BBA - Molecular and Cell Biology of Lipids 1868 (2023) 159334

Contents lists available at ScienceDirect

BBA - Molecular and Cell Biology of Lipids

journal homepage: www.elsevier.com/locate/bbalip

Review

Impact of sphingolipids on protein membrane trafficking

Auxiliadora Aguilera-Romero *, Rafael Lucena, Susana Sabido-Bozo, Manuel Muñiz *

Department of Cell Biology, Faculty of Biology, University of Seville, 41012 Seville, Spain
Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Spain

ARTICLE INFO

Keywords:
Sphingolipids
Protein sorting
Membrane trafficking
Secretory pathway
Endocytic pathway

ABSTRACT

Membrane trafficking is essential to maintain the spatiotemporal control of protein and lipid distribution within membrane systems of eukaryotic cells. To achieve their functional destination proteins are sorted and transported into lipid carriers that construct the secretory and endocytic pathways. It is an emerging theme that lipid diversity might exist in part to ensure the homeostasis of these pathways. Sphingolipids, a chemical diverse type of lipids with special physicochemical characteristics have been implicated in the selective transport of proteins. In this review, we will discuss current knowledge about how sphingolipids modulate protein trafficking through the endomembrane systems to guarantee that proteins reach their functional destination and the proposed underlying mechanisms.

1. Introduction

The eukaryotic cell is compartmentalized into membrane-bound organelles defined by a specific set of proteins and lipids. This compositional heterogeneity is maintained by an elaborated endomembrane system that builds up the secretory and endocytic pathways [1]. Along these pathways, each type of protein is transported and directed to its correct functional destination in a fundamental process known as sorting [2]. Deficiencies in this process cause protein mistargeting and induce defects in the establishment of cell polarity, immunity, and other physiological processes that have been associated with genetic pathologies and neurodegenerative diseases [3,4].

The sorting of proteins along the endomembrane systems is intrinsically linked to vesicle biogenesis. These lipid carriers are generated by cytosolic protein coats that bend the donor membrane, concomitantly sorting a selected group of cargo proteins, from resident proteins and incorporating them into the nascent vesicle. The interaction between cargos and coats can be direct, by specific recognition of sorting signals in the sequence of the protein, or indirect through receptor or adaptor proteins that link the coat complexes with the transported protein [2,5]. Because coat-based protein sorting is not the focus of this review, the reader is referred to [6–8] for a deeper understanding. The coat-driven hypothesis does not explain the existence of uncoated vesicles [9] or how cargo proteins lacking a known signal to interact with the coat machinery arrive at their destination [5,10]. Therefore, in addition to cargo capture by the vesicle coat, other mechanisms must contribute to protein sorting. In this sense, certain membrane lipids, such as sphingolipids, have been suggested to play a key role in protein sorting within endomembrane systems [11].

Due to their physicochemical characteristics, sphingolipids interact more favorably with each other and with sterols than with other lipids. This behavior has led to the proposal of a theoretical lipid-based mechanism where highly saturated sphingolipids and sterols can induce the formation of tightly and thicker packed membrane domains, named rafts, that float in a fluid bilayer of unsaturated glycerophospholipids. These lipid-ordered domains would then recruit and laterally segregate specific proteins, acting as sorting platforms for protein export in selective transport carriers [12]. The correlation between sphingolipid enrichment and the increase in the length of the transmembrane domain of proteins along the secretory pathway [13,14] suggests that hydrophobic mismatches between the lipid bed and the transmembrane region of the protein could operate the specific sorting of proteins into the ordered domains formed by sphingolipids and sterols. Proteins with shorter domains are found in the thin and relatively loosely packed ER membrane, while those with longer domains preferably localize in the thick and tightly packed membrane, enriched in sphingolipids, of the late secretory pathway [15]. In addition to the length, recent studies combining biophysics with molecular simulations indicate that for single-pass transmembrane proteins, a thinner surface area of the transmembrane domain and post-translational modifications, such as protein palmitoylation, are important parameters to promote lateral segregation of proteins in most ordered lipid domains [16].

* Corresponding authors.
E-mail addresses: auxi@us.es (A. Aguilera-Romero), mmuniz@us.es (M. Muñiz).

https://doi.org/10.1016/j.bbalip.2023.159334
Received 21 February 2023; Received in revised form 28 April 2023; Accepted 10 May 2023
Available online 17 May 2023

The original raft hypothesis considers proteins as passive passengers with minor impact on the lipid environment around them, being lipid-lipid interactions the main force that drives protein sorting. Nevertheless, the interplay between proteins and lipids has become more complex [17]. Integral membrane proteins have been proposed to determine membrane thickness instead of cholesterol [13,18]. That is, the transmembrane protein would recruit specific lipids around its transmembrane domain to avoid hydrophobic exposure and not the other way around as the raft hypothesis proposes. Notably, the multivalent binding of some toxins to the head of glycosphingolipids promotes local lipid reorganization and bending of the membrane [19]. The development of click chemistry combined with lipidomic analysis has identified transmembrane domains that specifically recognize a particular sphingolipid species [20]. And molecular simulation studies postulate that some transmembrane domains could facilitate the asymmetry of the lipid bilayer, by the local enrichment of specific lipids in one of the leaflets of the membrane [21,22]. Therefore, proteins might also sense and influence their lipidic environment, indicating that more sophisticated molecular interactions between lipids and proteins determine protein transport.

This review attempts to summarize recent insights into how the rich chemical diversity of sphingolipids affects the sorting and trafficking of proteins in endomembrane systems.

2. Sphingolipids in a shot

The main lipids proposed to coalesce to generate the mentioned raft domains are sterols, saturated glycerophospholipids, and sphingolipids [11]. Lateral segregation of lipids is inherent in sphingolipids because of their specific biophysical properties [23]. This special class of lipids comprises a small but critical fraction (10–20 %) of membrane lipids that build eukaryotic cells [24,25]. Their synthetic pathway starts when the enzyme serine palmitoyl transferase condenses a serine to a fatty acid to generate the backbone of all sphingolipids: an acyl amino alcohol or sphingoid base. This is attached to a fatty acid *via* the amino group at the Carbon-2 (C2) position to generate ceramides and to a head group *via* de Carbon-1 (C1) to produce complex sphingolipids [26,27]. The chemical diversity of sphingolipids is determined by the combinatorial power of these three components. Based on the polar head group, they are classified as ceramides, phosphosphingolipids, or glycosphingolipids. The phosphosphingolipids, *e.g.*, sphingomyelin in animals or inositol phosphoceramide (IPC) in plants and fungi, carry the polar head groups phosphocholine and phosphoinositol, respectively. The glycosphingolipids contain a variety of monosaccharides linked by specific glycosidic bonds. The sphingoid base usually consists of a straight-chain amino alcohol of 18 to 20 carbons that can be hydroxylated or bear some unsaturations. The amide-linked fatty acid increases the structural diversity of this family, with a chain length that can vary from 14 to 26 carbons and several degrees of unsaturation and hydroxylation [28,29]. Additionally, it has been described a non-canonical pathway of sphingolipid *de novo* synthesis, which uses an alanine or glycine instead of serine to give rise to a special type of toxic sphingolipids named deoxysphingolipids, which cannot be degraded by the canonical sphingolipid catabolism [30].

The biophysics of sphingolipids that explain its special behavior in membranes is determined by the following features: 1) the interfacial region that links the nonpolar hydrocarbon region to the polar head group of sphingolipids is prompted to create highly flexible hydrogen-bonded networks. The amide bond and the hydroxyl groups function as hydrogen donors or acceptors, enhancing hydrogen bonding between sphingolipids, which is not available in glycerophospholipids [31]. 2) Some sphingolipids species, such as that bearing long acyl chains from 22 to 26 carbons, are asymmetric: the sphingoid base extends less into the membrane than the fatty acid. This could create packing defects in the interior of the membrane that could be solved by interdigitation between sphingolipids or glycerophospholipids localized in opposite leaflets of the membrane bilayers [32]. 3) The fatty acyl chain of sphingolipids is more extended than monounsaturated phosphoglycerolipids; therefore, sphingolipids have a greater surface that will increase Van der Waals interactions between neighboring lipids [32,33]. 4) While ceramides have a unique capacity to flip-flop and induce the transbilayer movement of adjacent lipids [31], sphingolipids with complex head groups such as the oligosaccharide of gangliosides reduce trans-leaflet "flip-flop", trapping complex sphingolipids in the outer leaflet of cell membranes and promoting membrane asymmetry [34].

3. Sphingolipids in protein trafficking in the early secretory pathway

The secretory pathway synthesizes and regulates the delivery of membrane lipids and a third of the eukaryotic proteome to their proper subcellular localization [1]. At the beginning of this pathway, regulated bidirectional vesicular transport maintains homeostasis between the ER and the Golgi apparatus. Anterograde transport transfers the new proteins synthesized at the ER to the Golgi apparatus, and retrograde transport recycles back to the ER the machinery required for future rounds of transport. Two types of vesicles mediate these complementary routes; COPII vesicles carry out anterograde transport while COPI builds vesicles for retrograde transport. The role of sphingolipids in these steps is mainly related to the transport of a family of proteins anchored to the membrane by Glycosylphosphatidylinositol (GPI). However, its implication in the early stages could be greater, as the lack of the protein cTAGE5, which helps sort collagen in COPII carriers, made cells more sensitive to decreased sphingolipid levels [21].

In this section, we summarize two examples that highlight two different mechanisms by which sphingolipid diversity might impact the transport events occurring between the ER and the Golgi apparatus.

