LIPID TRAFFIC: FLOPPY DRIVES AND A SUPERHIGHWAY

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Abstract | Understanding how membrane lipids achieve their non-random distribution in cells is a key challenge in cell biology at present. In addition to being sorted into vesicles that can cross distances of up to one metre, there are other mechanisms that mediate the transport of lipids within a range of a few nanometres. These include transbilayer flip–flop mechanisms and transfer across narrow gaps between the endoplasmic reticulum and other organelles, with the endoplasmic reticulum functioning as a superhighway along which lipids can rapidly diffuse.

WOBBLE
Movement in which the lipid molecule partially dips into the opposite leaflet of the bilayer and then moves back to its original position without changing its longitudinal orientation.

PHOSPHOINOSITIDE
A phosphorylated derivative of the glycerolipid phosphatidylinositol. As three positions of the inositol ring can be phosphorylated independently of each other, there are seven possible phosphoinositides.

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doi:10.1038/nrm1591

Cellular membranes contain many more lipid species than are needed to form a bilayer (BOX 1). Moreover, the different lipid species are non-randomly distributed between subcellular organelles, as well as between the two leaflets of organelle membranes (FIG. 1). These observations match other data that show that lipids have functions beyond that of providing the backbone of cellular membranes. Lipids are increasingly being recognized as versatile and dynamic regulators of numerous cellular processes that range from cell growth, development and survival to intracellular signalling, cargo sorting and membrane trafficking. Consequently, the dynamics of membrane-lipid organization and its physiological implications is a rapidly expanding multidisciplinary field. The non-random lipid distribution in cells cannot be explained by local metabolism alone and is maintained despite extensive membrane trafficking between different organelles. So, at present, much attention is focused on the mechanisms by which cells impose selectivity and directionality on lipid movement.

Lipid traffic rules

A powerful interplay between *Saccharomyces cerevisiae* genetics and biochemistry in cell-free systems has generated detailed information on the mechanisms of intracellular protein transport[1]. In comparison, our understanding of how lipids are sorted and moved within cells is still rather limited. One of the difficulties is that lipids lack the type of 'address labels' that are used by proteins to reach their destination. Moreover, the traffic rules that apply to lipids are more diverse and less absolute than those for proteins, which makes it harder to translate them into molecular terms. If we ignore the motions that do not result in transport — such as the rotation of the entire molecule around its longitudinal axis and the WOBBLE — membrane lipids are subject to three kinds of movement: lateral diffusion in the two-dimensional plane of the membrane; flip–flop across the membrane into the opposite leaflet; and diffusion out of the membrane into the aqueous phase, followed by insertion into another membrane (FIG. 2).

Lateral movement. Lipids move in the lateral plane of the membrane with diffusion coefficients that are 10–100-fold larger than those of most membrane proteins, and they can cover an area of 0.1–1 $\mu m^2 s^{-1}$ in a cell that is typically 10–20 μm in each dimension[2]. Consequently, lipids would readily equilibrate between organelles that are connected by vesicular pathways. However, such mixing is not observed, and cells are capable of maintaining the unique protein and lipid compositions of their organelles. For proteins, the underlying process is clearly not ongoing local synthesis or breakdown. With the notable exception of PHOSPHOINOSITIDES[3], this is also true for lipids. For example, the enrichment of sphingolipids and sterols in the plasma membrane compared to the endoplasmic reticulum (ER) is not due to their synthesis in the plasma membrane, nor to their hydrolysis in the ER. Consequently, these lipids must be subject to extensive intracellular sorting. The question is, what molecular interactions are responsible for their sorting?

BIOGENIC MEMBRANE
A membrane that can synthesize lipids. Biogenenic membranes include the plasma membrane in Gram-positive bacteria, the inner membrane in Gram-negative bacteria and the endoplasmic reticulum in eukaryotes.

Transbilayer movement. The movement of lipids between the two membrane leaflets is known as flip–flop. In model membranes, flip–flop is slow for lipids that carry a polar head group, but is more rapid for lipids that lack one[4,5]. Half-times ($t_{1/2}$) range from seconds or minutes for diacylglycerol (DAG), ceramide and cholesterol to hours or days for phosphatidylcholine (PC), sphingomyelin and glycosphingolipids (GSLs; FIG. 3). However, a prerequisite for the expansion of BIOGENIC MEMBRANES, such as ER membranes, is that phospholipids that are produced in the cytosolic leaflet can flip to the other side at a rate that is close to that of their production. This led Bretscher[6] to postulate the involvement of a 'FLIPPASE'. ER flippases function independently of metabolic energy and catalyse the transverse movement of most phospholipid classes in both directions[7,8], and therefore promote a symmetrical lipid distribution across the bilayer.

By contrast, the paradigm for an asymmetric lipid distribution is the plasma membrane, where the aminophospholipids phosphatidylserine (PS) and phosphatidylenthanolamine (PE) are generally concentrated in the cytosolic leaflet (FIG. 1). This lipid asymmetry is generated and maintained by an energy-dependent flippase, known as the aminophospholipid TRANSLOCASE, that uses ATP hydrolysis to mediate a fast ($t_{1/2}$ of minutes) translocation of PS and PE from the EXOPLASMIC to the cytosolic leaflet of the bilayer[9]. The importance of keeping PS in the cytosolic leaflet becomes apparent when the plasma-membrane lipid asymmetry is disrupted by the action of a Ca^{2+}-activated phospholipid SCRAMBLASE[10]. PS that is externalized by this activity can trigger important physiological events, from blood coagulation to the recognition and clearance of apoptotic cells[11]. In recent years, a growing list of candidate lipid flippases has been identified, which has provided new opportunities to explore the mechanism and biological significance of lipid transport across cellular membranes.

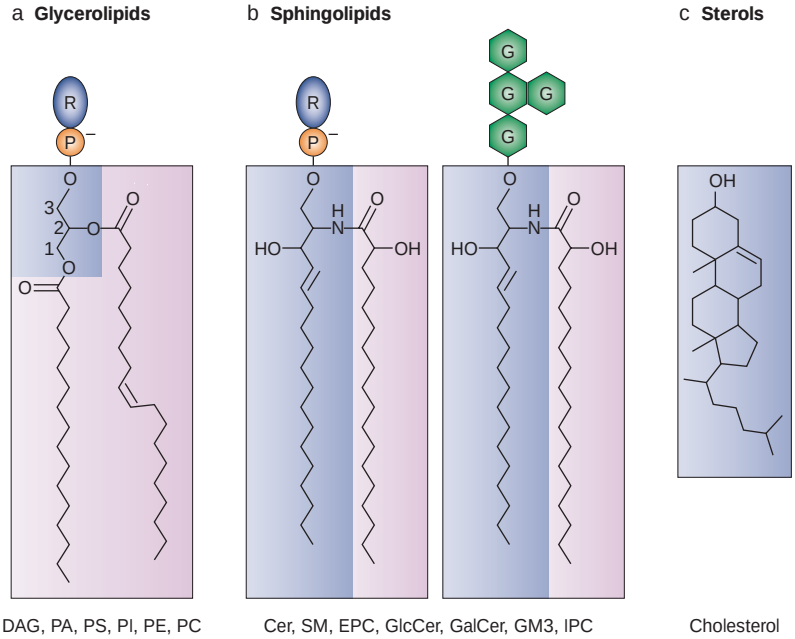
Monomeric exchange. Lipids can exchange between membranes as single molecules. Monomeric exchange involves the desorption of a lipid from a membrane into the aqueous phase and its insertion into another membrane. Spontaneous exchange rates, which are limited by the desorption step, are relatively rapid for short-chain or single-chain lipids (a $t_{1/2}$ of minutes for lysophosphatidylcholine), but there is no appreciable desorption of monomeric lipids that carry two long fatty-acid chains (a $t_{1/2}$ of days for DAG, PC and GSLs)[5,12] (FIG. 3). Despite this, the monomeric exchange of lipids is crucial as a transport mechanism for organelles like mitochondria, chloroplasts and lipid droplets that are not connected to vesicular pathways and that lack the synthetic machinery to generate their own full repertoire of lipids. This non-vesicular phospholipid trafficking can be highly efficient[13]. For example, mitochondria must import PC, PS and phosphatidylinositol (PI) to maintain their functional integrity[13]. Also, in some cell types, an intermediate step in the synthesis of PC — the single most abundant phospholipid — occurs in the inner mitochondrial membrane, whereas the other steps in the pathway occur in the ER. This indicates a bulk two-way flow of lipid in and out of mitochondria[14]. There is growing evidence that monomeric exchange has an even more general role in lipid transport between organelles, because phospholipid transport from the ER to the plasma membrane proceeds unperturbed when vesicular pathways are blocked by genetic manipulation or by metabolic poisons[15–17]. In addition, specific lipids are selectively

