HOW PROTEINS MOVE LIPIDS AND LIPIDS MOVE PROTEINS

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Cells determine the bilayer characteristics of different membranes by tightly controlling their lipid composition. Local changes in the physical properties of bilayers, in turn, allow membrane deformation, and facilitate vesicle budding and fusion. Moreover, specific lipids at specific locations recruit cytosolic proteins involved in structural functions or signal transduction. We describe here how the distribution of lipids is directed by proteins, and, conversely, how lipids influence the distribution and function of proteins.

EXOCYTIC PATHWAY
Secretory or membrane proteins are inserted into the endoplasmic reticulum. They are then transported through the Golgi to the *trans*-Golgi network, where they are sorted to their final destination.

ENDOCYTIC PATHWAY
Macromolecules are endocytosed at the plasma membrane. They first arrive in early endosomes, then late endosomes, and finally lysosomes where they are degraded by hydrolases. Molecules can recycle to the plasma membrane from early endosomes, and there are also connections with the exocytic pathway.

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Metabolic functions are often compartmentalized within membrane-bounded compartments in eukaryotic cells. Although each cellular membrane has a unique set of proteins for its specialized functions, its basic building blocks are lipids (BOX 1). Lipids provide mechanical stability and a strong tendency to form closed structures. At the same time, lipids allow a snug fit of membrane-spanning proteins within the membrane, and ensure sufficient flexibility for vesicle budding and membrane fusion. Bilayers made of the prototype membrane phospholipid phosphatidylcholine have all of these properties in the test tube. But many types of lipid are needed to determine precisely the physical properties of a given membrane in a cell. Moreover, cellular membranes contain many signalling lipids that transduce signals by interacting with specific proteins (BOX 2). Cells use both the structural and signalling properties of lipids to control membrane transport, thereby ensuring the unique molecular composition of each membrane-bounded compartment along the EXOCYTIC and ENDOCYTIC PATHWAYS.

To understand the biological functions of lipids, we must have insight into their physical properties in the cellular context, that is, in lipid mixtures. The activity of each lipid is determined by its local CONCENTRATION in time. As this parameter is determined by a balance between synthesis and hydrolysis, and by transport, we need to learn about these processes, the proteins involved, and how their activity is regulated. During evolution, the selective transport of proteins to specific cellular locations and the protein-mediated transport of lipids have evolved coordinately. It can therefore be expected that membrane proteins and lipids make up indissociable parts of an intricate sorting machinery.

Selectivity in lipid transport

The differences in lipid composition between cellular organelles cannot be explained by local metabolism alone. For example, the plasma membrane and the endoplasmic reticulum (ER) have different lipid compositions (BOX 3), despite the fact that some plasma membrane lipids are synthesized in the ER, and lipids are continuously transported between the two membranes (BOX 4). In addition to VESICULAR TRANSPORT, some lipids can rapidly equilibrate across membranes and between membranes by monomeric exchange (FIG. 1). Each transport mechanism would result in dissipation of the compositional differences unless it could transport certain lipids in one, and others in the opposite direction.