3.1. GPI-anchored protein sorting from the ER

GPI-APs are surface proteins conserved across eukaryotes with multiple functions; they participate in cell adhesion and communication, immune responses, directional growth, or reproduction [35–37]. The common feature of this striking family of proteins is the attachment to the external leaflet of the plasma membrane by a glycolipid motif, the GPI-anchor. This post-translational modification acts as an export signal that defines their destiny and leads GPI-APs to be trafficked separately from transmembrane proteins along the secretory pathway [38,39]. The synthesis of GPI-APs starts in the ER, where the newly-synthesized protein is covalently bound through a phosphoethanolamine to the glycan core of the anchor, which is linked to the luminal leaflet of the membrane by an unsaturated lipid (phosphatidylinositol or etherphosphatidylinositol). Once the protein is attached to the glycolipid, the immature unsaturated form of the anchor is remodeled to a saturated lipid that varies between phosphatidylinositol, etherphosphatidylinositol, or ceramide, depending on the organism [37,40]. Acquisition of these saturated lipids changes their physicochemical characteristics, increasing their appetence for more ordered domains and enhancing their segregation from other transmembrane proteins.

In budding yeast, the GPI-lipid undergoes complete structural remodeling in the ER. The mature lipid usually carries a long saturated glycerophospholipid or a ceramide with a very long-chain saturated fatty acid of 26 carbons (C26:0) [40]. Once remodeled, GPI-APs are segregated and sorted from transmembrane cargo proteins into special subdomains of the ER membrane called ER exit sites (ERES). When concentrated in these domains, they are packaged into specific COPII vesicles that transport them to the Golgi [41]. Although the exact mechanism that sorts GPI-APs from transmembrane proteins during ER export was unknown, a role for sphingolipids was suggested. Firstly, the GPI lipid remodeling allows the biochemical isolation of GPI-AP in detergent-resistant membrane fractions [42–44], which had been

proposed to reflect their clustering in domains enriched with ceramide. Secondly, ER export of GPI-APs requires ongoing ceramide synthesis [45–47]. Combining a yeast genetic system with super-resolution confocal live imaging microscopy (SCLIM), Rodriguez-Gallardo and collaborators were able to observe, *in vivo*, the entry of newly synthesized cargos into ERES at nanoscale dimensions. Using Gas1-GFP, a yeast model GPI-AP having a C26 ceramide-based GPI-lipid moiety [45] they showed that, *in vivo*, the C26 ceramide present in the ER membrane drives the specific clustering and sorting of GPI-APs into selective ERES different from those that contain other secretory proteins, such as the transmembrane plasma membrane protein Mid2 [22]. Decreasing the length of the n-acyl chain of cellular ceramide in the membrane from very long chain C26 to shorter C18-C16 disrupted Gas1-GFP clustering and rerouted this protein to Mid2 containing ERES. The observed lipid sorting mechanism was independent of the cytosolic coats and was mediated by their cargo-protein receptor, the p24 complex. GPI-APs are completely luminal proteins that require a protein that connects them to the cytosolic coats to ensure their loading in the nascent COPII vesicle. Interestingly, molecular dynamics simulations show that the transmembrane region of one of the yeast p24 family proteins, Emp24, concentrates ceramides around the cytosolic leaflet of the transmembrane helix. Therefore, it was proposed that the p24 complex would facilitate the segregation and sorting of Gas1 into specific ERES through concomitant interactions with C26 ceramides in both leaflets of the membrane. The free C26 ceramides enriched in the cytosolic leaflet of the membrane by the transmembrane domain could interdigitate with the C26 ceramides of the GPI-anchor in the luminal leaflet to solve packing defects. The ability of very long chain ceramides to interdigitate can remodel membranes by promoting high curvature and membrane bending [46]. This biophysical characteristic of very long chain ceramides could explain how the p24 complex remodels the membrane to facilitate the segregation and sorting of Gas1 into specific vesicles (Fig. 1). The presence of ceramides on both sides of the lipid bilayer seems to be key since sorting of Gas1 also requires a ceramide-type GPI-anchor [47].

It remains unclear whether this sorting mechanism in the ER is conserved in mammalian cells. Although a minor fraction of GPI-APs has been associated with detergent-resistant domains in the ER in a cholesterol-dependent manner [48,49], differential sorting of GPI-APs at the ER, as shown in yeast, has not yet been seen in mammalian cells [50]. Unlike yeast, which usually has a very long chain-ceramide-based GPI anchor, GPI-APs of mammalian cells carry an ether lipid [40] and remodeling of the GPI-lipid portion is carried out in the Golgi apparatus [43]. Nevertheless, a recent study found that ceramides and ether lipids are coregulated and that these two classes of lipids evolutionarily share some similar physicochemical properties and functions [21]. Further investigation is needed to study whether ether lipids in mammalian cells might function similarly to ceramides in the regulated export from the ER of GPI-APs.

3.2. Retrograde COPI transport of p24 proteins

The members of the family of p24 proteins are conserved type I transmembrane proteins that cycle between the ER and the Golgi apparatus. In addition to being the specific receptor for GPI-APs and other cargo proteins [51], they display other functions in the early secretory pathway. Incorporation of p24 proteins into COPII vesicles selectively promotes receptor-mediated sorting of secretory cargos [52,53], and are significant players in COPI vesicle biogenesis [54,55].

The association of these proteins with sphingolipids is not only related to the transport of GPI-APs; in mammals, the first intramembrane lipid binding pocket was characterized in the p24 member TMED2. The molecular species determining motif recognizes specifically a sphingolipid, sphingomyelin with an n-acyl chain of 18 carbons (SM-C18) [20]. The functional consequences of this binding have been related to the biogenic function of TMED2 in the formation of COPI vesicles. In the cell, TMED2 alternates between inactive monomeric and active dimeric forms [56]. Specific binding of SM-C18 to the transmembrane domain of TMED2 promotes its dimerization. Then, the dimeric form specifically recruits the first effectors of the COPI vesicle, Arf1 and COPI coats, with subsequent membrane deformation and cargo incorporation that finally built up the COPI carrier (Fig. 2) [56]. New data combining *in vivo*, *in vitro*, and *in silico* studies show that the SM-C18 binding pocket partially overlaps with a conserved sequence that appears key for self-transmembrane association. In this study, SM-C18 does not enhance but interferes with the dimerization propensity of TMED2 in a cholesterol-enriched membrane [57]. This new data show that the mechanism underlying SM-C18 role in p24 protein transport remains open for investigation.

The tight association between TMED2 and SM-C18 has been reinforced by the finding that a decrease in ether lipids specifically affects the amount of SM-C18 in HeLa cells. Ether lipids have been proposed to be enriched around the luminal region of the TMED2 transmembrane domain [21]. Therefore, a decrease of membrane ether lipids could impact the functioning of p24 complexes at the early secretory pathway which in turn would lead to the degradation of SM-C18 in the lysosome [21].

These results suggest that different binding mechanisms between lipids and proteins could play a significant role in the tuning of protein transport at the early stages of the secretory pathway [21].

4. Sphingolipids in protein trafficking at the late secretory pathway

Once cargo proteins arrive at the Golgi apparatus, they are eventually processed and distributed in transport carriers to their functional destination. In the following, we present the newest evidence supporting the role of sphingolipids in transport events at the latest steps of the secretory pathway, drawing attention to examples that show the

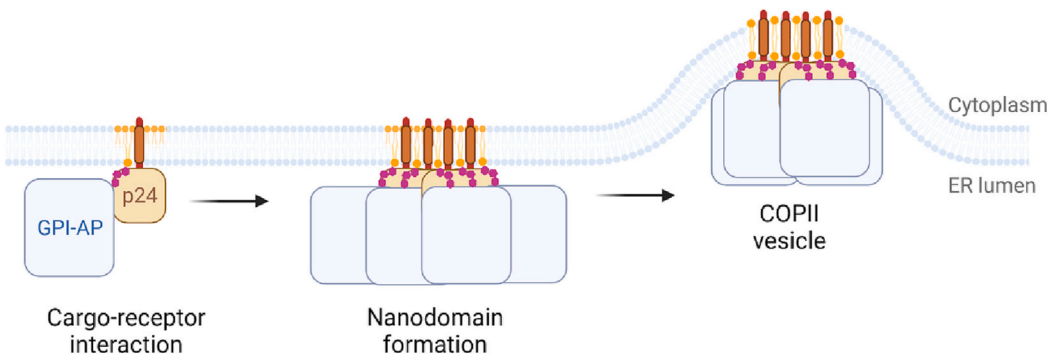

Fig. 1. Schematic representation of the proposed mechanism for the sorting of ceramide-containing GPI-APs (GPI-AP) into specific COPII vesicles in yeast. The p24 complex promotes local phase separation and acyl chain interdigitation by concentrating C26 membrane ceramides (orange) in the cytosolic leaflet and C26 ceramide-based GPI-APs in the luminal leaflet of the ER membrane. This leads to protein clustering and membrane curvature that segregates GPI-APs into discrete zones next to selective ERES, which leads to their incorporation into a specific COPII vesicle.

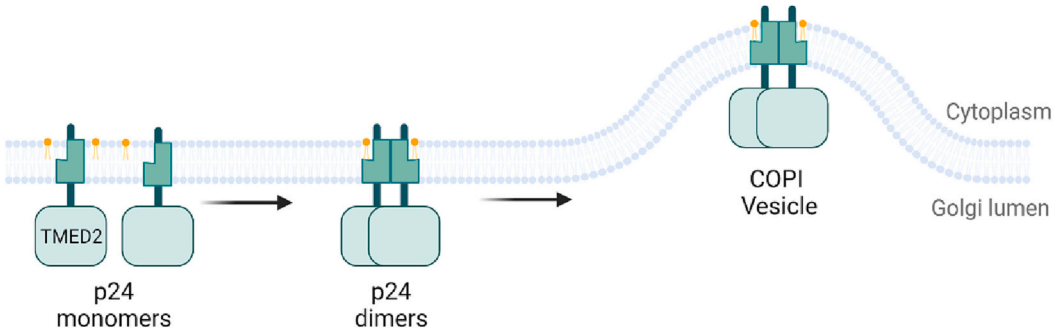

Fig. 2. Suggested mechanism for the sorting of p24 protein, TMED2, into COPI vesicles. The specific binding of SM-C18 (orange) to the transmembrane domain of TMED2 promotes its dimerization, which triggers the biogenesis of the COPI vesicle.

importance of sphingolipid chemical diversity in the sorting and destination of proteins.

