ELSEVIER

Membrane lipids and vesicular traffic

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Lipids were long considered to be passive passengers of carrier vesicles with the single role of sealing the transport container. We now know that specific phospholipids are required for efficient fusion, while others facilitate budding and fission. Moreover, the various polyphosphoinositides assist in the recruitment from the cytosol of proteins of the transport machinery. Finally, the segregation of membrane lipids into different fluid phases appears to serve as a 'lipid raft' mechanism for protein sorting at various stages of the secretory and endocytic pathways. The current challenge is to understand how proteins control the metabolism and subcellular localization, and thereby the activity, of the various lipids.

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Abbreviations
ER endoplamsic reticulum
GPI glycosylphosphatidylinositol

Introduction

A continuous stream of carrier vesicles interconnects all the organelles of eukaryotic cells apart from the mitochondria and peroxisomes. Membrane vesicles bud from one membrane, travel through the cytosol and fuse with a different membrane. They thereby carry membrane components and lumenal contents from one organelle to the next, or secrete the content into the extracellular environment (Figure 1). The matrix of cellular membranes is the classic fluid lipid bilayer, and as vesicular transport is bidirectional and fast as compared to the turnover of membrane components, one important question is how cells maintain the identities of the membranes of their organelles. Cellular membranes contain hundreds of lipid species that differ in their polar headgroups and fatty tails. This review addresses the question of how cells utilize this lipid variety in vesicular transport, and how proteins control the local lipid composition.

Overview of the relevant properties of lipids

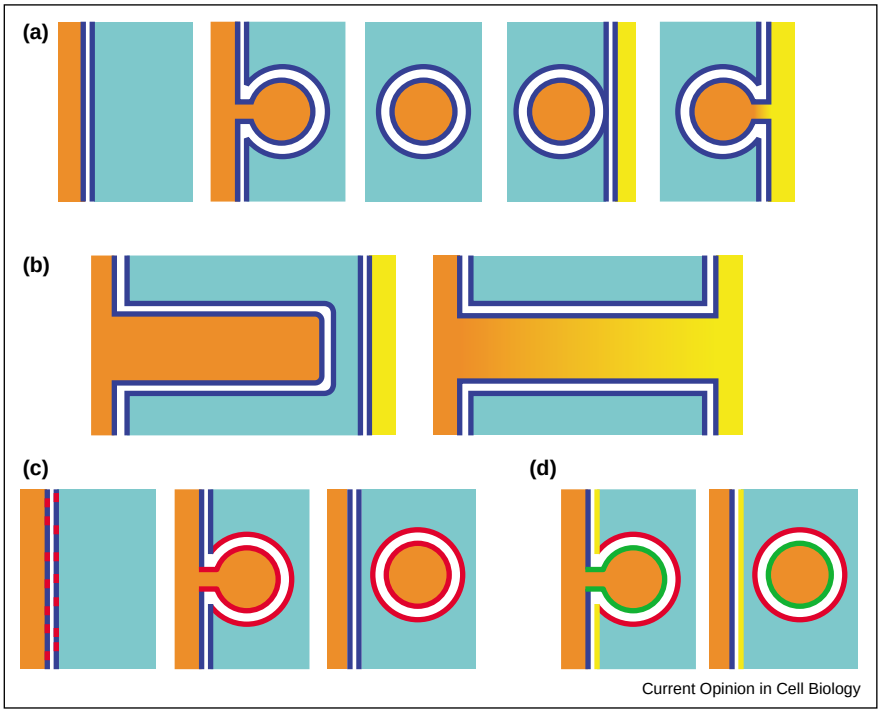
Lipid polymorphism and transmembrane asymmetry