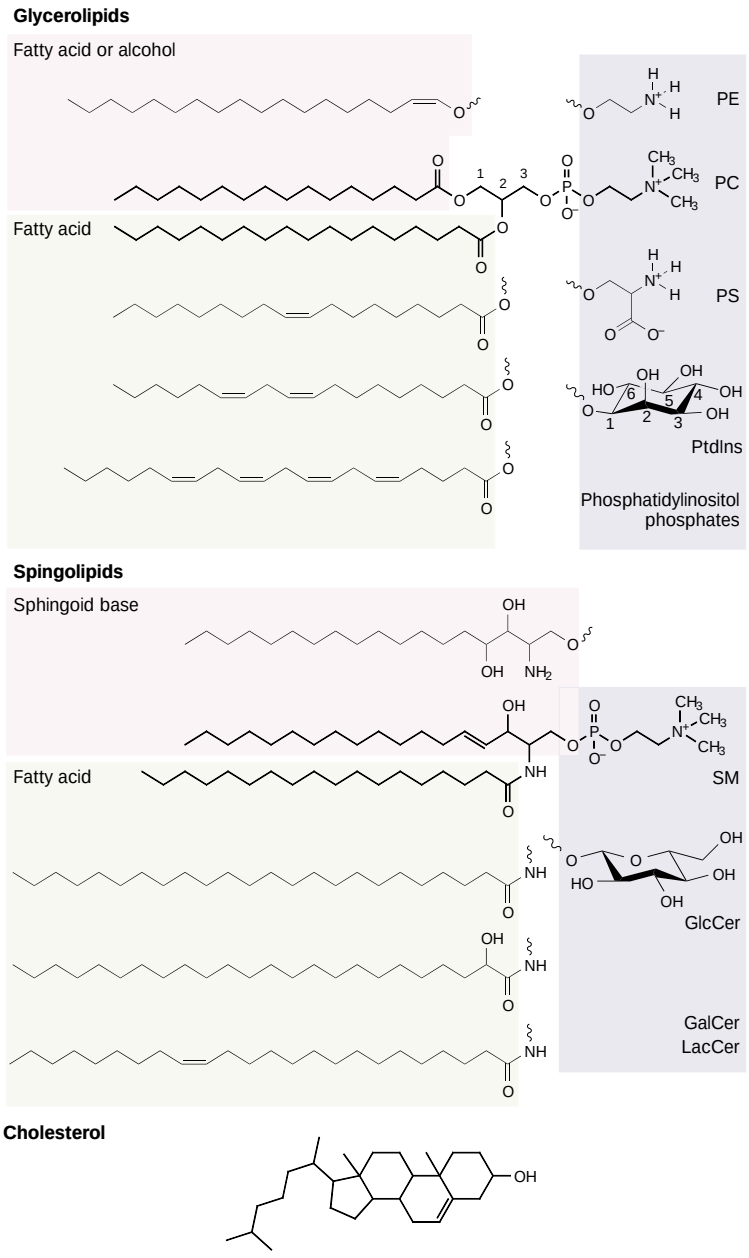
What lipids need to be transported and in which direction? First, during membrane growth, phospholipids must move across the membrane of the ER, mitochondria and peroxisomes, and glucosylceramide must move across the Golgi membrane for complex glycolipid biosynthesis. Second, phosphatidylserine and phosphatidylethanolamine must move to the cytosolic leaflet of the plasma membrane to maintain its transbilayer asymmetry. Third, along the exocytic and endocytic pathways, sphingolipids, saturated phospholipids and cholesterol need to be transported unidirectionally from the ER and Golgi

Box 1 | Structure of mammalian membrane lipids

The most abundant animal lipid, phosphatidylcholine (PC), consists of glycerol, two fatty acid chains on sn-1 and -2, and phosphate (phosphatidic acid, PA) carrying the head group choline. Various C16–C20 fatty acids can be esterified at sn-2, but, generally, C16:0 or C18:0 is esterified at sn-1 (diacylglycerol). A long-chain alcohol can be ether-bonded at sn-1 (alkyl-acylglycerol). In plasmalogens, the alcohol is unsaturated (alkenyl-acylglycerol). A *cis*-double bond kinks the ether and acyl chains and increases the membrane area of the lipid. PE, phosphatidylethanolamine; PS, phosphatidylserine.

Sphingolipids contain a C18–C20 sphingoid base, mostly sphingosine, and a fatty acid, amide-linked to the nitrogen (ceramide). In dihydroceramide, sphingosine lacks the *trans*-double bond (sphinganine). Phytoceramide contains phytosphingosine (C4-OH sphinganine). The fatty acid can be long, *cis*-unsaturated at C15, and hydroxylated at C2. SM, sphingomyelin; GalCer, galactosylceramide; GlcCer, glucosylceramide; LacCer, lactosylceramide. Yeast sphingolipids contain inositolphospho-phytoceramide with longer fatty acids, notably C26:0.