4.1. Protein export from the Trans-Golgi network

The Trans-Golgi network (TGN) is the major cargo sorting station of the secretory pathway. A heterogeneous group of cargo proteins is segregated and packed into lipid vesicles and directed to the apical or basolateral plasma membrane, endosomal compartments, or secretory vesicles [8]. The first evidence supporting a lipid-based protein sorting was the specific enrichment in sphingolipids and cholesterol of vesicles derived from the TGN [58]. Further studies found that, in mammalian cells, sphingomyelin is sorted into specific post-Golgi vesicles [59]. In plants, VLFA-sphingolipids (very long fatty acid sphingolipids) are specifically enriched in subdomains in the TGN [60] and ceramides participate in vesicle biogenesis at the TGN [61].

Two new studies support the role of sphingolipids in protein sorting at the late secretory pathway. On one side, sorting and subcellular distribution of the Down syndrome cell adhesion molecule (Dscam) proteins of *Drosophila melanogaster* is dependent on sphingolipid biosynthesis. Dscam proteins are essential for neuronal development at early stages because they are implicated in the self-avoidance of growing axons and dendrites, and proper axonal connectivity. Isoforms of these proteins that differ in the length of their transmembrane domain are sorted and transported by distinct vesicles to the dendritic or axonal membrane region of the neuron. Sphingolipids are major components of the vesicles that specifically transport Dscam proteins with longer transmembrane domains to the axon of the neuron (Fig. 3) [62]. On the other side, in polarized cells, the differential export of the lipoprotein lipase mediated by its protein receptor, syndecan-1, is also dependent on sphingomyelin-enriched vesicles. However, the surface area of the transmembrane domain of syndecan-1 and not its length is the driving force that sorts syndecan-1 and the bounded lipoprotein lipase in the sphingomyelin secretion pathway [33].

Another type of cargo protein classically related to sphingolipids in the late secretory pathway is the GPI-AP. As mentioned in the previous section, in yeast, sphingolipids play a crucial role in the sorting of ceramide-based GPI-APs at the ER. In mammals, however, a role for sphingolipids was initially proposed in the sorting of GPI-APs in the late secretory pathway. Inhibition of sphingolipid synthesis affects the apical delivery of GPI-APs in polarized cells [63] and remodeled GPI-APs have been found in detergent-resistant membranes [64], which suggests that they could cluster into sphingolipid-enriched domains [11]. However, contrary to the proposed hypothesis further experiments showed that the differential transport of GPI-APs to the apical plasma membrane from Golgi was independent of sphingolipids and remodeling of the GPI anchor to a saturated fatty acid [65,66]. Indeed, the lack of sphingolipids affected both GPI-APs and other transmembrane proteins targeted to the plasma membrane [67,68]. Therefore, sphingolipids might drive, in general, the apical *versus* basolateral sorting of plasma membrane proteins and not specifically of GPI-APs.

The clustering and apical sorting of GPI-APs have been associated with the n-glycosylation [65] and the oligomerization state of the protein [69]. GPI-AP homocluster formation, which subsequently facilitates their apical sorting, is calcium-dependent [70]. The underlying mechanism involves the soluble luminal Ca^{2+}-binding Golgi-resident protein, Cab45, an abundant cargo of mammalian sphingomyelin-enriched vesicles. Cab45 is a central regulator of cargo sorting at TGN that recognizes and concentrates other luminal cargos in a calcium-dependent manner, prior to vesicle formation [71]. The Cab45 function is regulated by the TGN-resident calcium/manganese pump, secretory pathway Ca^{2+}-ATPase pump type 1 (SPCA1), whose activity is regulated by sphingomyelin synthesis [72]. Thus, SPCA1 activity enhanced by synthesis and local enrichment of sphingomyelin within the TGN membrane would define TGN sorting domains and cargo exit sites (Fig. 4).

A mechanism that also suggests sphingolipid synthesis as a regulator

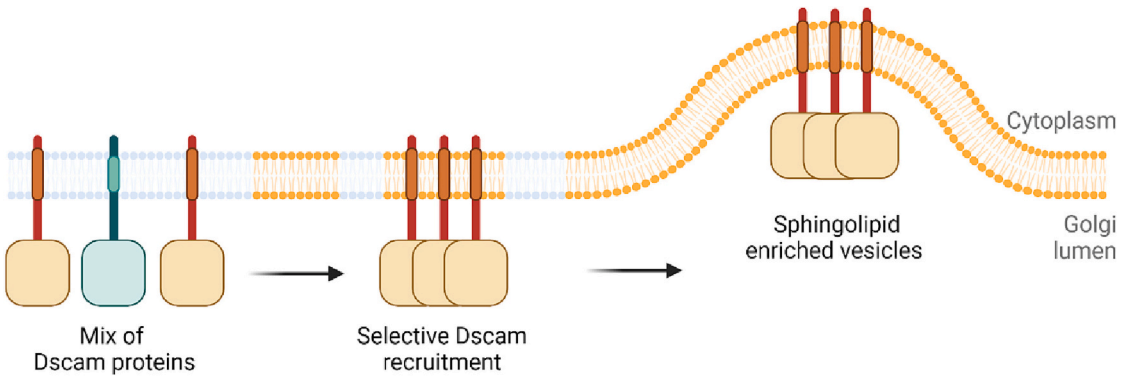

Fig. 3. Proposed sorting mechanism of Dscam proteins at the TGN. Dscam proteins with longer transmembrane domains would be recruited into enriched sphingolipid nanodomains (orange), promoting its sorting into sphingolipid-enriched vesicles.

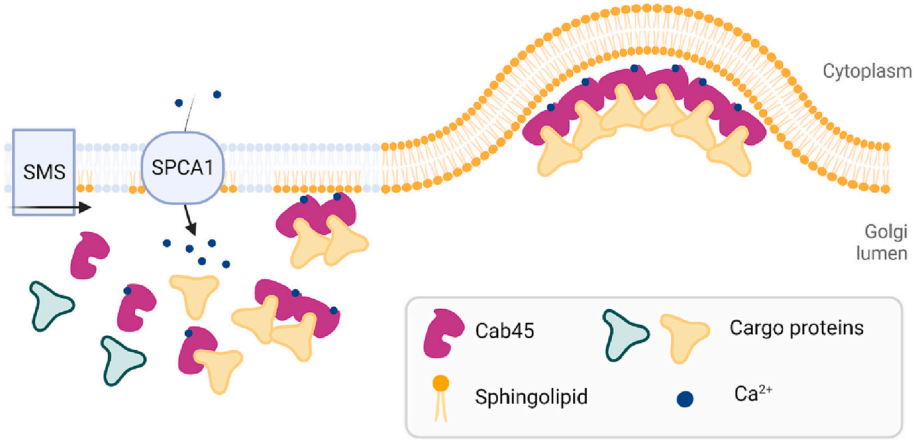

Fig. 4. Model of the Cab45-dependent protein sorting mechanism at the TGN. The synthesis of sphingomyelin produced by the sphingomyelin synthase (SMS) regulates the activity of the SPCA1 calcium pump, generating a local enrichment of calcium in the lumen of the TGN. The soluble Golgi protein Cab45 binds to calcium, oligomerizing and binding specific cargo molecules. These secretory cargos are then sorted for secretion into vesicles enriched in sphingomyelin.

of vesicular protein transport, has been described for the auxin carrier PIN2 in plants. Local synthesis of glycosylinositolphosporylceramides might moderate phosphoinositide-specific phospholipase C (PI-PLC) activity impacting PI4P homeostasis. The imbalance in PI4P metabolism specifically affects PIN2 sorting at distinct subdomains of the TGN [73]. But how is SPCA1 or PI-PLC regulated by sphingolipid synthesis? Deng and collaborators discuss the possibility that sphingomyelin acts as an agonist by binding to a site in SPCA1 that activates calcium pumping to the lumen of the TGN. If so, we could have a mechanism similar to that found in TMED2. SPCA1 might have a sphingolipid-binding domain that recognizes a specific specie of sphingomyelin or a product of its metabolism, such as diacylglycerol. How sphingomyelin signaling and its structural features are potentially involved in secretory cargo sorting remains to be elucidated.

Despite the multiple efforts to understand the role of sphingolipids in protein transport at the TGN, only a few studies address the importance of sphingolipids diversity in this process. Research on plants has pointed out that the chemical nature of sphingolipids determines the sorting mechanisms that occur in the trans-Golgi network of auxin transporters [74]. The family of auxin transporters, PIN, are essential proteins in plant physiology. The subcellular localization of these transmembrane proteins determines the directionality of the polar auxin transport and plays a key role in the asymmetric distribution of auxin and plant development [75]. Sorting into different subdomains of the TGN of two auxin carriers of *Arabidopsis thaliana* PIN1 and PIN2 seems to be dependent on sphingolipids. On the one hand, as mentioned before, the synthesis of very long-chain glycosylinositolphosporylceramides is implicated in PIN2 sorting at SV/TGN subdomain [73]. On the other hand, very long-chain ceramides have been proposed to modulate the specific sorting of PIN1 at RAB-A2a-positive/CCVs compartments in the TGN [74].