Box 1 | The three main classes of eukaryotic membrane lipids

Glycerolipids are based on glycerol (see figure, part **a**; blue shading) with two C16–C18 fatty-acid chains (pink shading) linked at sn-1 and -2 (which forms diacylglycerol (DAG)). A *cis*-double bond is usually present in the fatty acid that is linked at sn-2, which causes a kink in the acyl chain and decreases the packing density of the lipid. A phosphate (P) can be attached at sn-3 (which forms phosphatidic acid (PA)) and this phosphate can carry a head group (R) that is either neutral (which produces phosphatidylserine (PS) or phosphatidylinositol (PI)) and gives a net acidic charge, or basic (which forms phosphatidylethanolamine (PE) or phosphatidylcholine (PC)) and gives a neutral or zwitterionic lipid.

Sphingolipids are based on a C18 sphingoid base, which is usually sphingosine in animals (see figure, part **b**; blue shading), with a saturated C16–C26 fatty acid (pink shading) that is amide linked to the nitrogen (which forms ceramide (Cer)). In dihydroceramide, sphinganine replaces sphingosine (sphinganine lacks the *trans*-double bond between C4 and C5). Phytoceramide in plants and fungi contains phytosphingosine (C4-OH sphinganine) and a fatty acid that is usually hydroxylated at C2 (not shown). In animals, the sphingolipid head group (R in the left panel of part **b** of the figure) can be phosphocholine (which produces sphingomyelin (SM)) or phosphoethanolamine (to form ethanolaminephosphoryl ceramide (EPC)). Alternatively, in the case of glycosphingolipids, it can be glucose (to form glucosylceramide (GlcCer)) or galactose (to produce galactosylceramide (GalCer)), which can be further decorated by extra monosaccharides (all labelled G in the right panel of part **b** of the figure) to create a wide range of glycosphingolipids (for example, GM3). Plant and fungal sphingolipids typically contain a phosphoinositol head group (which forms inositolphosphorylceramide (IPC)) that is often mannosylated.

Sterols are based on a planar four-ring structure (see figure, part **c**), with cholesterol being the form that is present in mammals, ergosterol in fungi, and stigmasterol and sitosterol in plants.



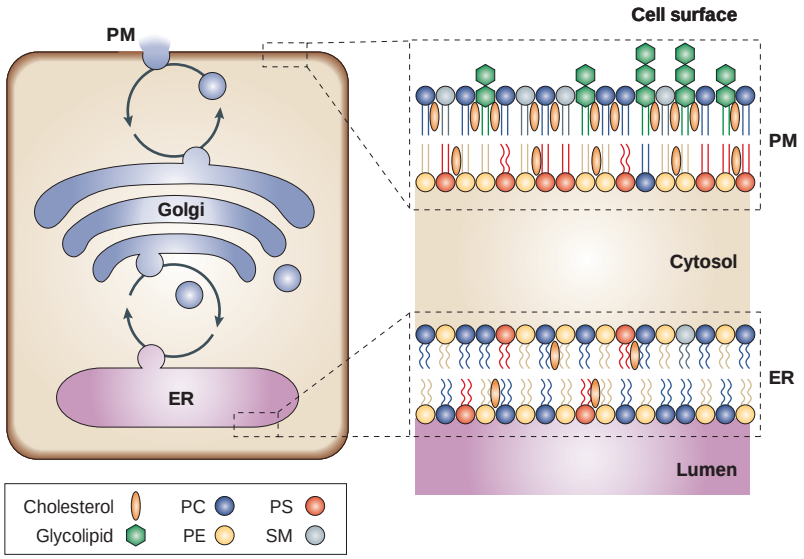


Figure 1 | **Membrane-lipid distributions.** Membrane lipids show non-random distributions between and within organelles that are connected by vesicular pathways[31,98–100]. The plasma membrane (labelled PM in the figure) is rich in sterols, sphingolipids and saturated glycerolipids (BOX 1), which, due to their high packing densities, promote bilayer rigidity and impermeability. For example, the rat liver plasma-membrane composition is: 30–40 mol% cholesterol; 10–15 mol% sphingomyelin (SM) and glycosphingolipids; 25 mol% phosphatidylcholine (PC); 15 mol% phosphatidylethanolamine (PE); 5 mol% phosphatidylserine (PS); and 5 mol% phosphatidylinositol (PI). In addition, the plasma membrane has an asymmetric lipid arrangement with the aminophospholipids concentrated in the cytosolic leaflet and the sphingolipids concentrated in the exoplasmic leaflet. The membrane topology of cholesterol is not known, but its location is probably determined by its high affinity for sphingolipids and saturated glycerolipids[22–24]. The endoplasmic reticulum (ER) membrane, on the other hand, shows a symmetric lipid distribution and primarily contains unsaturated glycerolipids that make the membrane flexible, and therefore facilitate the incorporation of newly synthesized proteins. For example, the composition of the ER membranes of rat liver is: 5 mol% cholesterol; 50–60 mol% PC; 25 mol% PE; and 10 mol% PI. Vesicular traffic between the ER and the plasma membrane passes through the Golgi, a multi-cisternal organelle in which lipid sorting must occur to prevent the randomization of the subcellular lipid distribution. This figure has been modified with permission from REF. 102 © (2003) Taylor and Francis Ltd.

delivered to points late in the EXOCYTIC PATHWAY by non-vesicular routes. Two examples have been particularly well studied: the delivery of ceramide from the ER directly to the *TRANS*-GOLGI[18,19]; and the delivery of PI from the ER to both the Golgi and the plasma membrane for conversion into phosphoinositides[20,21]. Both transport routes are highly conserved throughout evolution and are mediated by lipid-transfer proteins (LTPs). LTP-dependent lipid transport does not require metabolic energy; instead, it is driven by the flow of individual lipids down concentration gradients that are maintained by the removal or consumption of lipid in the acceptor compartment. Later, we return to the role of LTPs, and suggest that they can transport lipids with a uniquely high efficiency when they are anchored near narrow cytosolic gaps where the ER comes into contact with other organelles.

Sorting along endocytic and exocytic pathways

Lipid sorting. Given the bidirectional vesicular pathways that function between the ER and the plasma membrane, a remarkable feature of lipid organization in animal cells is the enrichment of sphingolipids and cholesterol in the plasma membrane (FIG. 1). Cholesterol is synthesized at the ER and can move spontaneously across the membrane and between two adjacent membranes as a monomer. Its location is probably determined by its high affinity for sphingolipids and saturated glycerolipids[22–24]. Sphingolipids (sphingomyelin and complex GSLs) are synthesized in the Golgi lumen and have no access to the cytosolic leaflet[25–27], which forces the conclusion that sphingolipids must be sorted — that is, selectively included in ANTEROGRADE-moving membrane carriers or excluded from RETROGRADE ones. According to the cisternal-maturation model (BOX 2), net anterograde transport could be achieved if sphingolipids were prevented from leaving the cisternae in which they are made. In support of this scenario, coatomer protein (COP)I-COATED VESICLES contain significantly reduced amounts of sphingomyelin and cholesterol in comparison to their parental Golgi membranes[28]. At present, the debate focuses on whether lipid sorting is achieved because the vesicle-budding machinery is recruited to phase-separated LIPID MICRODOMAINS, or because the generation of curvature (by coats or motor proteins) induces large-scale sorting by excluding lipid species that tend to form flat and rigid membranes (FIG. 4). Model-membrane studies show that lipid-microdomain formation can trigger membrane-budding and -fission events, which raises the possibility that cells exploit lipid immiscibility to drive membrane vesiculation[29]. However, a general consensus on the size, shape and dynamics of lipid microdomains in cellular membranes is lacking at present[30].

