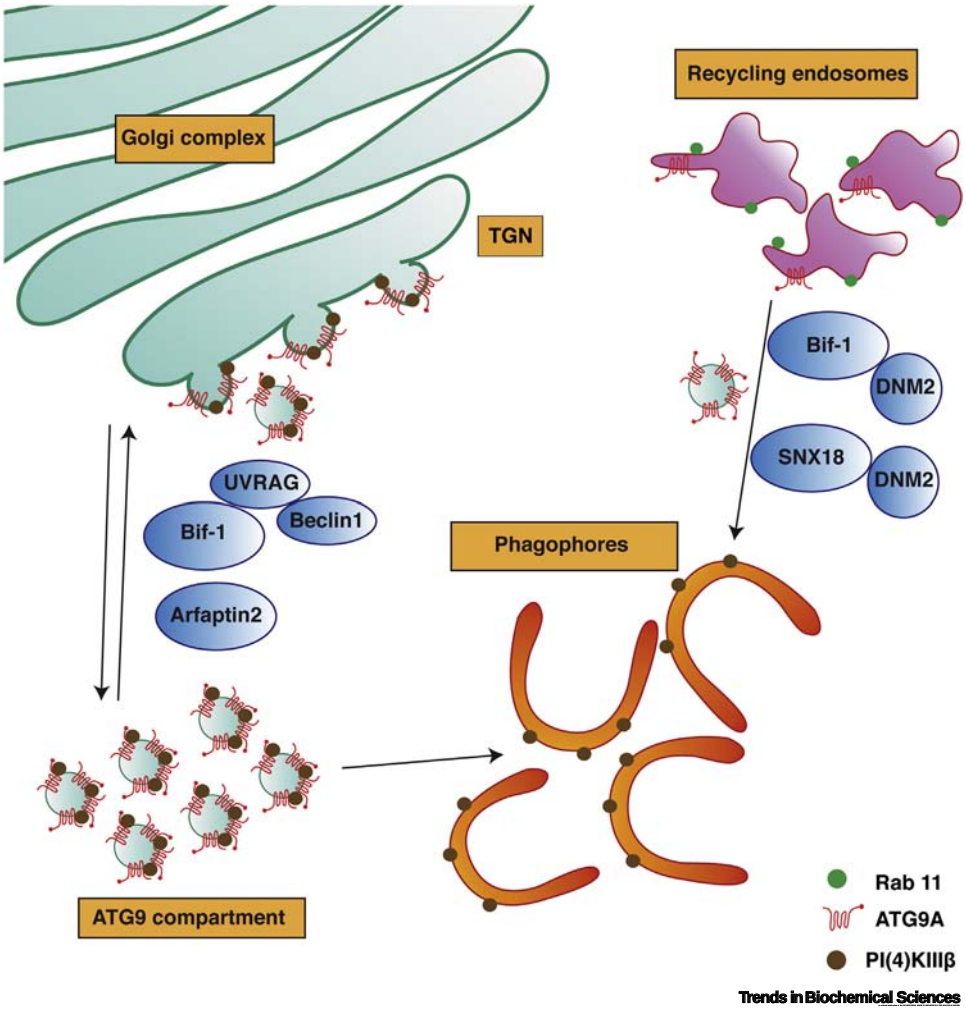


Figure 3. BAR-Domain Containing Proteins Control Autophagy-Related (ATG)-9A Vesicle Trafficking during Autophagy. ATG9A traffics between the Golgi complex, recycling endosomes, and the 'ATG9 compartment' to contribute to the formation of the autophagosome. Different BAR-domain containing proteins regulate its trafficking between the different compartments. ATG9A shuttling between the Golgi and the 'ATG9 compartment' is mediated by Bif-1 in complex with UV radiation-resistance associated (UVRAG), Beclin1, and Arfaptin2, which also controls the correct delivery of phosphatidylinositol-4-kinase (PI(4)K)-IIIβ to the phagophore through ATG9A vesicles. ATG9A trafficking from the recycling endosomes to the site of the nascent phagophores is controlled by Sorting nexin 18 (SNX18) and Bif-1 through the GTPase activity of dynamin 2 (DNM2).

reduced number of ULK1- and WIPI2-positive early autophagic structures, and LC3B-positive autophagosomes. Importantly, Arfaptin2 has been shown to drive the enrichment and activation of PI(4)P metabolizing enzymes on ATG9A vesicles [16]. Arfaptin2 also regulates the correct subcellular localization of PI(4)KIIIβ, the activation of the PI(4)KIIIβ by PKD2 phosphorylation at Ser294 [19,49], as well as the proper distribution of PI(4)P from the Golgi complex to the ATG9 compartment and the autophagosome initiation site. Active PI(4)KIIIβ is delivered by ATG9A vesicles to the autophagosome initiation site (Figure 3). Of note, together with PI(4)KIIIβ, PI(4)KIIα is also proposed to be delivered on ATG9A vesicles by Arfaptin2. However, PI(4)KIIα exclusively regulates the late stages of autophagy, suggesting that two distinct pools of PI(4)P can spatiotemporally regulate the different stages of the autophagosome formation.

Golgi to Autophagosome/Endolysosome Trafficking

PI(4)KIIα, required for the maturation of autophagosomes, is palmitoylated and resides in the TGN in basal conditions, but relocates to autophagosomes through interaction with GABARAPs (Figure 2) [15,56]. PI(4)KIIα and PI(4)P generated by this enzyme have been reported to be essential for autophagosome–lysosome fusion [15,57]. Interestingly, the trafficking of PI(4)KIIα from the TGN to autophagosomes might be dependent on the AP3 complex because PI(4)KIIα contains a conserved sorting signal (^{56}ERQPLL^{61}) necessary for its proper localization to endosomes and lysosomes (Figure 2Av) [58]. Furthermore, PI(4)KIIα was detected in ATG9A vesicles, and interacts with ATG9A under fed and starvation conditions [16]. This suggests that PI(4)KIIα traffics from the TGN to the autophagosome through AP3-derived ATG9A vesicles to increase PI(4)P production to promote autophagosome–lysosome fusion; however, ATG9A interaction with AP3 has not yet been described.