Sterols are defined by their planar and rigid tetracyclic ring. Animal membranes contain cholesterol and low quantities of related sterols such as 7-dehydrocholesterol. Cholesterol esters with fatty acids are storage lipids, just like triacylglycerol. Yeasts contain ergosterol, plants mainly stigmasterol and sitosterol.



LIPID CONCENTRATION
The density of a given lipid at a certain lateral position on one surface of a particular membrane, expressed here as mol% of total lipids. Mol% of phospholipids does not take into account the presence of glycosphingolipids and cholesterol.

VESICULAR TRANSPORT
Transport from one organelle to another, during which cargo-containing vesicles bud from the donor membrane and fuse with an acceptor membrane.

to the plasma membrane and endocytic membranes, whereas unsaturated phospholipids require transport in the opposite direction. Fourth, superimposed on this transport, in epithelial cells, glycosphingolipids and sphingomyelin must be enriched over phosphatidylcholine along the pathway reaching the apical surface, but not along the pathway to the basolateral surface. Last, phospholipids (and sometimes cholesterol) must be transported from the ER to mitochondria and peroxisomes, organelles that do not seem to be connected by vesicular transport. How does the cell maintain the differences in lipid composition between the various membranes and between the two bilayer leaflets of those membranes?

Energy-independent translocators. The spontaneous movement of amphipathic lipids across lipid bilayers has a typical half-time of hours (FIG. 2), which is very slow compared with lateral diffusion, whereby lipids move µm distances in seconds. The observation that phospholipids and their analogues translocate readily across the ER membrane (seconds to minutes) has led to the general concept that this movement is protein mediated, and first steps in the purification of a

Box 2 | Signalling lipids

One beautiful example of how cells use lipids for signalling is the phosphoinositide system, which has broad implications for signal transduction, cytoskeletal regulation and membrane dynamics. Its intricacy is illustrated by the known involvement of close to a hundred (isoforms of) kinases and phosphatases in the production and inactivation of signalling inositol lipids (BOX 1), and by the specific recognition of these lipids by receptor domains in at least another hundred proteins. In addition, a range of regulated phospholipase Cs generate free phosphoinositide headgroups and diacylglycerol (DAG), which themselves activate Ca^{2+} channels and protein kinases C. Furthermore, signalling lipids might change the local physical properties of the membrane. Similarly to DAG, ceramide produced by a sphingomyelinase during apoptosis might activate a specific protein kinase and phosphatase but it also grossly affects membrane properties[76]. As newly synthesized ceramide does not act as a signalling molecule, cells seem to separate metabolic pools from signalling pools of one and the same lipid. One other example is sphingosine-1-phosphate, the final intermediate in the breakdown of sphingolipids. It also binds to specific plasma membrane receptors involved in stress responses[77]. Related receptors for its glycerolipid homologue lysophosphatidic acid (LPA) have proliferative and/or morphological effects[78]. Other lipids that signal between cells through specific receptors are the cytokine platelet activating factor (PAF: alkyl-acetyl phosphatidylcholine) and phosphatidylcholine-derived peroxidation products with PAF activity, the 'eicosanoids' (prostaglandins, thromboxanes and leukotrienes), that are synthesized from arachidonic acid (C20:4) after its production from phospholipids by a regulated phospholipase A_2, and steroid hormones produced from cholesterol by cleavage of its side-chain in the mitochondria.

translocator, the ER flippase, have been reported[1]. Most studies indicate that the ER flippase might not require energy, might be bidirectional, and might not be specific for phosphatidylcholine. One other candidate substrate for this flippase is newly synthesized phosphatidylethanolamine, which has been shown to translocate to the lumen of the ER in 30 min[2,3]. Other phospholipids that must cross the ER membrane are the dolichol-phosphoglycolipids and glycosylphosphatidylinositols (GPI). In both cases, the initial steps of synthesis occur on the cytoplasmic surface[4,5], but the lipids must reach the lumenal side for *N*-glycosylation and GPI-anchoring, respectively.