Coupled lipid and protein sorting. The exclusion of sphingolipids and sterols from COPI vesicles will cause their progressive accumulation in maturing cisternae, and this process is probably coupled to a gradual depletion of unsaturated glycerolipids along the exocytic pathway[31]. This remodelling of the cisternal membrane will alter its biophysical properties and promote a more compact, thicker and less permeable bilayer. The enrichment in sterols and sphingolipids might explain the observed increase in bilayer thickness of the *trans*-Golgi and plasma membranes[31,32], which is most pronounced in the GSL-enriched APICAL MEMBRANE of polarized epithelial cells[31–33]. Bretscher and Munro[34] proposed a model for protein sorting in the Golgi, which is based on preferential interactions between lipids and transmembrane proteins that have the best-matching hydrophobic lengths. Golgi enzymes generally have shorter transmembrane domains than plasma-membrane proteins, and this property would prevent them from being included in the sphingolipid/sterol-rich membrane regions that move to the cell surface (FIG. 4). Consistent with this idea, lengthening the transmembrane domains of Golgi enzymes results in them moving to the plasma membrane[35], whereas shortening the transmembrane domains of plasma-membrane proteins results in them being retained in the Golgi[36].

FLIPPASE
A general term that refers to a protein, or protein complex, that facilitates the energetically unfavourable movement of the polar head group of a phospholipid or glycosphingolipid through the hydrophobic interior of a membrane.

However, the recent finding that, in rat liver cells, the basolateral membrane is actually the thinnest of all exocytic-pathway membranes indicates that hydrophobic matching to bilayer thickness is unlikely to function as a dominant mechanism for protein sorting[33]. Indeed, basolateral protein sorting typically relies on the recognition of discrete targeting motifs in the cytosolic tails of membrane proteins by adaptor-protein coats[37]. Apical protein sorting, on the other hand, is thought to be more dependent on the cooperation of weak interactions between GSLs and glycoproteins (for example, glycosylphosphatidylinositol (GPI)-ANCHORED PROTEINS) in the *trans*-Golgi membrane and lumen[38]. Whether coat proteins have a role in the formation of apical transport carriers remains unclear. Apical transport intermediates might form by the progressive maturation of *trans*-Golgi cisternae, which might be driven by the adaptor-protein-dependent removal of basolateral and endosomal/lysosomal material. Therefore, the sorting of apical cargo might occur through a 'sorting by retention' principle, which is based on the aggregative properties of apical-membrane proteins and lipids. As it would represent the terminal step in the cisternal-maturation process, such a mechanism would not require an apical coat complex. However, in polarized Madin–Darby-canine-kidney cells, newly synthesized GPI-anchored proteins are first delivered to the basolateral membranes, and are then internalized by a clathrin-independent pathway that leads to the apical membrane[39]. GPI-anchored proteins in liver cells also follow an indirect pathway to the apical membrane. The selective uptake of GPI-anchored proteins seems to occur through caveolae — flask-shaped plasma-membrane invaginations that are found on the basolateral surface of epithelial cells[39,40]. Interestingly, GSLs are also selectively internalized through caveolae[41]. So, in some epithelial cells, the sorting of newly synthesized GPI-anchored proteins and possibly also GSLs might not occur at the *trans*-Golgi network, but rather during internalization from the basolateral membrane.

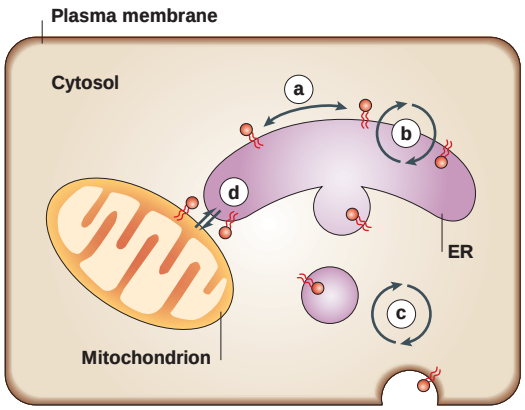


Figure 2 | **Mechanisms of lipid transport.** Lipids can diffuse laterally (step **a**; lateral movement) or transversely between the two leaflets of each organelle membrane (step **b**; transbilayer movement or flip–flop). As lipids form the backbone of a membrane, they are an integral part of vesicular carriers (step **c**; vesicular transport) that connect, for example, the endoplasmic reticulum (ER) and the plasma membrane indirectly through the Golgi (not shown). At the same time, lipids can be exchanged as monomers between the cytosolic surfaces of organelle membranes (step **d**; monomeric exchange). Monomeric exchange is especially relevant for organelles such as mitochondria and chloroplasts that are not connected to other organelles by vesicular pathways, and can be facilitated by lipid-transfer proteins. Maintaining the differences in lipid composition between organelles and between the two leaflets of individual organelle membranes requires selectivity and directionality in the various transport mechanisms. For simplicity in this figure, scaled-up lipids (red) are shown on top of single lines that represent the various membrane bilayers.

How cells connect the opposing sides of a bilayer

Apart from the need for cellular lipids to reach their membrane of destination, they have to adopt the right distribution over the two halves of the membrane. Transbilayer lipid movement is essential for cell growth and survival, and it is crucial for a balanced growth of the ER bilayer. The same is true for mitochondria, organelles that derive most of their membrane lipids from the ER through a non-vesicular pathway. Although the precise mechanism by which lipids are moved across cellular membranes remains to be established, it is generally accepted that this process is protein-mediated and involves both energy-dependent and energy-independent lipid flippases. The latter category includes ER flippases and plasma-membrane scramblases. These activities function as facilitators of transbilayer lipid movement, presumably by providing a membrane-spanning hydrophilic surface along which the polar head group can slide. Such facilitators can be selective for certain lipids (TABLE 1; see also online supplementary information S1 (table)), but they cannot move lipids against a concentration gradient or into a leaflet that is under high lateral pressure. Energy-dependent flippases, or translocases, on the other hand, can work against a gradient. A prime example is the aminophospholipid translocase, which is responsible for the selective sequestration of PS and PE in the cytosolic leaflet of the plasma membrane.

Energy-independent flippases. Unlike the situation in protein-free liposomes, phospholipid flip–flop across the ER bilayer is fast (the $t_{1/2}$ ranges from seconds to minutes) and is independent of metabolic energy[7,8]. Even though a consensus has been reached that lipid flip–flop in the ER is protein mediated, it is unclear whether it involves a dedicated flippase, a group of flippases, or the mere presence of proteins in the ER bilayer. The first steps in the purification of a bidirectional ER flippase have been reported[42]. However, the observation that peptides that mimic the α-helices of transmembrane proteins can stimulate phospholipid flip–flop in liposomes indicates that the ability to catalyse flip–flop in the ER is not necessarily restricted to one specific protein[43]. An exception concerns the translocation of dolichol-pyrophosphoryl oligosaccharides from the cytosolic to the lumenal leaflet of the ER, which is an obligatory step in *N*-linked protein glycosylation that is probably mediated by the Rft1 protein in *S. cerevisiae*[44] (TABLE 1). Furthermore, complex GSL synthesis in the

TRANSLOCASE
A lipid translocase is a flippase that uses ATP hydrolysis to catalyse the unidirectional movement of a lipid from one membrane leaflet to the other. Translocases help to create asymmetric lipid distributions across the bilayer. A well-known example is the aminophopsholipid translocase that is responsible for the selective sequestration of phosphatidylserine and phosphatidylethanolamine in the cytosolic leaflet of the plasma membrane.

EXOPLASMIC LEAFLET
The non-cytosolic leaflet of a membrane, which faces the extracellular space or the lumen of an organelle.

SCRAMBLASE
An energy-independent, bidirectional lipid flippase that, when activated by a transient rise in intracellular Ca^{2+} levels, disrupts lipid asymmetry by facilitating a rapid equilibration of lipids between the two membrane leaflets.

EXOCYTIC PATHWAY
Newly synthesized proteins that are destined for the cell surface are first imported by the endoplasmic reticulum, then move through the Golgi complex and, finally, are packaged into secretory vesicles that fuse with the plasma membrane.

TRANS-GOLGI
The Golgi apparatus is composed of a stack of flattened cisternae (bags), which is asymmetric. Post-endoplasmic-reticulum carriers fuse together to make the *cis* side of the Golgi, whereas the *trans* side is where post-Golgi carriers form.