Membrane lipids have unique geometrical shapes defined by their chemical structures [1,2]. The ubiquitous phospholipid phosphatidylcholine, which comprises 50% of the cellular lipids, is cylindrical: the headgroup cross-sectional area equals the cross-sectional area occupied by the fatty acyl tails. By contrast, the second most abundant lipid, phosphatidylethanolamine, is a cone, as is the endosomal lysobisphosphatidic acid [3•]. A monolayer of a cone-shaped lipid will adopt a negative curvature, as the headgroup ends pack more tightly than the tail ends. The same tension is created when the reversed cone lysophosphatidic acid is acylated [4,5], or, to an even greater degree, when its phosphate headgroup is removed by a phospholipase C to yield diacylglycerol [6]. Large headgroups, as in lysophospholipids, create positive monolayer curvature. Lysophospholipid concentrations can be (locally) increased by phospholipase A_2. Clearly, tension will only yield membrane curvature when the two membrane leaflets in the bilayer do not contain the same lipids. Curvature is also induced by minimal increases in the number of lipids in one leaflet as compared to the other [7]. Spontaneous lipid translocation across model membranes is slow but phosphatidic and lysobisphosphatidic acid translocation is induced by low pH (see [3•,7]. Besides inducing curvature, cone lipids strongly facilitate fusion and fission (which also includes a fusion step), whereas the cylindrical lipid phosphatidylcholine and the cylindrical combination of sphingomyelin plus cholesterol resist bending and fusion [8].

Lipid sorting by lateral heterogeneity and protein recruitment

Lipids in eukaryotic membranes are fluid. Lipids in the endoplamsic reticulum (ER) are generally unsaturated, whereas the saturated lipids in the other membranes are fluidized by cholesterol. Remarkably, the different mutual affinities of lipids can yield coexisting lipid phases of different fluidity (i.e. liquid-ordered versus disordered). Segregation occurs in liposomes with a plasma membrane lipid composition and, amazingly, one phase can spontaneously bud away from the other (Figure 1; [9]). In vesicular traffic, such buds would then need to contain specific proteins to target them to the correct organelle. The thickness of membranes matches the hydrophobic length of transmembrane domains [10•] and saturated and unsaturated lipid anchors localize proteins to different areas on the cytosolic plasma membrane

Figure 1



Vessicular transport. **(a)** Vesicular transport involves curving of the donor membrane, fission, transport through the cytosol, docking and fusion with a target membrane. **(b)** In some cases tubes are formed from a membrane. The tubes may fuse with a target membrane (the ER/Golgi [20] or the plasma membrane [41]). In endosomes, tubulo-vesicular structures bud from the end of the tube [6]. **(c)** The budding vesicle may have a different lipid composition from the rest of the membrane, and its content may differ from that of the original compartment. **(d)** In the case that a vesicle buds from an asymmetric membrane, both inner and outer leaflet of the budding vesicle may have a composition different from that of the original membrane leaflet. Lateral lipid segregation in each leaflet may be determined by the physical immiscibility of lipids alone or may require stabilization by protein oligomerization or curvature.

surface. Still, it remains essentially unknown what molecular properties determine the specific interactions between proteins and different lipid phases. Cytosolic proteins of the transport machinery are recruited in a highly regulated fashion via the various phosphoinositides, whose synthesis and degradation is time- and position-dependent [11], a topic not discussed here.

Different lipid combinations act at the various organelles

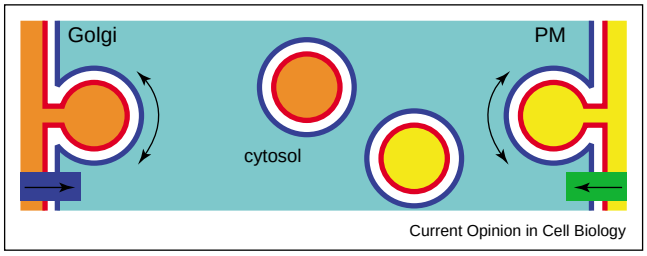
Aminophospholipid flip and budding

Because of its high levels of unsaturated lipids and low levels of sphingolipids and cholesterol, the ER membrane is highly flexible with a high rate of spontaneous transbilayer lipid translocation. Curvature in this membrane is generated by COPII coats. In the retrograde pathway, the COPI complex causes budding from Golgi membranes, and the complex is also able to bud vesicles from liposomes [12,13]. However, in the yeast Golgi, members of the Drs2p family of P-type ATPases appear to be essential for forming anterograde and retrograde transport vesicles [14]. In addition, inactivation of Drs2p ATPases at the Golgi and plasma membrane dramatically affects endocytosis [15]. Originally, the Golgi P-type ATPase Drs2p itself was tentatively identified as the aminophospholipid translocator maintaining the cytosolic orientation of phosphatidylserine and phosphatidylethanolamine at the plasma membrane, but new evidence ascribes this function to the two plasma membrane members of the family [15]. There are over a dozen Drs2p-like P-type ATPases in humans; their involvement in vesicular transport (Figure 2) supports the idea that translocation of lipid mass to the cytosolic surface may drive curvature and budding [7]. Lately, ABC transporters have also been found to be involved in lipid translocation, but in the opposite, exoplasmic direction. Whether ABC transporters affect budding is presently unclear.