A similar energy-independent translocation towards the lumen of an organelle has been observed for the glycosphingolipid glucosylceramide in the Golgi. On the other hand, the complex glycolipids synthesized from glucosylceramide in the lumenal leaflet were unable to translocate back to the cytosolic leaflet[6,7]. The protein responsible for glucosylceramide translocation towards the lumen of the Golgi has not been identified. Even for fatty acids, predicted to move easily across membranes, a family of fatty acid transport proteins (FATPs) has been identified, that facilitate the uptake of exogenous fatty acids across the plasma membrane[8]. Finally, a scramblase might be responsible for bidirectional lipid transport across the plasma membrane during signalling events and apoptosis. As a consequence, phosphatidylserine, which is normally restricted to the cytoplasmic leaflet (BOX 3), now appears on the cell surface, where it stimulates blood clotting and functions as a recognition signal for the engulfment of apoptotic cells by macrophages. Moreover, sphingomyelin, which is normally restricted to the exoplasmic leaflet, moves to the cytosolic surface where it is degraded by sphingomyelinase, inducing various physiological effects (BOX 2). Although a candidate scramblase has been cloned[9], its functional activity awaits rigorous characterization.

Energy-dependent translocators. Energy-independent translocators allow lipids to equilibrate between the two leaflets of the bilayer. Net movement in one direction can be driven by a difference in concentration that might be maintained by continuing synthesis or by the presence of a sink for the lipid on one side of the membrane. By contrast, other translocators can use energy, mostly ATP, to move a lipid up a concentration gradient and thereby create transbilayer lipid asymmetry (BOX 3). The latter type has been found mainly in plasma membranes. Energy-dependent translocation of lipids was first shown for the aminophospholipids phosphatidylserine and phosphatidylethanolamine[10]. The protein responsible, the plasma membrane aminophospholipid translocase, is thought to be the main activity that changes the symmetric lipid distribution found in the ER to the asymmetric lipid arrangement found in mammalian plasma membranes. It reduces the exposure of phosphatidylserine on the cell surface and, through a net movement of lipid molecules from the outer into the cytoplasmic bilayer leaflet of the plasma membrane, it increases the lateral pressure in that leaflet, possibly driving endocytosis[11]. The best candidate protein for aminophospholipid translocase activity is the P-type ATPase named ATPase II[12], but convincing evidence for its ability to move lipids across bilayers is still lacking. It has been suggested that the action of this aminophospholipid flippase is counteracted by an outward-directed energy-dependent floppase[13].

ATP-binding cassette (ABC) TRANSPORTERS have been implicated in lipid translocation primarily on the basis of genetic evidence: mice having null-alleles for the liver-specific ABC transporter Mdr2 (ABCB4) cannot secrete phosphatidylcholine into their bile[14]. The suggestion that ABCB4 translocates phosphatidylcholine to the outer leaflet of the plasma membrane was supported by biochemical evidence. In parallel with Mdr2, an energy-independent phosphatidylcholine translocator might be present in the bile canalicular membrane[15]. Unexpectedly, the closely related MDR1 P-glycoprotein (ABCB1), the main mammalian MULTIDRUG TRANSPORTER, also translocated short-chain lipid analogues across the plasma membrane[16], including the short-chain phosphatidylcholine platelet-activating factor[17,18]. Whereas MDR1 probably does not translocate natural phosphatidylcholine, it translocates both analogues of glucosylceramide[16] and natural glucosylceramide (R.J. Raggers and G.v.M., unpublished data) across the plasma membrane. MDR1 might also translocate glucosylceramide in the Golgi in parallel with the energy-independent translocation mentioned in the previous section[6,7].

Other ABC transporters are involved in sterol transport. The impaired efflux of cholesterol from the plasma membrane to exogenous HDL (reverse cholesterol transport) in Tangier disease is due to mutations in ABC1 (ABCA1). ABC1 might extrude cholesterol from

GPI
The general function of GPI anchors is to attach proteins to membranes, possibly to specific domains therein. The anchor is made of one molecule of phosphatidylinositol to which a carbohydrate chain is linked through the C-6 hydroxyl of the inositol, and is linked to the protein through an ethanolamine phosphate moiety.