ANTEROGRADE/RETROGRADE
Terms that signify the direction in which vesicles move. Cargo that is destined for the cell surface moves in the anterograde direction, and movement in the opposite direction, namely from the cell surface toward the endoplasmic reticulum, is retrograde.

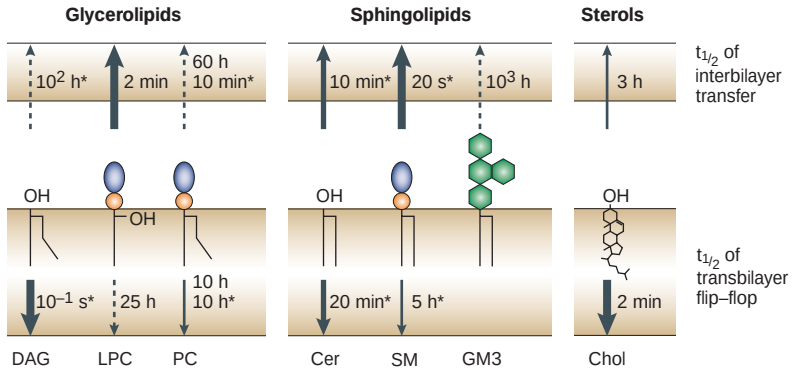


Figure 3 | **Interbilayer and transbilayer lipid movement.** The figure shows the half-times ($t_{1/2}$) for spontaneous interbilayer and transbilayer lipid movement[5,12,101]. Larger arrows signify fast movement, whereas slower movement is indicated by dashed arrows. Concomitant with an increased size or polarity of its head group or a decreased hydrophobicity of its backbone, the lipid transfers more readily between two opposing membranes (interbilayer movement), but flips less easily across the membrane (transbilayer movement). The flip–flop rate of polar lipids, such as phosphatidic acid or phosphatidylglycerol, increases when the negative charge is neutralized by protonation. Chain saturation and a close packing of lipids (for example, in liquid-ordered microdomains) increase membrane viscosity and lower both rates of movement. The $t_{1/2}$ of transbilayer flip–flops or interbilayer transfers that are marked with an asterisk have been determined using short chain (C5) dimethyl-bodipy-labelled lipid analogues. For more information on the lipid representations shown here, see BOX 1. GM3 is a simple and widely distributed glycosphingolipid. Cer, ceramide; Chol, cholesterol; DAG, diacylglycerol; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; SM, sphingomyelin.

Golgi lumen requires the transbilayer movement of the precursor glucosylceramide, which is synthesized on the surface of the *cis*-Golgi[25]. Whether this energy-independent transport is mediated by a dedicated monohexylsphingolipid flippase is unclear[27].

The hypothesis that the mere presence of transmembrane helices is sufficient to induce rapid flip–flop only holds for specific organelles. For example, flip–flop in the plasma membrane is a slow process[9,27], even though this organelle contains many different transmembrane proteins. This might be related to the finding that helix-induced flip–flop is strongly inhibited by cholesterol[43], which is an abundant component of the plasma membrane. Cholesterol might exert its inhibitory effect by causing an increased packing of the acyl chains through which the polar head group has to travel. The gradual increase in cholesterol levels along the exocytic pathway might therefore function as a regulatory device to switch the mechanism of transbilayer lipid movement from a constitutive flip–flop in the ER to a more tightly controlled translocation in the plasma membrane[43]. This would facilitate the establishment of an asymmetrical transbilayer lipid arrangement. In red blood cells, a rise in intracellular Ca^{2+} causes the disruption of lipid asymmetry by activating a scramblase that mediates the rapid equilibration of phospholipids between the two plasma-membrane leaflets. A red-blood-cell-derived membrane protein, PLSCR1 (TABLE 1), was found to be capable of triggering Ca^{2+}-dependent flip–flop in reconstituted proteoliposomes[45]. However, red blood cells from *Plscr1*$^{-/-}$-knockout mice did not show any defect in the externalization of aminophospholipids on elevation of intracellular Ca^{2+} concentrations[46]. As PLSCR1 belongs to a protein family (TABLE 1), the absence of a phospholipid-scrambling defect in these knockout mice might be due to redundancy. Finally, although fatty acids are predicted to move easily across lipid bilayers, a family of fatty-acid transporters has been identified (TABLE 1) that facilitates the import of exogenous fatty acids across the plasma membrane[47].

Energy-dependent inward flippases. The most well-studied example of an energy-dependent flippase is the aminophospholipid translocase (APLT), which hydrolyses ATP to energize the fast inward translocation of PS and PE across the plasma membrane. APLT was first detected in the human red-blood-cell membrane by Devaux and co-workers[9], but APLTs have now been found in the plasma membranes of many nucleated cells, as well as in the membranes of secretory vesicles and the *trans*-Golgi[10,48,49]. Some cell types show a similar inward transport of PC and might therefore contain either a PC-specific translocase in addition to the APLT or an inward translocase of different specificity that translocates both aminophospholipids and PC[48,50]. Prime candidates for APLTs are members of a conserved subfamily of P-TYPE ATPASES called P4 ATPases[50–52] (TABLE 1). P4 ATPases in humans have been implicated in FAMILIAL INTRAHEPATIC CHOLESTASIS[53] and ANGELMAN SYNDROME[54], but it is not yet known which cellular processes are affected in these diseases. However, P4 ATPases in *S. cerevisiae* reside in different organelles of the exocytic pathway and have a crucial role in the formation of plasma-membrane- and Golgi-derived transport vesicles[50,55,56]. Precisely how P4 ATPases participate in vesicle biogenesis is unclear. One possibility is that a high concentration of aminophospholipids in the cytosolic leaflet is required for the efficient recruitment of the vesicle-budding machinery. Alternatively, P4-ATPase-dependent lipid pumping might facilitate vesicle budding by expanding the cytosolic leaflet of the membrane at the expense of the exoplasmic leaflet[57]. Interestingly, stimulation of APLT activity seems to be sufficient to trigger endocytic-vesicle formation in red blood cells[58], which lack the machinery for generating coated vesicles. Members of a newly identified protein family — the

COPI-COATED VESICLES
Transport vesicles that bud from the Golgi due to the assembly of a cytosolic coat protein known as coatomer protein (COP)I.

LIPID MICRODOMAINS
Lateral lipid assemblies that form spontaneously in the bilayer due to a differential miscibility of membrane lipids. Microdomains are typically enriched in sphingolipids and sterols, and have been postulated to acquire specific functions by concentrating or excluding specific membrane proteins. Conversely, membrane proteins might affect microdomain size, composition and lifespan.

APICAL MEMBRANE
The region of the plasma membrane of an epithelial cell that faces the lumen. The region that is connected to the underlying tissue is known as the basolateral membrane.

GPI-ANCHORED PROTEINS
Proteins that are primarily found at the cell surface and that are attached to the bilayer by means of a lipid anchor, which is composed of glycosylphosphatidylinositol (GPI).

Box 2 | Cisternal-maturation model

This model proposes that anterograde transport through the Golgi stack is mediated by the cisternae. In brief, a *cis*-cisterna arises through the fusion of vesicular intermediates from the endoplasmic reticulum. It then matures from a *cis*- to a *trans*-cisterna as a result of the exchange of Golgi-associated enzymes through small coatomer protein (COP)I-coated vesicles, which are moving in a retrograde fashion. Cisternal maturation would explain the anterograde transport of procollagen and other large molecules that do not readily fit into the 50-nm COPI vesicles. However, as COPI vesicles are unlikely to move exclusively in a retrograde fashion, and because not all secretory cargo passes through the Golgi stack at the same rate, it is believed that COPI vesicles also contribute to anterograde transport.