Sphingolipid/cholesterol domains and sorting

Glycerophospholipids, cholesterol and the sphingolipid backbone ceramide are synthesized at the ER. Whereas ceramide reaches the first enzyme of glycolipid synthesis, glucosylceramide synthase, in the early Golgi by vesicular transport, its transport to the Golgi sphingomyelin synthase, which was recently identified as SMS1 [16•,17•],

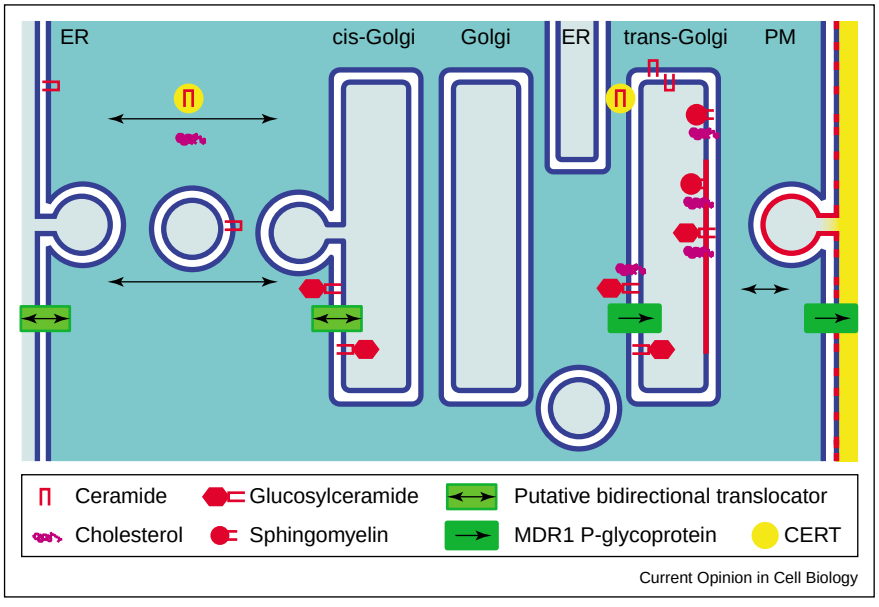
Figure 2



Lipid translocators may induce curvature. Different P-type ATPases at specific locations are involved in the translocation of aminophospholipids towards the cytosolic leaflet of cellular membranes and may provide the driving force for the budding reaction in rigid membranes. Because the lateral pressure will be felt all along the closed membrane, the translocator does not have to reside in the bud itself. PM, plasma membrane. The blue and green boxes indicate two different P-type ATPases that translocate aminophospholipids from the exoplasmic membrane leaflet (red lines) towards the cytosolic leaflet (blue lines) and thereby generate lateral pressure in the cytosolic leaflet (double-headed arrows). The difference in lateral pressure between the two leaflets may be the driving force behind budding.

occurs by an independent pathway via the ceramide transfer protein CERT [18••]. SMS1 colocalized with the trans-Golgi marker sialyltransferase [16•], which positions the major site of sphingolipid synthesis in the trans-Golgi. Considering the bidirectional vesicular connection between the ER and the plasma membrane (Figure 3), the most remarkable feature of the lipid organization in mammalian cells is the enrichment of sphingolipids and cholesterol in the late Golgi, plasma membrane and endosomes. Cholesterol spontaneously moves between and across membranes as a monomer. Its location is determined by its high affinity for sphingolipids and saturated glycerophospholipids. A key issue, then, is what drives the sphingolipid/cholesterol complex towards the plasma membrane. The lumenal orientation of the sphingolipids forces the conclusion that the complex must be specifically included in the forward pathway or excluded from retrograde vesicles (Figure 3). Indeed, COPI-coated Golgi vesicles had reduced levels of sphingomyelin and cholesterol [19]. These vesicles may bud from pre-existing domains. Alternatively, the generation of curvature (e.g. by coats) may induce large-scale sorting by specifically including the unsaturated lipids in the forming bud. In this way, highly curved retrograde tubules [20] could also release unsaturated lipids from the Golgi. Whether a similar sorting reaction occurs on the cytosolic surface, excluding the cytosolically oriented phosphatidylserine from the retrograde pathway, remains to be determined, as the lipid organization on the cytosolic side of lumenal sphingolipid-cholesterol domains is unknown.