4.2. Extracellular vesicle formation

Sphingolipids have also been shown to play a role in exosome biogenesis. Exosomes are extracellular vesicles that function in intercellular communication, allowing cells to exchange specific cargoes, including proteins, lipids and RNAs [76]. As early endosomes mature into late endosomes or multivesicular endosomes, cargoes are sorted on the endosomal membrane, which then buds inward to form intraluminal vesicles. These vesicles are secreted as exosomes into the extracellular space when multivesicular endosomes fuse with the plasma membrane [77]. Mechanisms controlling cargo selection and intraluminal vesicle budding can be dependent or independent of the endosomal sorting complex required for transport (ESCRT). An ESCRT-independent mechanism of exosome biogenesis involves the generation of ceramide from sphingomyelin by the neutral sphingomyelinase II (nSMase2) [78]. Trajkovic et al. showed that in a mouse oligodendroglial cell line, ceramide produced by nSMase2 is required for the sorting of proteolipid protein (PLP) into exosomes enriched in ceramide. Consistently, the same study also showed that ceramide generation by nSMase2 in giant unilamellar vesicles (GUVs) leads to the formation of intravesicular membranes. The authors propose that locally produced ceramide could then form ceramide-enriched microdomains, which recruit and laterally segregate specific cargo and simultaneously bend inward the endosomal membrane by imposing a spontaneous negative curvature due to the characteristic cone-shape of ceramide. Interestingly, it has been shown that the ceramide produced by the nSMase2 not only stimulates exosome biogenesis but also their secretion by decreasing endosomal acidification [79]. This study suggests that ceramide disrupts the assembly of the multimeric vacuolar-ATPse proton pump, required for endosomal acidification, by specifically sequestering and incorporating the vacuolar-ATPase subunit V0A1 into exosomes. Likewise, an analogous neutral sphingomyelinase-dependent mechanism has been recently identified to drive a non-conventional exosome biogenesis pathway at the nuclear membrane in activated neutrophils. In these cells, ceramide generated by neutral sphingomyelinase 1 (nSMase1) is required to form non-conventional exosomes at the nuclear envelope containing the signaling lipid mediator leukotriene B4 and its synthesizing enzymes [80].

4.3. Sphingolipids in virus assembly

The human immunodeficiency virus 1 (HIV-1) belongs to the family of enveloped viruses. This virus depends on host cell lipids to fulfill their assembly and replication functions. The envelope of HIV-1 is enriched in different phosphoinositides, phosphatidylserine, sphingomyelin, and cholesterol. The proposed mechanism for the sorting of these specific host lipids and the viral-associated proteins implies lipid-based phase partitioning induced by the viral protein Gag. This protein binds the inner membrane of the PM through its myristoyl chain and interactions with negatively charged lipids such as phosphoinositides. The multimerization of the protein facilitates lipid partitioning that eventually results in transbilayer coupling of acyl chains promoting the formation of more ordered domains. Subsequently, lipids and proteins with affinity for ordered lipid environments would be sorted on both leaflets of the bilayer into the assembly site [81]. Little is known about the impact of sphingomyelin in this protein sorting event. Because sphingomyelin is one of the major constituents of the viral membrane, Tafesse and coworkers decided to evaluate whether the acyl chain length of the

sphingomyelin regulates the assembly, release and replication of the virus. They used a cell lacking the ceramide synthase 2 that has a strong decrease in levels of long-chain (C22–24) sphingomyelins and hexosylceramide lipids. Their results reveal that very long-chain sphingomyelins are not essential for sorting proteins into the membrane envelope but they do have an important role in downstream functions such as fusion and replication of the virus [82].

5. Sphingolipids in protein trafficking at the endocytic pathway

Eukaryotic cells interact with their environment through the plasma membrane. The lipid and protein composition of this selective barrier is controlled by the endocytic pathway that also internalizes material from the extracellular environment. The endocytic processes couple mechanical deformation and bending of specific membrane domains to protein cargo capture to finally produce the transport carrier. The bending of the membrane can be coat-dependent or independent [83]. Although the clathrin coat-mediated vesicle pathway is by far the best characterized of the endocytosis processes, in this review, the focus will be on the studies that claim a role for sphingolipids in the sorting and transport of cargo in clathrin-independent carriers.

5.1. A tale of toxins, viruses, and galectins

The discovery of the sphingolipid binding domain of HIV-1, PrP (prion) and beta-amyloid peptide (Alzheimer) that recognizes the oligosaccharide moiety of galactosyl ceramides and sphingomyelins [84] uncover the potential of the polar head groups of sphingolipids as regulators of protein activities and transport.

A special group of glycosphingolipid binders is the bacterial Shiga toxin (ST) and Cholera toxin (CT), and the fold capsid protein VP1 of Simian virus 40 [19,85,86]. These homopentameric proteins function as lectins recognizing the plasma membrane by the specific binding between their sphingolipid pockets and the oligosaccharide moiety of complex glycosphingolipids (Table 1). Once the recognition is established, they transit the endocytic pathway to reach the ER. The mechanism underlying their transport and sorting from plasma membrane to endosome and subsequently to Golgi and ER is still unclear. Later work in the field offers an interesting glimpse into the role that sphingolipids might play. The multiple binding between the glycosphingolipid receptor and the protein pentamer induces glycosphingolipid reorganization, membrane bending, and the formation of narrow tubular pits from which endocytic carriers are originated in a clathrin-independent manner (Fig. 5) [85,87,88].

Molecular simulation studies have shown that ST, CT, and VP1 are inherently capable of initiating membrane bending events. Through the multiple binding to the polar head of the glycosphingolipids, the pentamer acquires the optimal orientation to force membrane bending. This happens because the glycosphingolipid pockets are localized at the edges of the pentamer and slightly over the membrane plane. The binding to the polar head of the glycosphingolipids pushes down the membrane at the center of the pentamer while the binding sites might pull the membrane upward, leading to curvature [89–91]. Despite the intrinsic capacity of the pentamers to deform the membrane, the latest studies show that the acyl chain length and saturation of the glycosphingolipid receptor play a role in the recognition, sorting and final subcellular fate of toxins and viruses. Therefore, as shown for the LecA lectins of *Pseudomonas aeruginosa* and the ST B-subunit, the lipid environment and the acyl chain composition of glycosphingolipids influence the presentation of the head of the glycosphingolipid receptor to the protein, affecting its recognition and subsequent binding to the membrane [92,93]. The composition of the acyl chain also affects the trafficking of these particles: VP1 endocytosis at the plasma membrane depends on long and saturated glycosphingolipids [87]; independent clathrin carriers are induced by ST when binding to long unsaturated glycosphingolipids [85] and the final destination of CT relies on the characteristics of the bonded glycosphingolipid (Table 1) [94,95]. Schmieder et al. [98] introduced an appealing concept [98] to explain the sorting of glycosphingolipids in the plasma membrane-endosome network. The authors define a new structural organizing principle that mediates the sorting of the glycosphingolipid, monosialotetrahexosylganglioside (GM1) in a cholesterol-dependent manner. The C14* motif, as the authors named it, comprises the 14 plus one saturated hydrocarbons extending from the amide bond at the water bilayer interface. This long-saturated motif can easily align with cholesterol promoting lateral segregation of lipids that sorts GM1 in subdomains of the early endosome from where they are trafficked to the lysosome or brought back to the PM. Moreover, this lateral segregation could involve transbilayer interactions [96]. The contribution of this model is the consideration of the length and the position of the unsaturation with respect to the outer membrane in the fatty acid as important parameters to define the complex sphingolipid distribution in cellular membranes. This opens exciting perspectives to better understand the functional meaning of sphingolipid diversity in the endocytic pathway.

The considerable advances in the study of toxins and virus particles trafficking have greatly contributed to understand the role of sphingolipids in clathrin-independent endocytosis. The current pool of evidence indicates that the interplay between proteins and lipids is essential to trigger endocytosis and, subsequently, to reach the intracellular destination, but who initiates the process? On the one hand, a lipid environment is needed for the correct presentation of the head groups of

Table 1
Summary of sphingolipids involved in sorting and vesicular transport of proteins along the endomembrane system.

Transport event	Protein	Sphingolipid	Organism	Ref
Specific ERES	Gas1p	Membrane C26-ceramide and ceramide motif of the GPI-anchor	Saccharomyces cerevisiae	[22,47]
COPI vesicles at cis Golgi	TMED2	C18-Sphingomyelin	Mammals (HeLa cells)	[20]
SM-derived vesicles at TGN	Cab45	Synthesis of sphingomyelin at TGN	Mammals (HeLa cells/)	[70,72]
SM-derived vesicles at TGN	Syndecan-1 and Lipoprotein lipase	Sphingomyelin	Mammals (HeLa cells)	[33]
Axonal membrane	Dscam (TM2)	Sphingolipids	Drosophila melanogaster (Neurons)	[62]
CCVs/TGN	PIN1/AUX1	Alpha-hydroxylated ceramide with an acyl-chain longer than 18 carbons	Arabidopsis thaliana	[74]
SVs/TGN	PIN2	C24-and C26-acyl chain alpha-hydroxylated Glycosylinositolphosporylceramides	Arabidopsis thaliana	[60,73]
Lipid carriers at the PM	VP1 protein	C18- monosialotetrahexosylganglioside (GM1)	Simian virus 40	[87]
Transit to ER	CTxB	C16:1 GM1	Vibrio cholerae	[94]
Transport to Lysosome	CTxB	GM1 with C14* motif	Vibrio cholerae	[95]
Lipid carriers at the PM	STxB	Globotriaosylceramide (Gb3) with long acyl chains	Shigella dysenteriae	[85,103]
Clathrin-independent carriers	Galectine-3	Glycosphingolipid	Mammlas (Mouse embryonic fibroblasts)	[101]