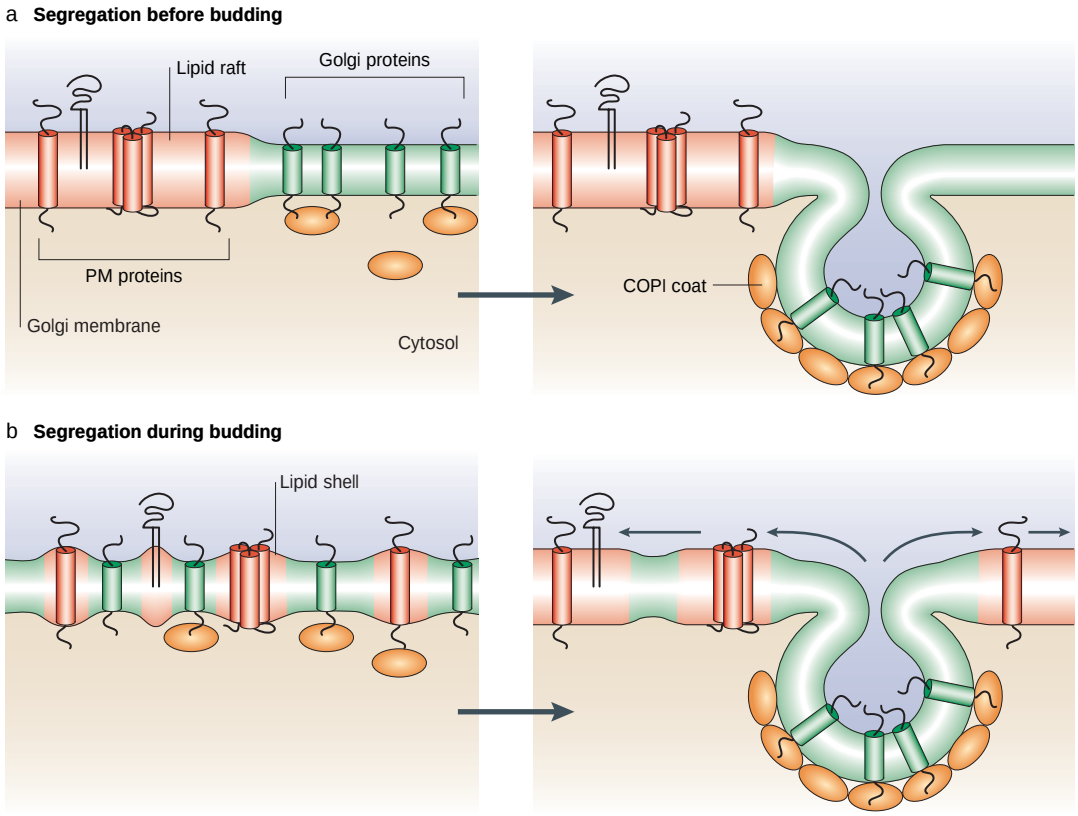


Figure 4 | **Models for the coupled sorting of lipids and proteins in the Golgi.** Coatomer protein (COP)I-coated vesicles, which are involved in recycling Golgi proteins, contain lower sphingolipid and sterol levels than the cisternal membranes from which they bud. **a** | This lipid segregation might occur because ongoing sphingolipid synthesis triggers a phase separation and generates sphingolipid/sterol-poor domains (green) that function as donor sites for COPI vesicles and sphingolipid/sterol-rich domains (red; lipid rafts) that do not ('segregation before budding' model). **b** | Alternatively, the coat machinery might influence the lipid composition at the bud site by imposing a high curvature on the membrane. This would lead to the selective exclusion of sphingolipids, sterols and other lipids that tend to form flat and rigid membranes ('segregation during budding' model). Due to their short membrane spans, Golgi proteins would segregate away from the thick sphingolipid/sterol-rich membrane regions and preferentially partition into the glycerolipid-rich COPI vesicles. Plasma membrane (PM) proteins, on the other hand, do not have access to COPI vesicles, either because their membrane anchors are favoured energetically by an ordered sphingolipid/sterol-rich environment (which is found in lipid rafts/shells) or because of the preferential partitioning of Golgi proteins at sites of vesicle budding. The retention of plasma-membrane proteins in Golgi cisternae would result in their unidirectional transport toward the cell surface by cisternal maturation (BOX 2). This figure has been modified with permission from REF. 102 © (2003) Taylor and Francis Ltd.

P-TYPE ATPASES
A ubiquitous family of polytopic membrane proteins that use the energy of ATP to transport ions across cellular membranes against a concentration gradient. A distinctive biochemical feature of these pumps is an acid-stable phosphorylated aspartate residue that forms during the pumping cycle, and the phosphorylated (P) intermediate gives the family its name.

FAMILIAL INTRAHEPATIC CHOLESTASIS
A rare inherited condition in children, in which patients are unable to secrete bile from the liver, which often progresses to an irreversible scarring of the liver (cirrhosis) within the first decade of life.

ANGELMAN SYNDROME
A neurological disorder, also named 'happy puppet' syndrome, in which severe learning difficulties are associated with a characteristic facial appearance and behaviour.

OUTER-SEGMENT DISC MEMBRANE
The outer segment of rod cells contains a stack of discs, which are each formed by a closed membrane in which the photoreceptor rhodopsin is embedded.

APOLIPOPROTEINS
Proteins that form scaffolds for extracellular lipoprotein particles that carry lipids (triglycerides and cholesterol esters) between the liver and peripheral tissues.

TANGIER DISEASE
A genetic disorder that is characterized by a defect in the efflux of cholesterol and its associated esters to high-density lipoproteins. The disease was first identified in a five-year-old inhabitant of the island of Tangier, located off the coast of Virginia, USA.

CDC50 family — probably represent integral components of the P4-ATPase-dependent translocation machinery[59], with a function similar to that of the β-subunit of X^+, K^+-ATPases (TABLE 1).

Another type of putative energy-dependent inward flippase is the ATP-binding cassette (ABC) transporter ABCA4. This photoreceptor-cell-specific transporter has been associated with Stargardt macular dystrophy, a retinal degenerative disease that is accompanied by the defective transport of retinal PE derivatives from the lumenal to the cytosolic leaflet of the OUTER-SEGMENT DISC MEMBRANE[60].

Energy-dependent outward flippases. The study of multidrug resistance in cancer cells and in *S. cerevisiae* led to the identification of ABC transporters that have a role in the outward movement of lipids across the plasma membrane. Mice that lack the liver-specific ABC transporter ABCB4 are unable to extrude PC into the bile[61], a process that involves the outward translocation of PC and its subsequent desorption from the cell surface. A closely-related human ABC transporter, ABCB1, and its functional counterparts in *S. cerevisiae* translocate various short-chain lipid analogues to the outer face of the plasma membrane[62,63], and the overexpression of these translocases causes endogenous aminophospholipids to accumulate at the cell surface[50].

The impaired efflux of cholesterol across the plasma membrane to exogenous APOLIPOPROTEINS in TANGIER DISEASE is caused by mutations in ABCA1. Given its role in PS exposure on apoptotic cells and macrophages that are carrying out phagocytosis, ABCA1 might affect cholesterol transport indirectly by translocating PS towards the cell surface[64]. Mutations in ABCG5 and ABCG8, two further candidate sterol/phospholipid translocases, cause sitosterolaemia, a disease that affects the intestinal absorption and biliary excretion of dietary sterols[65]. Furthermore, the import of long-chain fatty acids or

ADRENOLEUKODYSTROPHY
An inherited metabolic disorder in which the myelin sheath on nerve fibres is lost and the adrenal gland degenerates, which leads to progressive neurological disability and death. People with adrenoleukodystrophy accumulate high levels of very-long-chain fatty acids in their brain and adrenal cortex, because the fatty acids are not broken down in a normal manner.

PERIPHERAL MEMBRANE PROTEINS
Proteins that have an affinity for a membrane because they bind to a membrane receptor (either another membrane protein or a lipid head group). They do not integrate into the hydrophobic core of the bilayer.

acyl-coenzyme-A molecules into peroxisomes requires the ADRENOLEUKODYSTROPHY-associated transporter ABCD1 (REF. 66). Finally, recent work in *S. cerevisiae* identified a novel membrane protein, Rsb1, that has an essential role in the ATP-dependent efflux of long-chain bases across the plasma membrane[67]. Rsb1 does not belong to the ABC-transporter family and lacks an obvious homologue in humans. In addition, even though Rsb1 contains a predicted ATP-binding motif, it is unclear whether the protein functions as a transport ATPase.