ABC TRANSPORTERS
Large protein family of transporters that contain an ATP-binding cassette. They hydrolyse ATP and transfer a diverse array of small molecules across membranes.

MULTIDRUG TRANSPORTER
Energy-dependent efflux pump that is responsible for decreased drug accumulation in multidrug-resistant cells. Multidrug resistance is an acquired simultaneous resistance to a wide spectrum of drugs arising from the administration of drugs typically over long periods.

HDL
The smallest type of lipoprotein found in blood plasma, which functions in reverse transport from tissues to the liver.

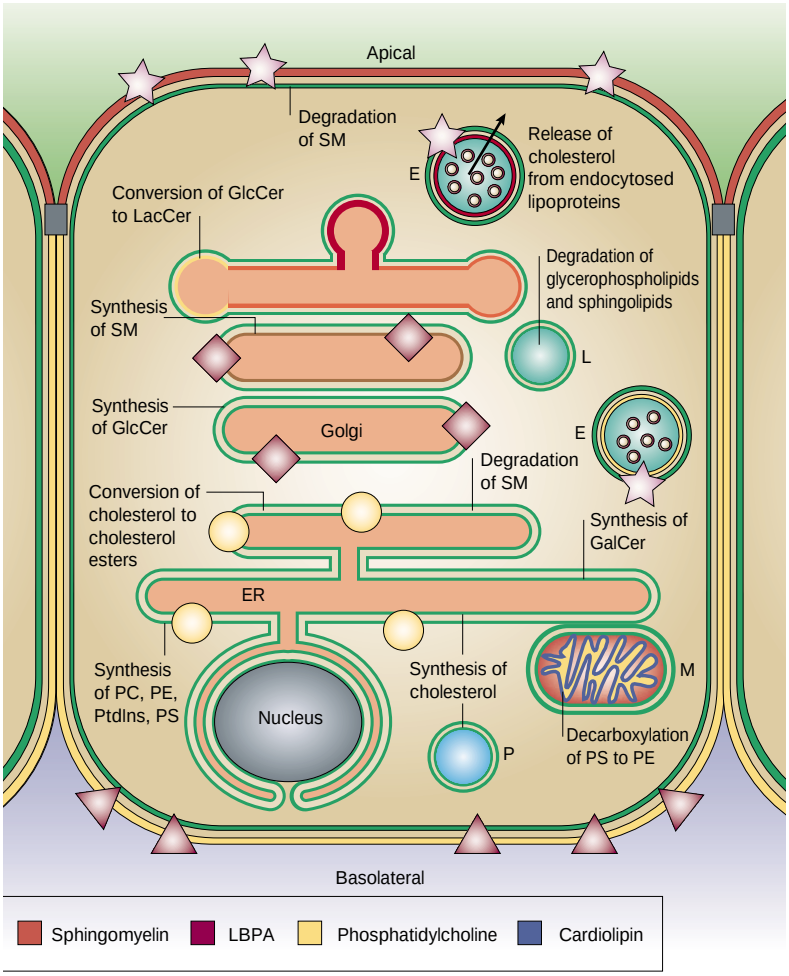
DISC
The phototransduction apparatus in the outer segment of rod cells contains a stack of discs, each formed by a closed membrane in which rhodopsin molecules are embedded.