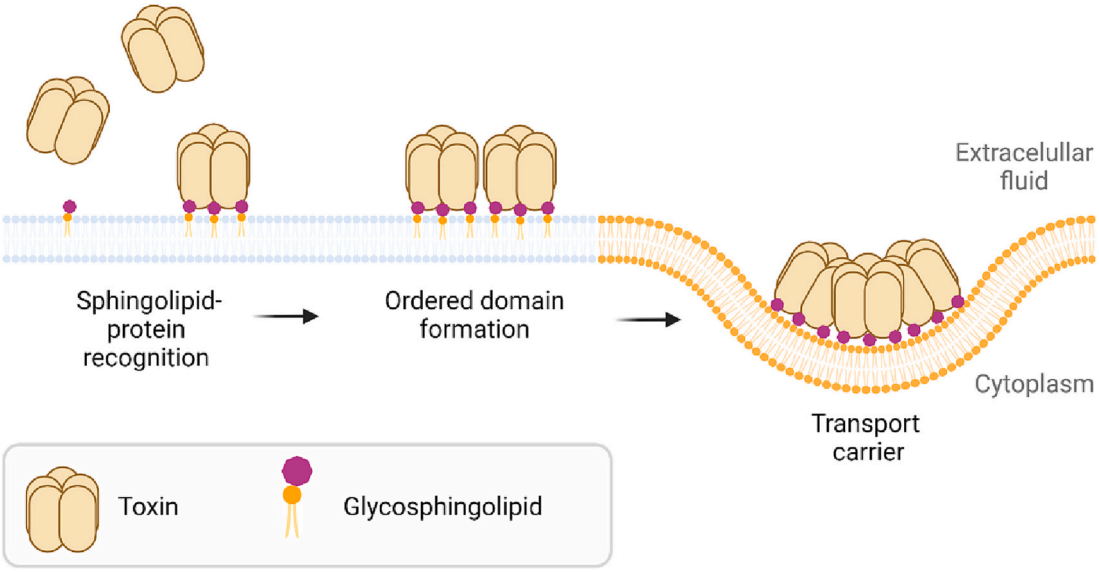

Fig. 5. Model for toxin sorting into endocytic carriers at the plasma membrane. Multivalent binding of the toxin to the head of specific glycosphingolipids induces membrane curvature and stabilizes ordered membrane domains (orange), which enhances the bending of the membrane to finally generates the transport carrier.

receptors to the protein [92,93] and the spontaneous assembly of glycosphingolipids into transient nanoclusters in the plasma membrane [95,96] may facilitate the multiple binding of sphingolipid receptors, promoting the curvature-inductive properties of the protein [88,94,95]. On the other hand, proteins can act as nanoscale lipid clustering devices that override the requirements for spontaneous nanocluster formation and create larger and more stable nanodomains that lead to coat-independent formation of endocytic membrane invaginations [87,96]. These apparently contradictory data might indicate that the order of events responds to the assembly of specific compositional environments programmed to achieve optimal protein trafficking [97]. For example, based on evidence of CT transport [94,95] glycosphingolipid variants capable of forming spontaneous nanoclusters could facilitate the assembly of domains for proteins targeted at the lysosomal compartment. On the contrary, functional domains directed to TGN or ER might rely mainly on the ability of the protein to fine-tune the lipid template and initiate the transport event. In summary, lipids and proteins work cooperatively to fully achieve optimal trafficking, and unraveling the mechanisms that modulate the creation of functional transport membrane domains is an important task for the future.

Similar mechanisms could be operating for cellular lectins as Galectins. They are galactose-binding lectins involved in many physiological roles and related to protein transport [98–100]. Particularly galectin-3 can, upon oligomerization, bind glycosphingolipids and drive membrane bending and construction of tubular endocytic pits [101]. A hypothesis has been proposed that compiles the knowledge obtained from toxins, viruses, and cellular lectins. The “GlycoLipid-Lectin” hypothesis aims to provide a conceptual framework to explain the mechanisms underlying the cargo incorporation and biogenesis of clathrin-independent endocytic carriers. This hypothesis postulates that lectins specifically recognize the hydrophilic heads of glycolipids inducing membrane deformation to finally construct endocytic pits [102].

6. Concluding remarks

Membrane compartmentalization defines eukaryotic life. The unique chemical composition of membrane organelles is continuously adapted to fulfill their function and maintain cell homeostasis. Consequently, cells conduct intense and dynamic trafficking of proteins and lipids along their endomembrane systems. To achieve their final destination, proteins must dive into heterogeneous membrane landscapes to be segregated from the resident proteins of the biogenic compartment and transported to and maintained at their functional site. This sorting process involves intricate protein interactions. However, as presented in this review, a more active role has been assigned to lipids in protein membrane trafficking.

Although sphingolipids were promptly connected with the specific transport of proteins in the late secretory pathway, their precise role in the chain of transport events remains unclear. Technical advances have brought new life into our understanding, and we are beginning to catch the first glimpses of the fascinating interplay between this special type of lipids and proteins to achieve efficient transport along membrane systems. The most evident conclusion is that there is no unique strategy by which sphingolipids may promote or regulate the correct arrival of the protein to its destination. First, sphingolipids could play a structural role by generating exclusive domains that facilitate the sorting of cargo proteins and the formation of the lipid carrier. Second, they might be modulators of the transport process, acting as signaling molecules that define the location, timing, and direction of the transport event. From the mechanistic point of view, the ability of sphingolipids to coalesce with sterols and other saturated lipids creating a more ordered and thicker lipid domain has led to propose that sphingolipid lateral segregation could be one of the driving forces in coat-independent transport events. However, proteins might actively use the emergent properties of sphingolipids to promote their specific transport; facilitating lipid-lipid interactions by the concentration of specific sphingolipids around their transmembrane domain or by stabilizing local sphingolipid nanodomains through binding to the head groups. Complementary, sphingolipids could regulate the sorting and transport of proteins, through specific one-to-one interactions with the protein, either by imposing a specific orientation of proteins towards the membrane or by enhancing a distinct conformational state of the protein in the membrane. In summary, efficient membrane trafficking implies a cooperative clustering of proteins and lipids, where lipids and proteins impact each other to create the specific requirements for each of the transport events that tune up the spatiotemporal distribution of proteins in response to cellular needs.

Understanding the nature and function of sphingolipids in membrane trafficking is an immense challenge. Despite the exciting recent advances, the molecular mechanisms of action remain poorly explored and, as presented in this review, just a few works have studied the importance of sphingolipid diversity. Only an interdisciplinary approach where cell biology, biochemistry and chemical biology

synergize with biophysics and modeling would provide a better comprehension of the sphingolipid-protein interplay in the traffic of proteins along the membranes.

Credit authorship contribution statement

MM: Funding acquisition. MM and AAR: Conceptualization. AAR, SSB and RL: writing original draft and editing. AAR: visualization and preparation of figures. Figures were created with Biorender. All authors approved the final version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

The authors would like to thank Dr. Howard Riezman for his essential contributions to the field of protein and lipid transport in membrane systems and for critical reading of this review. This work is supported by grant PID2020-119505GB-I00/AEI/10.13039/501100011033 to M.M., by grants co-financed by the Junta de Andalucía and the ERDF PY20_01240 and US-1380893 to M.M., and by "VI Own Research Plan" of the University of Seville VIPPIT-2020-I.5 to M.M. This work was also supported by the Ministry of Education, Culture, and Sport (MECD) fellowship to S.S.-B. and PAIDI 2020 (P18-FRJ-1132) co-financed by Programa Operativo FEDER 2014-2020 from Junta de Andalucía to R.L.