Lipid-transfer proteins

Lipid-transfer-protein families. Several protein families have been discovered that can transport lipid monomers across the aqueous phase[68] (TABLE 2; see also online supplememtary information S2 (table)). Each LTP tends to bind to a specific lipid with a 1:1 stochiometry, using a deep hydrophobic pocket that sometimes has a hinged cover (BOX 3). Structurally different LTPs seem to use a common solution to the problem of extracting a lipid from a membrane. In the open conformation, the hinged cover projects away from the protein, and the hydrophobic portion of the cover that lined the pocket now projects outwards[69,70]. This allows an LTP in the open conformation to embed itself partially into the cytosolic leaflet of the bilayer. Hydrophobic patches near the opening to the binding pocket might initiate the docking of LTPs[71]. Genome-wide surveys have shown that there are large families of proteins that contain lipid transfer (LT) domains (TABLE 2). Many of these proteins are much larger than the LT domain itself and contain domains with activities that are unrelated to lipid transfer (TABLE 2) — for example, GTPase regulators and transcription factors[72,73]. This indicates that some proteins that contain LT domains might function as lipid sensors rather than as lipid carriers.

Lipid-transfer-protein targeting. LTPs are generally thought to be cytosolic, as they were originally purified from cytosolic activities[68], which fits with their function in transferring lipids across the cytosol. However, it seems more probable that LTPs are PERIPHERAL MEMBRANE PROTEINS. To exchange lipids between membranes, they must function at membranes[69,70]. LTPs are therefore likely to depend on accurate membrane targeting for their activity, as is true for other peripheral membrane proteins[74]. However, in contrast to most peripheral membrane proteins that target one compartment, LTPs might be expected to contain two separate targeting signals, one for the donor compartment and one for the acceptor compartment, so that they can efficiently shuttle lipids between the two. Evidence that LTPs are not purely cytosolic has accumulated over recent years, and a number of them show a dual-targeting capability (TABLE 2). A crucial point that is often overlooked is that the binding of a lipid ligand deep inside an internal pocket is unlikely to be responsible for the membrane targeting of an LTP, because the lipid is extracted from the membrane when it binds to the LTP. So, immediately following binding, any anchoring to the membrane is lost. We therefore expect LTPs to have specific targeting determinants that interact with other membrane components (either lipids or proteins). Some of these targeting determinants are probably present on the external surface of the LT domains, about which little is known. In some cases, targeting determinants have now been found in domains that are separate from the LT domain (TABLE 2).

Table 1 | Candidate lipid flippases*

Protein type	Human homologue‡	Yeast homologue‡	Substrate	Biological role
Energy-independent flippases				
ER flippases	AAH43595	Rft1	$Man_5GlcNAc_2$-PP-Dol	*N*-linked glycosylation
Ca^{2+}-induced scramblases	PLSCR1/2/3/4	YJR100C	PLs	Blood coagulation, mast-cell activation, clearance of apoptotic cells
Fatty-acid transporters	FATP1/2/3/4/5/6	Fat1	VLCFAs	Energy homeostasis
Energy-dependent inward flippases				
P4 P-type ATPases	ATP8A1/A2, ATP8B1/B2/B3/B4, ATP9A/B, ATP10A/B/D, ATP11A/B/C	Dnf1, Dnf2, Dnf3, Drs2, Neo1	PS, PE (PC)	Maintenance of lipid asymmetry, transport-vesicle formation, fertility, bile secretion
CDC50-family members	Q9NV96, AAH09006, XP_090844	Cdc50, Ros3, Crf1	PS, PE (PC)	P4 P-type-ATPase stability, localization and function
ABC transporter	ABCA4	–	Retinal-PE	Vision
Energy-dependent outward flippases				
ABC transporters	ABCA1 ABCB1/MDR1 ABCB4/MDR3 ABCD1 ABCG1/G5/G8	– Pdr5, Yor1 – Pxa1, Pxa2 –	PLs, cholesterol PLs, GSLs PC VLCFAs PLs, cholesterol	Sterol homeostasis Multidrug resistance Bile secretion Fatty-acid β-oxidation Sterol homeostasis
Others	?	Rsb1	LCBs	Sphingolipid homeostasis

*A further version of this table that includes all of the relevant references is available as online supplementary information S1 (table). ‡Some human and yeast proteins were identified solely by sequence similarity and have not yet been characterized functionally. ABC, ATP-binding cassette; ER, endoplasmic reticulum; GSLs, glycosphingolipids; LCBs, long-chain bases; $Man_5GlcNAc_2$-PP-Dol, $mannose_5$-$acetylglucosamine_2$-pyrophosphatidyldolichol; MDR, multidrug resistance; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PLs, phospholipids; PS, phosphatidylserine; VLCFAs, very-long-chain fatty acids.

PARASITOPHOROUS VACUOLE
A membrane-bound organelle that contains an intracellular parasite, such as *Toxoplasma gondii*. Although the membrane that surrounds this organelle is derived from the host, it is modified by the parasite to facilitate its survival and growth.

INTEGRAL MEMBRANE PROTEIN
A protein with a moiety (either a covalently linked lipid or a transmembrane domain) that is integrated into the hydrophobic core of the bilayer.

Membrane contact sites

The microanatomy of the ER. As discussed above, LTPs shuttle lipids from a donor compartment (usually the ER) across cytosolic gaps to an acceptor compartment, and to do this with maximum efficiency, they probably function as peripheral membrane proteins on both compartments. A crucial feature for this process is the microanatomy of the ER. The ER forms zones of apposition — or membrane contact sites (MCSs) — at which it comes within 10 nm of almost every other membrane compartment. Among the numerous subspecialized regions in the ER (there are at least 16; REF. 75) are MCSs with mitochondria, the plasma membrane, chloroplasts, lipid droplets, the *trans*-Golgi, endosomes, lysosomes and PARASITOPHOROUS VACUOLES[14,76–80]. In each case, the ER comes close to, but does not fuse with, the other compartment. Importantly, there is evidence for lipid trafficking across most of these sites[14,18,19,76,81–85]. MCSs have often been overlooked because of the ubiquitous nature of the ER. Therefore, when ER-like membranes co-purify with another organelle in biochemical studies, they tend to be dismissed as contaminants. Similarly, in ultrastructural studies, the proximity of an organelle to the ER might be assumed to be a random event. However, when they are studied in depth, MCSs have been shown to be genuine, specialized organelle–organelle contact sites that are formed by protein–protein bridges[81,86]. There is only one example for which the molecular composition of the inter-organelle bridge is known: the nucleus–vacuole junction in *S. cerevisiae*. This is a large MCS between the cytosolic leaflet of the vacuolar limiting membrane and the cytosolic leaflet of the outer nuclear envelope[87], which is topologically similar to the ER–endosome MCSs that are found in higher eukaryotic cells[78]. The structural requirement for this MCS is a single INTEGRAL MEMBRANE PROTEIN of the outer nuclear envelope that binds to a single protein on the surface of the vacuole[79]. Therefore, two different organelles can be brought together at MCSs by simple protein bridges.

In the light of the many different types of MCS, we would like to speculate that the ER network is more than just a ubiquitous structure that extends to all parts of the cytoplasm, in that it might also indirectly link most intracellular membranes. The ER might therefore function as a conduit for lipids and other small molecules

Table 2 | Candidate lipid-transfer proteins*

LT domain	LT-domain-containing protein‡	Lipid bound§	Target membrane or membranes‖	LT-domain-containing -protein domain structure¶
CRAL/TRIO	Sec14	PI, PC	Golgi	LT
	CRALBP	11-*cis*-retinal/retinol	ER	LT
	TAP (SPF)	Squalene, vitamin E, PI	ND	LT–jelly-roll
	TTP	Vitamin E	LE	LT
	Sfh1–5	PI (not Sfh1)	Various	LT
PITP	PITPα	PI, PC	PM	LT
	PITPβ	PI, PC	Golgi, PM	LT
	RdgBαI and II	PI, PC	ER, Golgi	LT–FFAT–PYKi
	RdgBβ	PI, PC	Nucleus	LT
StART	StAR	Cholesterol	Mitochondria	LT
	MLN64	Cholesterol	LE	LT–TMD x 4
	CERT (GPBP)	Ceramide	ER, Golgi	PH–FFAT–LT
	PCTP	PC	Mitochondria	LT
ORP	OSBP, ORP4	25-hydroxcholesterol	ER, Golgi	PH–FFAT–LT
	Kes1, ORP2	Anionic PLs#	Golgi	LT
	ORP1	Anionic PLs#	ER, LE	ANK–PH–FFAT–LT
	Osh1	ND	NVJ, Golgi	ANK–PH–FFAT–LT
	Osh2	ND	ER, PM	ANK–PH–FFAT–LT
	Osh3	ND	ER, PM	GOLD–PH–FFAT–LT
	ORP3/6/7	ND	ER, PM	PH–FFAT–LT
	ORP9	ND	ER, Golgi	PH–FFAT–LT
	ORP5/8	ND	ND	PH–LT–TMD
GLTP	GLTP1	galactosyl/glucosyl lipids	ND	LT
	FAPP2	galactosyl/glucosyl lipids	Golgi	PH–LT
SCP2 (nsLTP)	SCP2	FA, acyl-CoA, PL, sterol	Peroxisomes, lysosomes, mitochondria	LT
	SCP-x	FA, acyl-CoA, PL, sterol	Peroxisomes	3-oxoacyl-CoA thiolase–LT
	UNC-24	ND	PM	SPFH–LT