Interestingly, the Golgi is able to have a role in autophagosome and late endosome fusion with lysosomes independently of vesicular traffic. The *medial*- and *trans*-Golgi localized protein Golgi reassembly stacking protein 55 (GRASP55) is *O*-GlcNAcylation and involved in the formation of Golgi stacks (together with GRASP65). However, under glucose deprivation, *O*-GlcNAcylation of GRASP55 is reduced, and GRASP55 relocalizes to autophagosomes and lysosomes through an interaction with LC3-II and LAMP2. This interaction is thought to create a tether between autophagosomes and lysosomes to induce their fusion [59].

Endolysosomal Pathway and Autophagy

Recycling endosomes and endosomal function are crucial to provide additional membrane sources for autophagosome formation and maturation. Underlying this crucial function is the connection between the Golgi complex and endosomes, which supports ATG9A vesicle trafficking.

Recycling Endosomes, Rab11, and the TRAPPIII Complex Regulate ATG9A Activity

The Rab11 effector, TBC1D14, a member of the Tre-2/Bub2/Cdc16 domain-containing RabGAPs, has been identified as a regulator of starvation-induced autophagy [60]. Overexpression of TBC1D14 induces tubulation of ULK1-positive recycling endosomes (REs), which is dependent on active Rab11, preventing their function in the autophagosome formation. TBC1D14 interacts with the trafficking protein particle III (TRAPPIII) complex through TRAPPC8, the mammalian ortholog of the yeast-specific autophagy subunit Trs85 [61]. TBC1D14 and TRAPPIII are required for activation of Rab1, localized to the Golgi complex; disruption of the TRAPP complex induces ATG9A dispersal. Thus, TRAPPIII, Rab1, and Rab11 are crucial for proper trafficking between the Golgi and the endosomal compartment, allowing a steady-state cycling of ATG9A to support the initiation of autophagosome formation. Interestingly, overexpression of SNX18 (also important for ATG9A trafficking) tubulates Rab11-positive REs [54,55], promoting the formation of ATG16L1 and LC3-II-positive membranes. Both TBC1D14 and SNX18 can shuttle between the REs and the Golgi complex, suggesting that this trafficking pathway, which may support the delivery of additional Golgi components (lipids or proteins) as well as ATG9A, is required for autophagosome formation.

Retromer Complex in ATG9A Trafficking and Autophagy

Recycling of membrane proteins and/or receptors from the endosomal network to Golgi or PM requires the Retromer complex [62–64]. Several regulators of this complex, such as TBC1D5 and the WASP and Scar homolog (WASH) complex, participate in autophagy. During autophagy, TBC1D5 relocates from Retromer and binds to LC3-II on autophagosomes via a LIR motif, potentially acting as a switch between endocytosis and autophagy [65,66]. Furthermore,

TBC1D5 and Retromer can interact with ATG9A and AP2 and influence ATG9A trafficking and autophagy [33].

Parkinson's disease can be caused by an autosomal-dominant mutation in VPS35 (a Retromer subunit) (D620N) [67], which destabilizes the WASH–Retromer interaction and, therefore, leads to autophagy dysregulation. Interestingly, ATG9A trafficking is also dysregulated in VPS35 mutant cells [68]. However, the role of retromer in ATG9A trafficking is still not clear, because depletion of VPS26 does not affect ATG9A redistribution under starvation, even though they partially colocalize [3].

As mentioned earlier, the WASH complex, involved in actin assembly on endosomes [69,70], regulates Retromer activity. However, the role of WASH in autophagy is still controversial: it has been reported to be necessary not only for autophagosome formation [68], but also as a negative regulator [71,72]. WASH knockdown decreases autophagy and alters ATG9A localization, suggesting a possible entrapment of ATG9A in REs due to Retromer dysregulation [68]. WASH has also been reported to regulate autophagy through control of ubiquitination of both AMBRA1 and Beclin1 [components of the PI(3)KC3 complex], which are essential for promoting VPS34 PI(3) kinase activity and autophagy [71,72].

Outstanding Questions

How do ERTGoCS and lipid trafficking to and from the Golgi affect autophagy?

Could PI-metabolizing enzymes regulate distinct stages of autophagy or recruit specific autophagy mediators, for example, through the regulation of different pools of PI(4)P?

What cellular stimuli mediate the recognition of ATG9A by different adaptor (AP) complexes for its trafficking?

Do the BAR-domain containing proteins, Arfaptin2, BIF-1, and SNX18, cooperate in the regulation of ATG9A vesicle trafficking?

Concluding Remarks

The Golgi complex has been long recognized as one of the most diverse organelles because of its central functions in the cell; it can distil traffic from the ER, moderate ERTGoCS, be a receiver for endosomal activity, and provide properly modified lysosomal enzymes. These functions are in addition to its essential role in glycosylation of proteins. Here, we have highlighted the role of the Golgi in autophagy, in particular, in the regulation of ATG9A localization. One future challenge is to unpick the well-documented effects on autophagy resulting from the perturbation of Golgi–endosomal trafficking to uncover direct mechanistic insights. This will provide knowledge to understand how the Golgi complex controls both proteins and lipids to regulate autophagy and will no doubt further reinforce the critical role of the Golgi complex (see Outstanding Questions).

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