References

[1] J.S. Bonifacino, B.S. Glick, The mechanisms of vesicle budding and fusion, Cell. 116 (2004) 153–166, https://doi.org/10.1016/S0092-8674(03)01079-1.
[2] R. Schekman, C. Barlowe, S. Bednarek, J. Campbell, T. Doering, R. Duden, M. Kuehn, M. Rexach, T. Yeung, L. Orci, Coat proteins and selective protein packaging into transport vesicles, Cold Spring Harb. Symp. Quant. Biol. 60 (1995) 11–21, https://doi.org/10.1101/sqb.1995.060.01.004.
[3] R. Di Martino, L. Sticco, A. Luini, Regulation of cargo export and sorting at the trans-Golgi network, FEBS Lett. 593 (2019) 2306–2318, https://doi.org/10.1002/1873-3468.13572.
[4] X. Wang, T. Huang, G. Bu, H. Xu, Dysregulation of protein trafficking in neurodegeneration, Mol. Neurodegener. 9 (2014) 31, https://doi.org/10.1186/1750-1326-9-31.
[5] A.D. Gillon, C.F. Latham, E.A. Miller, Vesicle-mediated ER export of proteins and lipids, Biochim. Biophys. Acta 1821 (2012) 1040–1049, https://doi.org/10.1016/j.bbalip.2012.01.005.
[6] C. Barlowe, A. Helenius, Cargo capture and bulk flow in the early secretory pathway, Annu. Rev. Cell Dev. Biol. 32 (2016) 197–222, https://doi.org/10.1146/annurev-cellbio-111315-125016.
[7] J. Dancourt, C. Barlowe, Protein sorting receptors in the early secretory pathway, Annu. Rev. Biochem. 79 (2010) 777–802, https://doi.org/10.1146/annurev-biochem-061608-091319.
[8] Y. Guo, D.W. Sirkis, R. Schekman, Protein sorting at the trans-Golgi network, Annu. Rev. Cell Dev. Biol. 30 (2014) 169–206, https://doi.org/10.1146/annurev-cellbio-100913-013012.
[9] N.C. Walworth, P.J. Novick, Purification and characterization of constitutive secretory vesicles from yeast, J. Cell Biol. 105 (1987) 163–174, https://doi.org/10.1083/jcb.105.1.163.
[10] C. Barlowe, Signals for COPII-dependent export from the ER: what's the ticket out? Trends Cell Biol. 13 (2003) 295–300, https://doi.org/10.1016/s0962-8924(03)00082-5.
[11] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature. 387 (1997) 569–572, https://doi.org/10.1038/42408.
[12] D. Lingwood, K. Simons, Lipid rafts as a membrane-organizing principle, Science. 327 (2010) 46–50, https://doi.org/10.1126/science.1174621.
[13] H.J. Sharpe, T.J. Stevens, S. Munro, A comprehensive comparison of transmembrane domains reveals organelle-specific properties, Cell. 142 (2010) 158–169, https://doi.org/10.1016/j.cell.2010.05.037.
[14] J.C.M. Holthuis, A.K. Menon, Lipid landscapes and pipelines in membrane homeostasis, Nature. 510 (2014) 48–57, https://doi.org/10.1038/nature13474.
[15] L.G. Welch, S. Munro, A tale of short tails, through thick and thin: investigating the sorting mechanisms of Golgi enzymes, FEBS Lett. 593 (2019) 2452–2465, https://doi.org/10.1002/1873-3468.13553.
[16] J.H. Lorent, B. Diaz-Rohrer, X. Lin, K. Spring, A.A. Gorfe, K.R. Levental, I. Levental, Structural determinants and functional consequences of protein affinity for membrane rafts, Nat. Commun. 8 (2017) 1219, https://doi.org/10.1038/s41467-017-01328-3.
[17] I. Levental, E. Lyman, Regulation of membrane protein structure and function by their lipid nano-environment, Nat Rev Mol Cell Biol. 24 (2023) 107–122, https://doi.org/10.1038/s41580-022-00524-4.
[18] M.Ø. Jensen, O.G. Mouritsen, Lipids do influence protein function-the hydrophobic matching hypothesis revisited, Biochim. Biophys. Acta 1666 (2004) 205–226, https://doi.org/10.1016/j.bbamem.2004.06.009.
[19] A.K. Kenworthy, S.S. Schmieder, K. Raghunathan, A. Tiwari, T. Wang, C.V. Kelly, W.I. Lencer, Cholera toxin as a probe for membrane biology, Toxins (Basel). 13 (2021) 543, https://doi.org/10.3390/toxins13080543.
[20] F.-X. Contreras, A.M. Ernst, P. Haberkant, P. Björkholm, E. Lindahl, B. Gönen, C. Tischer, A. Elofsson, G. von Heijne, C. Thiele, R. Pepperkok, F. Wieland, B. Brügger, Molecular recognition of a single sphingolipid species by a protein's transmembrane domain, Nature. 481 (2012) 525–529, https://doi.org/10.1038/nature10742.
[21] N. Jiménez-Rojo, M.D. Leonetti, V. Zoni, A. Colom, S. Feng, N.R. Iyengar, S. Matile, A. Roux, S. Vanni, J.S. Weissman, H. Riezman, Conserved functions of ether lipids and sphingolipids in the early secretory pathway, Curr. Biol. 30 (2020) 3775–3787.e7, https://doi.org/10.1016/j.cub.2020.07.059.
[22] S. Rodriguez-Gallardo, K. Kurokawa, S. Sabido-Bozo, A. Cortes-Gomez, A. Ikeda, V. Zoni, A. Aguilera-Romero, A.M. Perez-Linero, S. Lopez, M. Waga, M. Araki, M. Nakano, H. Riezman, K. Funato, S. Vanni, A. Nakano, M. Muñiz, Ceramide chain length-dependent protein sorting into selective endoplasmic reticulum exit sites, Sci. Adv. 6 (2020) 8237–8248, https://doi.org/10.1126/sciadv.aba8237.
[23] F.M. Goñi, A. Alonso, Effects of ceramide and other simple sphingolipids on membrane lateral structure, Biochim. Biophys. Acta 1788 (2009) 169–177, https://doi.org/10.1016/J.BBAMEM.2008.09.002.
[24] Y.A. Hannun, L.M. Obeid, Sphingolipids and their metabolism in physiology and disease, Nat Rev Mol Cell Biol. 19 (2018) 175–191, https://doi.org/10.1038/nrm.2017.107.
[25] C. Körner, F. Fröhlich, Compartmentation and functions of sphingolipids, Curr. Opin. Cell Biol. 74 (2022) 104–111, https://doi.org/10.1016/j.ceb.2022.01.006.
[26] A.H. Futerman, H. Riezman, The ins and outs of sphingolipid synthesis, Trends Cell Biol. 15 (2005) 312–318, https://doi.org/10.1016/j.tcb.2005.04.006.
[27] Y.A. Hannun, L.M. Obeid, Principles of bioactive lipid signalling: lessons from sphingolipids, Nat Rev Mol Cell Biol. 9 (2008) 139–150, https://doi.org/10.1038/nrm2329.
[28] J.T. Hannich, K. Umebayashi, H. Riezman, Distribution and functions of sterols and sphingolipids, Cold Spring Harb. Perspect. Biol. 3 (2011) 1–14, https://doi.org/10.1101/cshperspect.a004762.
[29] T. Harayama, H. Riezman, Understanding the diversity of membrane lipid composition, Nat Rev Mol Cell Biol. 19 (2018) 281–296, https://doi.org/10.1038/nrm.2017.138.
[30] M.A. Lone, T. Santos, I. Alecu, L.C. Silva, T. Hornemann, 1-Deoxysphingolipids, Biochim Biophys Acta Mol Cell Biol Lipids. 1864 (2019) 512–521, https://doi.org/10.1016/j.bbalip.2018.12.013.
[31] A. Alonso, F.M. Goñi, The physical properties of ceramides in membranes, Annu. Rev. Biophys. 47 (2018) 633–654, https://doi.org/10.1146/annurev-biophys-070317-033309.
[32] A.C. Carreira, A.E. Ventura, A.R.P. Varela, L.C. Silva, Tackling the biophysical properties of sphingolipids to decipher their biological roles, Biol. Chem. 396 (2015) 597–609, https://doi.org/10.1515/hsz-2014-0283.
[33] E.L. Sundberg, Y. Deng, C.G. Burd, Syndecan-1 mediates sorting of soluble lipoprotein lipase with sphingomyelin-rich membrane in the Golgi apparatus, Dev. Cell 51 (2019) 387–398.e4, https://doi.org/10.1016/j.devcel.2019.08.014.
[34] L. Cantù, E. Del Favero, S. Sonnino, A. Prinetti, Gangliosides and the multiscale modulation of membrane structure, Chem. Phys. Lipids 164 (2011) 796–810, https://doi.org/10.1016/j.chemphyslip.2011.09.005.
[35] M.C. Simon, J. Kusch, Communicative functions of GPI-anchored surface proteins in unicellular eukaryotes, Crit. Rev. Microbiol. 39 (2013) 70–78, https://doi.org/10.3109/1040841X.2012.691459.
[36] M.G. Paulick, C.R. Bertozzi, The glycosylphosphatidylinositol anchor: a complex membrane-anchoring structure for proteins, Biochemistry. 47 (2008) 6991–7000, https://doi.org/10.1021/bi8006324.
[37] N. Desnoyer, R. Palanivelu, Bridging the GAPs in plant reproduction: a comparison of plant and animal GPI-anchored proteins, Plant Reprod. 33 (2020) 129–142, https://doi.org/10.1007/s00497-020-00395-9.
[38] M. Muñiz, C. Zurzolo, Sorting of GPI-anchored proteins from yeast to mammals–common pathways at different sites? J. Cell Sci. 127 (2014) 2793–2801, https://doi.org/10.1242/jcs.148056.
[39] M. Muñiz, H. Riezman, Trafficking of glycosylphosphatidylinositol anchored proteins from the endoplasmic reticulum to the cell surface, J. Lipid Res. 57 (2016) 352–360, https://doi.org/10.1194/jlr.R062760.
[40] T. Kinoshita, M. Fujita, Biosynthesis of GPI-anchored proteins: special emphasis on GPI lipid remodeling, J. Lipid Res. 57 (2016) 6–24, https://doi.org/10.1194/jlr.R063313.