*A further version of this table that includes all of the relevant references is available as online supplementary information S2 (table). ‡Lipid-transfer (LT) proteins are subdivided into families on the basis of their LT-domain homologies. §This column shows the nature of the lipid that is solubilized by the LT domain, where known. ‖This column shows the known intracellular locations of the full-length protein, if known. ¶Shows the primary structure of identifiable domains other than the LT domain, when other such domains are present. #ORP domains (in ORP1/2 and Kes1) have been shown to bind anionic phospholipids (PLs), which indicates that this might be a general feature of ORP domains. However, these lipids are not solubilized and probably bind to a site other than that used by, for example, oxysterol. ANK, ankyrin-repeat region; CERT, ceramide-transfer protein; CRAL, cellular retinaldehyde; CRALBP, CRAL-binding protein; ER, endoplasmic reticulum; FA, fatty acid; FFAT, two phenylalanines in an acidic tract; GLTP, glycolipid-transfer protein; GOLD, Golgi dynamics; GPBP, Goodpasture's antigen-binding protein (an older name for CERT); LE, late endosomes; ND, not determined; nsLTP, non-specific LT protein; NVJ, nucleus–vacuole junction; ORP, OSBP-related protein; OSBP, oxysterol-binding protein; Osh, OSBP homologue; PC, phosphatidylcholine; PCTP, PC-transfer protein; PH, pleckstrin homology; PI, phosphatidylinositol; PITP, PI-transfer protein; PM, plasma membrane; PYKi, PYK2 interacting; RdgB, retinal degeneration type B; SCP2, sterol carrier protein-2; Sfh, Sec14 homologue; SPF, supernatant protein factor; SPFH, 'stomatin, prohibitin, flotillin, HflK/C'; StAR, steroidogenic-acute-response protein; StART domain, StAR-related LT domain; TAP, tocopherol-associated protein; TMD, transmembrane domain; TTP, tocopherol-transfer protein. Vitamin E is also known as α-tocopherol.

Box 3 | Lipid-transfer proteins

Lipid-transfer proteins (LTPs) were originally purified from cytosolic fractions that catalysed the transfer of lipids between donor and acceptor vesicles *in vitro*. In the structures that are available for LTPs, the lipid-binding pocket is largely lined with hydrophobic residues that stabilize the internal lipid. However, this large hydrophobic surface can render the protein quite unstable when no lipid is present[71]. Therefore, on purification from cell lysates, many LTPs contain various hydrophobic molecules, in particular, the detergents that are used in the purification process[70]. However, *in vivo*, each LTP specifically transfers a limited range of lipids, which is determined by both the head group and the backbone of the lipid (TABLE 2). LTPs can therefore distinguish between a glycerolipid and sphingolipid with the same head group[19]. In some cases, one LTP interacts with two lipids — for example, phosphatidylinositol (PI)-transfer proteins (PITPs) also bind phosphatidylcholine (PC). Interestingly, unlike other LTPs, PITPs are never found in their lipid-free (or apo) form. This indicates that it is extremely energetically unfavourable for the binding pocket to be occupied by water, which would result in the protein being unable to carry out the net transfer of lipid if it did not have this dual specificity. By also binding to PC, the PITP can shuttle PI in one direction and return carrying PC, so that it functions as a PI/PC exchange protein. This activity has developed twice in evolution: members of the mammalian PITP family are functionally interchangeable with the *Saccharomyces cerevisiae* PITP Sec14 (REF. 97), even though they share no primary sequence homology and actually bind PI in the opposite orientation in their binding pockets[69,70].

that cross MCSs. For example, in cells in which all the PE is synthesized in mitochondria, this lipid can diffuse throughout the ER to be taken up by other organelles after it has crossed one MCS to access the ER membrane[14,81] (FIG. 5a). An analogy for this mode of molecular transport is to think of each of the various organelles as a computer and to imagine that these computers are linked together by a network (superhighway) that is equivalent to the ER. The uploading and downloading of information at individual computers is equivalent to the molecular transport that occurs across the MCSs that are linked to this network.

But what evidence is there that the ER functions as a conduit in this manner? First, in a subject outside lipid transport, the ER functions as a conduit for the intracellular transport of Ca^{2+}, which is imported across the plasma membrane into the ER and is then passed to a subpopulation of mitochondria[88]. Second, there is increasing evidence that LTPs function at MCSs (see below). Targeting to MCSs might be the inevitable result of a protein having two separate targeting signals (FIG. 5b), such as those that are found in CERT[19] (ceramide transfer protein; TABLE 2). Although it is a speculative idea at present, a single LTP might simultaneously engage receptors on opposite sides of an MCS (FIG. 5c). Such bivalent binding would greatly enhance the AVIDITY of membrane targeting and, once bound across an MCS, an LTP could shuttle lipids across the cytosolic gap (that is, the LTP domain could cross from the donor to the acceptor compartment without changing its membrane anchorage). This would lead to an enormous gain in efficiency compared to a situation in which diffusion was required between the donor and acceptor compartments (FIG. 5b,c).

Lipid-transfer proteins at membrane contact sites. In support of the speculative model described above, there are several examples of LTPs that have been localized to MCSs. Three *S. cerevisiae* homologues of oxysterol-binding protein (OSBP) — Osh1, Osh2 and Osh3 — have been localized to MCSs[89]. These three Osh proteins and most human OSBP homologues contain a novel motif that facilitates ER targeting[89]. The motif, which consists of two phenylalanines in an acidic tract (the FFAT motif), binds with micromolar affinity to the cytosolic domain of vesicle-associated-membrane protein-associated protein (VAP), a highly conserved integral ER protein. In addition, the *Drosophila melanogaster* PI-transfer protein RdgBα (so called because mutations in this protein cause retinal degeneration type B) was shown some years ago to localize to a subpopulation of ER membranes that underlie the highly invaginated plasma membrane of photoreceptor cells in *D. melanogaster*[90]. The crucial function of RdgBα resides in its PI-transfer domain, which is required to re-supply PI to the plasma membrane after the hydrolysis of PI-4,5-bisphosphate in response to light[91]. The mechanism by which RdgBα targets the plasma membrane is still unknown, but it also contains a FFAT motif[89], which might be required for its ER targeting (TABLE 2). Other work in *S. cerevisiae* by Voelker and co-workers indicates that Sfh4, an LTP that is related to Sec14, functions together with the enzyme PS decarboxylase-2 (Psd2) to acquire PS across narrow intermembrane gaps[92].

LTPs make up a relatively high proportion of the few known MCS proteins. As a result, we predict that other LTPs with the same domain structure as Osh1–3 might also function at MCSs. One example is CERT, which shuttles ceramide between the ER and the *trans*-Golgi[19] and has the same two targeting domains as the Osh proteins — that is, a FFAT motif and a pleckstrin-homology (PH) domain that targets the *trans*-Golgi network[89,93]. This configuration would allow CERT to shuttle ceramide between these two organelles across cytosolic gaps. In addition, its targeting domains would allow CERT to specifically target the Golgi–ER MCS that was originally identified as the GOLGI–ER–LYSOSOME (GERL) compartment by Alex Novikoff 40 years ago[94]. OSBP, another LTP that shares the same targeting domains as Osh1–3, was originally defined as a Golgi protein using light microscopy[95]. However, we propose that it might target the ER–Golgi MCS, much as the *S. cerevisiae* OSBP homologues target other MCSs[89]. Interestingly, two OSBP-related proteins (ORP5 and ORP8) and MLN64 — a steroidogenic-acute-response-protein-related lipid-transfer (StART)-domain-containing protein, which is a cholesterol-binding protein of the late endosome[96]

AVIDITY
The overall measure of binding between a multivalent ligand and its receptors. It was originally defined for antibodies, for which avidity refers to the overall strength of binding between multivalent antigens and antibodies.