Figure 3



Lipid transport between ER and Golgi. A bidirectional vesicular transport pathway connects the ER and Golgi, although tubulation is also observed [20]. Cholesterol spontaneously exchanges between membranes. Ceramide can be transported by the transfer protein CERT [18••], which is required for reaching the sphingomyelin synthase 1 in the trans Golgi [16•,17•]. Glucosylceramide synthesized on the cytosolic surface of the cis-Golgi, which utilizes a different pool of ceramide, spontaneously translocates across the membrane to be converted to lactosylceramide and the ganglioside GM3. Translocation to the lumen of the trans-Golgi via the ABC transporter MDR1 may be specific for a glucosylceramide pool destined for the synthesis of neutral glycolipids [42]. PM, plasma membrane. Rafts (red lines, indicating sphingolipid/cholesterol composition) in lumenal leaflets of organellar membranes (blue lines, indicating glycerophospholipid composition) serve to sort sphingolipids, cholesterol and proteins towards the plasma membrane.

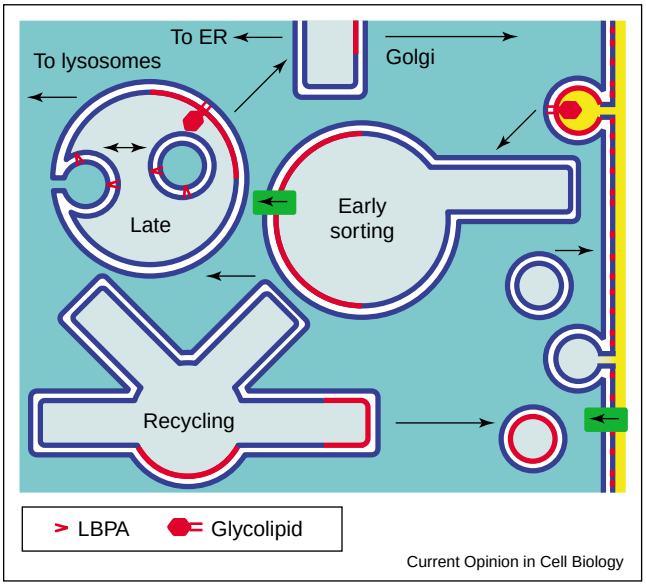
Sorting of lipid-anchored proteins

A special class of plasma membrane proteins are those anchored to the membrane by a lipid tail. What is the molecular mechanism that enriches glycosylphosphatidylinositol (GPI)-anchored proteins on the outer plasma membrane surface and acylated and prenylated proteins at the inner plasma membrane surface? GPI-anchored proteins are apparently excluded from retrograde transport, probably because they distribute with the sphingolipids. Still, whether GPI-anchored proteins are clustered on the cell surface is a matter of debate [21–23]. As GPI-anchored proteins are mainly sorted in the Golgi and endosomes, it seems relevant to study local clustering in these organelles. Acylated and prenylated proteins occupy different areas on the cytosolic surface that probably reflect ordered and disordered lipid domains, respectively [24]. Therefore, lipid rafts are likely to mediate the sorting of lipid-anchored proteins to the plasma membrane.

Lipid-binding proteins and vesicle flux

Unexpectedly, anterograde transport at the Golgi is exquisitely sensitive to changes in the cholesterol concentration [25,26], possibly because this leads to improper segregation of lipids and proteins [27] or to incorrect disposition of proteins on the cytosolic surface. In yeast, membrane flux through the exocytic pathway is regulated by cytosolic Sec14 [28], a family of cytosolic phosphatidylcholine/phosphatidylinositol transfer proteins that via their dual specificity may act as sensors of lipid composition and adapt lipid metabolism. Whereas one mammalian phosphatidylinositol transfer protein, PITPα, may have a related function and reports back to the nucleus [29], the highly homologous Golgi-associated PITPβ is essential for growth [30]. PITPβ binds sphingomyelin, which is unexpected as this lipid is thought to be confined to the Golgi lumen.