[41] M. Muñiz, P. Morsomme, H. Riezman, Protein sorting upon exit from the endoplasmic reticulum, Cell. 104 (2001) 313–320, https://doi.org/10.1016/s0092-8674(01)00215-x.
[42] M. Fujita, M. Umemura, T. Yoko-O, Y. Jigami, PER1 is required for GPI-phospholipase A2 activity and involved in lipid remodeling of GPI-anchored proteins, Mol. Biol. Cell 17 (2006) 5253–5264, https://doi.org/10.1091/MBC.E06-08-0715.
[43] Y. Maeda, Y. Tashima, T. Houjou, M. Fujita, T. Yoko-o, Y. Jigami, R. Taguchi, T. Kinoshita, Fatty acid remodeling of GPI-anchored proteins is required for their raft association, Mol. Biol. Cell 18 (2007) 1497–1506, https://doi.org/10.1091/MBC.E06-10-0885.
[44] G.A. Castillon, A. Aguilera-Romero, J. Manzano-Lopez, S. Epstein, K. Kajiwara, K. Funato, R. Watanabe, H. Riezman, M. Muñiz, The yeast p24 complex regulates GPI-anchored protein transport and quality control by monitoring anchor remodeling, Mol. Biol. Cell 22 (2011) 2924–2936, https://doi.org/10.1091/MBC.E11-04-0294.
[45] T. Yoko-O, D. Ichikawa, Y. Miyagishi, A. Kato, M. Umemura, K. Takase, M. Ra, K. Ikeda, R. Taguchi, Y. Jigami, Determination and physiological roles of the glycosylphosphatidylinositol lipid remodelling pathway in yeast, Mol. Microbiol. 88 (2013) 140–155, https://doi.org/10.1111/mmi.12175.
[46] S.N. Pinto, L.C. Silva, A.H. Futerman, M. Prieto, Effect of ceramide structure on membrane biophysical properties: the role of acyl chain length and unsaturation, Biochim. Biophys. Acta 1808 (2011) 2753–2760, https://doi.org/10.1016/j.bbamem.2011.07.023.
[47] S. Rodriguez-Gallardo, S. Sabido-Bozo, A. Ikeda, M. Araki, K. Okazaki, M. Nakano, A. Aguilera-Romero, A. Cortes-Gomez, S. Lopez, M. Waga, A. Nakano, K. Kurokawa, M. Muñiz, K. Funato, Quality-controlled ceramide-based GPI-anchored protein sorting into selective ER exit sites, Cell Rep. 39 (2022), 110768, https://doi.org/10.1016/j.celrep.2022.110768.
[48] C. Bonnon, M.W. Wendeler, J.-P. Paccaud, H.-P. Hauri, Selective export of human GPI-anchored proteins from the endoplasmic reticulum, J. Cell Sci. 123 (2010) 1705–1715, https://doi.org/10.1242/jcs.062950.
[49] D. Sarnataro, S. Paladino, V. Campana, J. Grassi, L. Nitsch, C. Zurzolo, PrPC is sorted to the basolateral membrane of epithelial cells independently of its association with rafts, Traffic. 3 (2002) 810–821, https://doi.org/10.1034/j.1600-0854.2002.31106.x.
[50] A.-S. Rivier, G.A. Castillon, L. Michon, M. Fukasawa, M. Romanova-Michaelides, N. Jaensch, K. Hanada, R. Watanabe, Exit of GPI-anchored proteins from the ER differs in yeast and mammalian cells, Traffic. 11 (2010) 1017–1033, https://doi.org/10.1111/j.1600-0854.2010.01081.x.
[51] R. Aber, W. Chan, S. Mugisha, L.A. Jerome-Majewska, Transmembrane emp24 domain proteins in development and disease, Genet Res (Camb). 101 (2019), e14, https://doi.org/10.1017/S0016672319000090.
[52] N. Gomez-Navarro, A. Melero, X.-H. Li, J. Boulanger, W. Kukulski, E.A. Miller, Cargo crowding contributes to sorting stringency in COPII vesicles, J. Cell Biol. 219 (2020), https://doi.org/10.1083/jcb.201806038.
[53] S. Lopez, A.M. Perez-Linero, J. Manzano-Lopez, S. Sabido-Bozo, A. Cortes-Gomez, S. Rodriguez-Gallardo, A. Aguilera-Romero, V. Goder, M. Muñiz, Dual Independent roles of the p24 complex in selectivity of secretory cargo export from the endoplasmic reticulum, Cells. 9 (2020) 1295, https://doi.org/10.3390/cells9051295.
[54] D.U. Gommel, A.R. Memon, A. Heiss, F. Lottspeich, J. Pfannstiel, J. Lechner, C. Reinhard, J.B. Helms, W. Nickel, F.T. Wieland, Recruitment to Golgi membranes of ADP-ribosylation factor 1 is mediated by the cytoplasmic domain of p23, EMBO J. 20 (2001) 6751–6760, https://doi.org/10.1093/emboj/20.23.6751.
[55] A. Aguilera-Romero, J. Kaminska, A. Spang, H. Riezman, M. Muñiz, The yeast p24 complex is required for the formation of COPI retrograde transport vesicles from the Golgi apparatus, J. Cell Biol. 180 (2008) 713–720, https://doi.org/10.1083/jcb.200710025.
[56] D.U. Gommel, A.R. Memon, A. Heiss, F. Lottspeich, J. Pfannstiel, J. Lechner, C. Reinhard, J.B. Helms, W. Nickel, F.T. Wieland, Recruitment to Golgi membranes of ADP-ribosylation factor 1 is mediated by the cytoplasmic domain of p23, EMBO J. 20 (2001) 6751–6760, https://doi.org/10.1093/EMBOJ/20.23.6751.
[57] S. Pannwitt, M. Stangl, D. Schneider, Lipid binding controls dimerization of the coat protein p24 transmembrane Helix, Biophys. J. 117 (2019) 1554–1562, https://doi.org/10.1016/j.bpj.2019.09.021.
[58] R.W. Klemm, C.S. Ejsing, M.A. Surma, H.-J. Kaiser, M.J. Gerl, J.L. Sampaio, Q. de Robillard, C. Ferguson, T.J. Proszynski, A. Shevchenko, K. Simons, Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network, J. Cell Biol. 185 (2009) 601–612, https://doi.org/10.1083/jcb.200901145.
[59] Y. Deng, F.E. Rivera-Molina, D.K. Toomre, C.G. Burd, Sphingomyelin is sorted at the trans Golgi network into a distinct class of secretory vesicle, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 6677–6682, https://doi.org/10.1073/pnas.1602875113.
[60] V. Wattelet-Boyer, L. Brocard, K. Jonsson, N. Esnay, J. Joubès, F. Domergue, S. Mongrand, N. Raikhel, R.P. Bhalerao, P. Moreau, Y. Boutté, Enrichment of hydroxylated C24-and C26-acyl-chain sphingolipids mediates PIN2 apical sorting at trans-Golgi network subdomains, Nat. Commun. 7 (2016), https://doi.org/10.1038/ncomms12788.
[61] J.M. Duran, F. Campelo, J. van Galen, T. Sachsenheimer, J. Sot, M.V. Egorov, C. Rentero, C. Enrich, R.S. Polishchuk, F.M. Goñi, B. Brügger, F. Wieland, V. Malhotra, Sphingomyelin organization is required for vesicle biogenesis at the Golgi complex, EMBO J. 31 (2012) 4535–4546, https://doi.org/10.1038/emboj.2012.317.
[62] G. Goyal, J. Zheng, E. Adam, G. Steffes, M. Jain, K. Klavins, T. Hummel, Sphingolipid-dependent Dscam sorting regulates axon segregation, Nat. Commun. 10 (2019) 813, https://doi.org/10.1038/s41467-019-08765-2.
[63] C. Lipardi, L. Nitsch, C. Zurzolo, Detergent-insoluble GPI-anchored proteins are apically sorted in fischer rat thyroid cells, but interference with cholesterol or sphingolipids differentially affects detergent insolubility and apical sorting, Mol. Biol. Cell 11 (2000) 531–542, https://doi.org/10.1091/mbc.11.2.531.
[64] Y. Maeda, Y. Tashima, T. Houjou, M. Fujita, T. Yoko-o, Y. Jigami, R. Taguchi, T. Kinoshita, Fatty acid remodeling of GPI-anchored proteins is required for their raft association, Mol. Biol. Cell 18 (2007) 1497–1506, https://doi.org/10.1091/mbc.e06-10-0885.
[65] G.A. Castillon, L. Michon, R. Watanabe, Apical sorting of lysoGPI-anchored proteins occurs independent of association with detergent-resistant membranes but dependent on their N-glycosylation, Mol. Biol. Cell 24 (2013) 2021–2033, https://doi.org/10.1091/mbc.E13-03-0160.
[66] N. Jaensch, I.R. Corrêa, R. Watanabe, Stable cell surface expression of GPI-anchored proteins, but not intracellular transport, depends on their fatty acid structure, Traffic. 15 (2014) 1305–1329, https://doi.org/10.1111/tra.12224.
[67] O.V. Vieira, P. Verkade, A. Manninen, K. Simons, FAPP2 is involved in the transport of apical cargo in polarized MDCK cells, J. Cell Biol. 170 (2005) 521–526, https://doi.org/10.1083/jcb.200503078.
[68] N.S. Imjeti, S. Lebreton, S. Paladino, E. de la Fuente, A. Gonzalez, C. Zurzolo, N-glycosylation instead of cholesterol mediates oligomerization and apical sorting of GPI-APs in FRT cells, Mol. Biol. Cell 22 (2011) 4621–4634, https://doi.org/10.1091/mbc.E11-04-0320.
[69] S. Paladino, D. Sarnataro, R. Pillich, S. Tivodar, L. Nitsch, C. Zurzolo, Protein oligomerization modulates raft partitioning and apical sorting of GPI-anchored proteins, J. Cell Biol. 167 (2004) 699–709, https://doi.org/10.1083/jcb.200407094.
[70] S. Lebreton, S. Paladino, D. Liu, M. Nitti, J. von Blume, P. Pinton, C. Zurzolo, Calcium levels in the Golgi complex regulate clustering and apical sorting of GPI-APs in polarized epithelial cells, Proc. Natl. Acad. Sci. U. S. A. 118 (2021), https://doi.org/10.1073/pnas.2014709118.
[71] J. von Blume, A.-M. Alleaume, G. Cantero-Recasens, A. Curwin, A. Carreras-Sureda, T. Zimmermann, J. van Galen, Y. Wakana, M.A. Valverde, V. Malhotra, ADF/cofilin regulates secretory cargo sorting at the TGN via the Ca2+ ATPase SPCA1, Dev. Cell 20 (2011) 652–662, https://doi.org/10.1016/j.devcel.2011.03.014.
[72] Y. Deng, M. Pakdel, B. Blank, E.L. Sundberg, C.G. Burd, J. von Blume, Activity of the SPCA1 calcium pump couples sphingomyelin synthesis to sorting of secretory proteins in the trans-Golgi network, Dev. Cell 47 (2018) 464–478.e8, https://doi.org/10.1016/j.devcel.2018.10.012.
[73] Y. Ito, N. Esnay, M.P. Platre, V. Wattelet-Boyer, L.C. Noack, L. Fougère, W. Menzel, S. Claverol, L. Fouillen, P. Moreau, Y. Jaillais, Y. Boutté, Sphingolipids mediate polar sorting of PIN2 through phosphoinositide consumption at the trans-Golgi network, Nat. Commun. 12 (2021) 4267, https://doi.org/10.1038/s41467-021-24548-0.
[74] J.E. Markham, D. Molino, L. Gissot, Y. Bellec, K. Hématy, J. Marion, K. Belcram, J.-C. Palauqui, B. Satiat-Jeunemaître, J.-D. Faure, Sphingolipids containing very-long-chain fatty acids define a secretory pathway for specific polar plasma membrane protein targeting in Arabidopsis, Plant Cell 23 (2011) 2362–2378, https://doi.org/10.1105/tpc.110.080473.
[75] S. Naramoto, Polar transport in plants mediated by membrane transporters: focus on mechanisms of polar auxin transport, Curr. Opin. Plant Biol. 40 (2017) 8–14, https://doi.org/10.1016/j.pbi.2017.06.012.
[76] D.M. Pegtel, S.J. Gould, Exosomes, Annu. Rev. Biochem. 88 (2019) 487–514, https://doi.org/10.1146/annurev-biochem-013118-111902.
[77] G. van Niel, G. D'Angelo, G. Raposo, Shedding light on the cell biology of extracellular vesicles, Nat Rev Mol Cell Biol. 19 (2018) 213–228, https://doi.org/10.1038/nrm.2017.125.
[78] K. Trajkovic, C. Hsu, S. Chiantia, L. Rajendran, D. Wenzel, F. Wieland, P. Schwille, B. Brügger, M. Simons, Ceramide triggers budding of exosome vesicles into multivesicular endosomes, Science. 319 (2008) 1244–1247, https://doi.org/10.1126/science.1153124.
[79] D. Choezom, J.C. Gross, Neutral sphingomyelinase 2 controls exosome secretion by counteracting V-ATPase-mediated endosome acidification, J. Cell Sci. 135 (2022), https://doi.org/10.1242/jcs.259324.
[80] S.B. Arya, S. Chen, F. Jordan-Javed, C.A. Parent, Ceramide-rich microdomains facilitate nuclear envelope budding for non-conventional exosome formation, Nat. Cell Biol. 24 (2022) 1019–1028, https://doi.org/10.1038/s41556-022-00934-8.
[81] P. Sengupta, A.Y. Seo, H.A. Pasolli, Y.E. Song, M.C. Johnson, J. Lippincott-Schwartz, A lipid-based partitioning mechanism for selective incorporation of proteins into membranes of HIV particles, Nat. Cell Biol. 21 (2019) 452–461, https://doi.org/10.1038/s41556-019-0300-y.
[82] E. Barklis, A. Alfadhli, J.E. Kyle, L.M. Bramer, K.J. Bloodsworth, R.L. Barklis, H. C. Leier, R.M. Petty, I.D. Zelnik, T.O. Metz, A.H. Futerman, F.G. Tafesse, Ceramide synthase 2 deletion decreases the infectivity of HIV-1, J. Biol. Chem. 296 (2021), 100340, https://doi.org/10.1016/j.jbc.2021.100340.
[83] G.J. Doherty, H.T. McMahon, Mechanisms of endocytosis, Annu. Rev. Biochem. 78 (2009) 857–902, https://doi.org/10.1146/annurev.biochem.78.081307.110540.
[84] R. Mahfoud, N. Garmy, M. Maresca, N. Yahi, A. Puigserver, J. Fantini, Identification of a common sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins, J. Biol. Chem. 277 (2002) 11292–11296, https://doi.org/10.1074/jbc.M111679200.