GOLGI–ER–LYSOSOME
(GERL). This term, which has fallen into disuse, was used by Alex Novikoff to describe what is, at present, called the *trans*-endoplasmic reticulum (ER) — that is, flattened ER cisternae that are intercalated between cisternae of the *trans*-Golgi. Claims of direct membrane continuity between GERL and adjacent lysosomes have not been substantiated.

— contain transmembrane helices (TABLE 2). Although these LTPs with transmembrane helices have not yet been studied at a functional level, they are puzzling. In particular, how could an integral membrane protein shuttle lipids across a cytosolic gap? This issue would be solved if the LTP functions at an MCS, where being embedded in one of the membranes would, according to the model presented in FIG. 5c, promote lipid transport.

Conclusions

Research on how cells move lipids across and between their bilayers is becoming a new branch of cell biology, in which the transport processes that are observed can be attributed to the functions of specific proteins. The list of proteins that have been proposed to drive lipid flip–flop is expanding rapidly and has boosted new investigations into the origin and biological

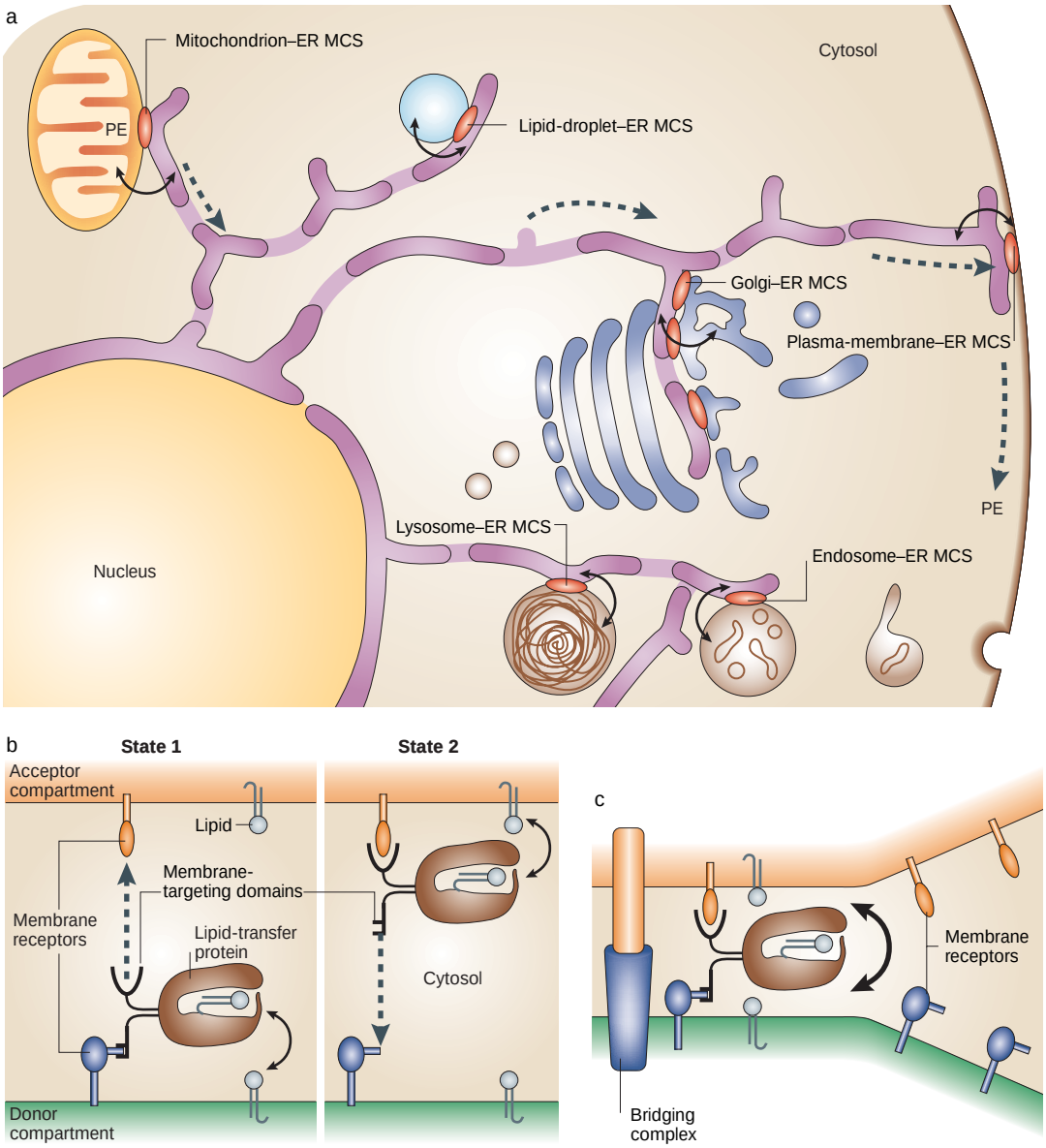


Figure 5 | **Lipid traffic at membrane contact sites.** The endoplasmic reticulum (ER) forms a network for the facilitated diffusion of material between other organelles. **a** | A lipid-transport network based on the ER. The ER forms a wide range of membrane contact sites (MCSs; red), and lipids are selectively transported (curved arrows) across MCSs by lipid-transfer proteins (LTPs). By crossing one MCS into the ER, diffusing through the ER, and then crossing a second MCS to exit the ER, lipids can use the ER as a superhighway for their intracellular transport between two sites. For example, phosphatidylethanolamine (PE) is synthesized in the mitochondrial matrix, and might move to the plasma membrane by a non-vesicular route that involves two MCSs — mitochondrion–ER and ER–plasma-membrane (see the dashed arrows). **b** | An LTP shuttling across an MCS. The two potential states of an LTP bound at an MCS are shown, and its diffusion across the cytosolic gap is indicated by dashed arrows. Lipid is exchanged at both membranes (curved arrows), which allows transport down a concentration gradient. **c** | An LTP that bridges an MCS. If an LTP can interact with both of its membrane receptors simultaneously, this will increase the avidity of membrane binding. The absence of a diffusion step would increase the efficiency of lipid transfer (larger curved arrow). This figure has been modified with permission from REF. 103 © (2003) Elsevier.

significance of transbilayer lipid asymmetry. An exciting development concerns the functional link between P4-ATPase-dependent lipid transport and vesicle formation in *S. cerevisiae*, which might prove useful in unravelling the molecular basis of the human brain and liver disorders that are caused by a perturbation of P4-ATPase function. However, the functional reconstitution of these proteins into chemically defined proteoliposomes will be necessary to provide direct evidence for their ability to move lipids across the bilayer. Such reconstitution has only been achieved in rare cases and, at present, poses a pressing issue that needs to be addressed to better understand the dynamics of biomembranes in relation to health and disease. It will be equally challenging to understand the molecular and functional organization of MCSs, a topic that is rapidly moving to centre stage in lipid cell biology. Evidence so far is compatible with a model in which LTPs are active at a large number of MCSs that are between different organelles and the ER. The idea of the ER functioning as a passive, long-range, highly efficient, pan-cellular lipid-distribution system together with numerous short-range lipid-transport mechanisms is, in some way, analogous to the contrasting roles of the information superhighway and floppy drives.

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Acknowledgements

We apologize for the omission of many significant papers that could not be cited or discussed due to space limitations. Work in the authors' laboratories is funded by grants from the Utrecht University High Potential Programme and the Netherlands Organization for Scientific Research (J.C.M.H.), and from The Wellcome Trust, the Biotechnology and Biological Sciences Research Council, and Fight For Sight (T.P.L.).

Competing interests statement

The authors declare no competing financial interests.

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