Figure 4



Endocytic lipid sorting. Glycolipids (with probably sphingomyelin and cholesterol) aggregate in the plasma membrane and the endosomes, and recycle to the plasma membrane via recycling endosomes and partially via late endosomes and Golgi [34]. Sphingolipids and cholesterol may be present as sizeable domains only when stabilized, e.g. by curvature. However, the lateral localization of the sphingolipids and cholesterol in the endocytic membranes is unknown. Lateral sphingolipid sorting in transcytosis maintains the enrichment of (glyco)sphingolipids in the apical membrane of epithelial cells [43]. The nature of the transport intermediates is not known. Vesicles bud into late endosomes by a process requiring the conical lipid lysobisphosphatidic acid, LBPA [3•]. Both LBPA and cholesterol are enriched in the internal membranes. However, whereas LBPA is enriched in lysosomes, cholesterol is depleted [44]. In some cases, these vesicles fuse back with the outer membrane [45]. Plasma membranes and endosomes contain P-type ATPases of the lipid translocator family. Sphingolipids travel from endosomes towards the Golgi and the plasma membrane. This must involve rafts (red lines).

Selective endocytosis

Endocytosis (Figure 4) may be driven by lipid translocation and actin-based mechanisms, but the primary machinery for protein sorting utilizes (clathrin) protein coats. By contrast, the preferential uptake of glycolipids by caveolae [31] requires lipid-based aggregation [32]. Glycolipids are used as endocytotic receptors by toxins, viruses and bacteria. Lipid rafts seem to play a major role in receptor signaling and endocytosis (not necessarily via caveolae), a process possibly regulated by integrins [33]. The glycolipid rafts appear to concentrate in the pathways from the early endosomes to the recycling endosomes and to the late endosomes, whence the rafts reach the Golgi [34], a transport pathway followed by glycolipid-binding toxins en route to the ER [35]. Transport of both sphingolipids and cholesterol to the Golgi is interrupted under conditions of lipid storage in endocytotic organelles (as seen in many lysosomal storage diseases) [36]. Cholesterol removal from the endosome depends on the NPC1 protein, which contains a sterol-sensing domain. Like its plasma membrane counterpart NPC1L1 [37•], it transports cholesterol (and possibly sphingolipids [38]) towards the cytosol by a mechanism that is unclear but may involve vesiculation. Cholesterol also modulates vesicular traffic at and after the early endosomes via annexin II [39,40].

Conclusions

Eukaryotic cells use the physical properties of their individual membrane lipid classes at specific steps in vesicle traffic. The local concentration of these lipids is regulated by an army of enzymes and translocators and appears to be one parameter in regulating the membrane flux through the various pathways. In addition, the spontaneous segregation of lipid mixtures into different phases appears to be one of the basic mechanisms by which proteins and lipids are sorted towards their

destinations. How the concentrations of lipids in each organelle, the sizes of organelles and the lipid fluxes between them fit in with the complex spatial and temporal network of lipid metabolism is a challenge that can only be addressed by combining our present molecular approaches with the integrative power of systems biology. This field, which may be termed cellular lipidomics, will greatly benefit from the rapid development of sensitive, high-throughput and lipid-wide methodology for lipid quantitation that is to be expected as a consequence of similar developments in genomics and proteomics over the last decade.

Update

The occurrence of two GPI-proteins in separate plasma membrane domains of different lipid composition [47] and the finding of lipid-anchored proteins in cholesterol-independent microdomains on the cytosolic surface (see [48]) illustrate that the reality of the organization of biomembranes is not explained by the mere notion of sphingolipid/cholesterol rafts. A specific function of sphingolipids on cytosolic surfaces is suggested by the fact that the cytosolic protein FAPP2, which contains a glycolipid binding domain, plays a role in regulating membrane flow between the Golgi and the plasma membrane [49]. Finally, whereas cholesterol is assumed to move passively across and between membranes, this transport appears to be mediated by intricate machineries. Cholesterol transport out of endo-/lysosomes requires both the soluble cholesterol-binding NPC2 protein in the lumen and the putative cholesterol transporter NPC1 in the membrane, which turn out to work in concert [50]. Lipids and proteins have been well-studied, but a lot remains to be learnt about how they team up in cell membranes.

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