[85] W. Römer, L. Berland, V. Chambon, K. Gaus, B. Windschiegl, D. Tenza, M.R. E. Aly, V. Fraisier, J.C. Florent, D. Perrais, C. Lamaze, G. Raposo, C. Steinem, P. Sens, P. Bassereau, L. Johannes, Shiga toxin induces tubular membrane invaginations for its uptake into cells, Nature 450 (7170) (2007), https://doi.org/10.1038/nature05996, 450 (2007) 670–675.
[86] Y.-J. Chen, X. Liu, B. Tsai, SV40 hijacks cellular transport, membrane penetration, and disassembly machineries to promote infection, Viruses. 11 (2019) 917, https://doi.org/10.3390/v11100917.
[87] H. Ewers, W. Römer, A.E. Smith, K. Bacia, S. Dmitrieff, W. Chai, R. Mancini, J. Kartenbeck, V. Chambon, L. Berland, A. Oppenheim, G. Schwarzmann, T. Feizi, P. Schwille, P. Sens, A. Helenius, L. Johannes, GM1 structure determines SV40-induced membrane invagination and infection., Nat. Cell Biol. 12 (2010) 11–18; sup pp 1-12. doi:https://doi.org/10.1038/ncb1999.
[88] A.M. Kabbani, K. Raghunathan, W.I. Lencer, A.K. Kenworthy, C.V. Kelly, Structured clustering of the glycosphingolipid GM1 is required for membrane curvature induced by cholera toxin, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 14978–14986, https://doi.org/10.1073/pnas.2001119117.
[89] W. Pezeshkian, L.J. Nåbo, J.H. Ipsen, Cholera toxin B subunit induces local curvature on lipid bilayers, FEBS Open Bio. 7 (2017) 1638–1645, https://doi.org/10.1002/2211-5463.12321.
[90] W. Pezeshkian, A.G. Hansen, L. Johannes, H. Khandelia, J.C. Shillcock, P.B. S. Kumar, J.H. Ipsen, Membrane invagination induced by Shiga toxin B-subunit: from molecular structure to tube formation, Soft Matter 12 (2016) 5164–5171, https://doi.org/10.1039/c6sm00464d.
[91] R. Kociurzynski, S.D. Beck, J.B. Bouhon, W. Römer, V. Knecht, Binding of SV40's viral capsid protein VP1 to its glycosphingolipid receptor GM1 induces negative membrane curvature: a molecular dynamics study, Langmuir. 35 (2019) 3534–3544, https://doi.org/10.1021/ACS.LANGMUIR.8B03765/SUPPL_FILE/LA8B03765_SI_001.PDF.
[92] T. Schubert, T. Sych, J. Madl, M. Xu, R. Omidvar, L.J. Patalag, A. Ries, K. Kettelhoit, A. Brandel, Y. Mely, C. Steinem, D.B. Werz, R. Thuenauer, W. Römer, Differential recognition of lipid domains by two Gb3-binding lectins, Sci. Rep. 10 (2020) 9752, https://doi.org/10.1038/s41598-020-66522-8.
[93] C.A. Lingwood, A. Manis, R. Mahfoud, F. Khan, B. Binnington, M. Mylvaganam, New aspects of the regulation of glycosphingolipid receptor function, Chem. Phys. Lipids 163 (2010) 27–35, https://doi.org/10.1016/J.CHEMPHYSLIP.2009.09.001.
[94] D.J.-F. Chinnapen, W.-T. Hsieh, Y.M. te Welscher, D.E. Saslowsky, L. Kaoutzani, E. Brandsma, L. D'Auria, H. Park, J.S. Wagner, K.R. Drake, M. Kang, T. Benjamin, M.D. Ullman, C.E. Costello, A.K. Kenworthy, T. Baumgart, R.H. Massol, W. I. Lencer, Lipid sorting by ceramide structure from plasma membrane to ER for the cholera toxin receptor ganglioside GM1, Dev. Cell 23 (2012) 573–586, https://doi.org/10.1016/j.devcel.2012.08.002.
[95] S.S. Schmieder, R. Tatituri, M. Anderson, K. Kelly, W.I. Lencer, Structural basis for acyl chain control over glycosphingolipid sorting and vesicular trafficking, Cell Rep. 40 (2022), https://doi.org/10.1016/j.celrep.2022.111063.
[96] S. Arumugam, S. Schmieder, W. Pezeshkian, U. Becken, C. Wunder, D. Chinnapen, J.H. Ipsen, A.K. Kenworthy, W. Lencer, S. Mayor, L. Johannes, Ceramide structure dictates glycosphingolipid nanodomain assembly and function, Nat. Commun. 12 (2021) 3675, https://doi.org/10.1038/s41467-021-23961-9.
[97] L. Johannes, C. Wunder, P. Bassereau, Bending "on the rocks"—a cocktail of biophysical modules to build endocytic pathways, Cold Spring Harb. Perspect. Biol. 6 (2014), https://doi.org/10.1101/CSHPERSPECT.A016741.
[98] M.P. Mathew, J.G. Donaldson, Distinct cargo-specific response landscapes underpin the complex and nuanced role of galectin-glycan interactions in clathrin-independent endocytosis, J. Biol. Chem. 293 (2018) 7222–7237, https://doi.org/10.1074/jbc.RA118.001802.
[99] A. Ivashenka, C. Wunder, V. Chambon, R. Sandhoff, R. Jennemann, E. Dransart, K. Podsypanina, B. Lombard, D. Loew, C. Lamaze, F. Poirier, H.-J. Gröne, L. Johannes, M. Shafaq-Zadah, Glycolipid-dependent and lectin-driven transcytosis in mouse enterocytes, Commun Biol. 4 (2021) 173, https://doi.org/10.1038/s42003-021-01693-2.
[100] D. Delacour, C.I. Cramm-Behrens, H. Drobecq, A. Le Bivic, H.Y. Naim, R. Jacob, Requirement for galectin-3 in apical protein sorting, Curr. Biol. 16 (2006) 408–414, https://doi.org/10.1016/j.cub.2005.12.046.
[101] R. Lakshminarayan, C. Wunder, U. Becken, M.T. Howes, C. Benzing, S. Arumugam, S. Sales, N. Ariotti, V. Chambon, C. Lamaze, D. Loew, A. Shevchenko, K. Gaus, R.G. Parton, L. Johannes, Galectin-3 drives glycosphingolipid-dependent biogenesis of clathrin-independent carriers, Nat. Cell Biol. 16 (2014) 595–606, https://doi.org/10.1038/ncb2970.
[102] L. Johannes, C. Wunder, M. Shafaq-Zadah, Glycolipids and lectins in endocytic uptake processes, J. Mol. Biol. 428 (2016) 4792–4818, https://doi.org/10.1016/j.jmb.2016.10.027.
[103] S. Arab, C.A. Lingwood, Intracellular targeting of the endoplasmic reticulum/nuclear envelope by retrograde transport may determine cell hypersensitivity to verotoxin via globotriaosyl ceramide fatty acid isoform traffic, J. Cell. Physiol. 177 (1998) 646–660, https://doi.org/10.1002/(SICI)1097-4652(199812)177:4<646::AID-JCP15>3.0.CO;2-B.