the membrane. Alternatively, it might accelerate cholesterol translocation from the cytosolic to the outer leaflet of the plasma membrane, as this process is normally slow with a measured half-time of 1–2 h in the erythrocyte membrane[19] (FIG. 2). ABC1 might translocate cholesterol indirectly through translocation of phosphatidylserine towards the outer leaflet of the plasma membrane, in a process that seems to be activated by Ca^{2+} (REF. 20). Interestingly, sitosterolaemia, a disease characterized by increased intestinal absorption and decreased biliary excretion of dietary sterols, is caused by mutations in ABCG5 and ABCG8, suggesting they are also, directly or indirectly, sterol translocators[21]. ABCR (ABCA4) is a rod-photoreceptor-specific ABC transporter, which seems to translocate all-*trans*-retinal, possibly covalently linked to phosphatidylethanolamine (*N*-retinylidene-phosphatidylethanolamine), from the lumenal side of the DISC membrane towards the cytosolic side[22,23]. If, indeed, some ABC transporters translocate lipids towards the cytosolic leaflet, the aminophospholipid translocator that translocates phosphatidylserine and phosphatidylethanolamine towards the cytosolic side might actually be an ABC transporter. Finally, import of (very) long-chain fatty acids or acyl-CoAs into peroxisomes requires the adrenoleukodystrophy ABC-transporter ABCD1.

Niemann-Pick type C (NPC) is a lipid storage disease caused by defective transport of cholesterol from late endosomes. NPC is caused by mutations in NPC1 or NPC2. The NPC1 protein is a proton-motive-force-driven lipid transporter[24], which has a typical sterol-sensing domain consisting of five transmembrane helices, but it probably does not translocate cholesterol. Because sphingoid bases accumulate in NPC lysosomes, and exogenous sphinganine induces an NPC phenotype[25], NPC1 might function to transport sphingosine from the lumenal to the cytosolic leaflet of lysosomes. The NPC2 protein, HE1, is a cholesterol-binding protein that localizes to the lysosomal lumen[26] and could be involved in transport of cholesterol between intralysosomal vesicles and the limiting membrane.

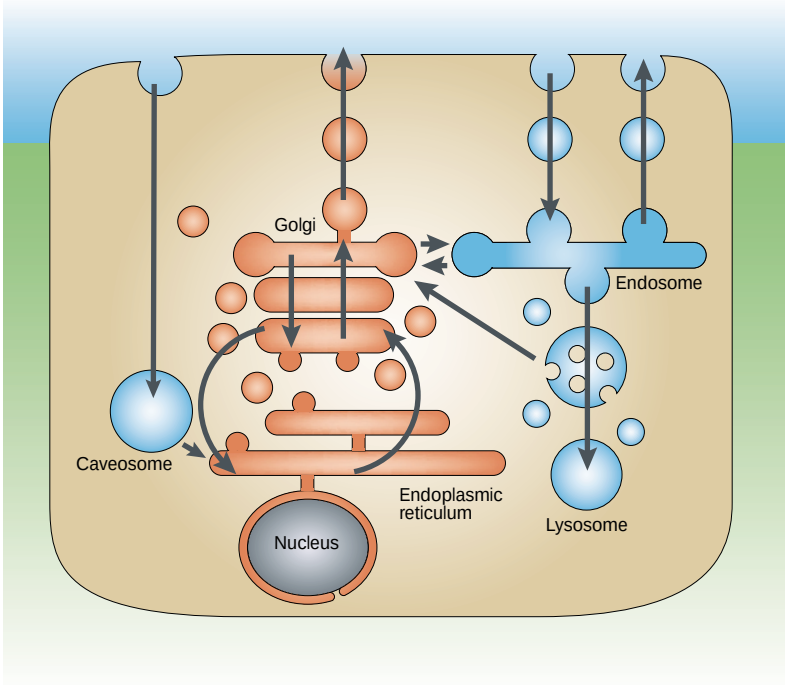
Proteins involved in monomeric lipid transport. Lipids can exchange between membranes as single molecules. As cellular membranes are separated by an aqueous phase, the lipid must desorb from the membrane into the aqueous phase, diffuse across it, and insert into the opposite membrane. Spontaneous exchange rates, which are limited by the desorption step, are low for most membrane lipids (FIG. 2). Proteins might stimulate lipid exchange between membranes by bringing membranes into contact as proposed for the ER and mitochondria[27] and the ER and *trans* Golgi[28]. Alternatively, lipid transfer proteins might provide a hydrophobic binding site and act as carriers. Cytosolic proteins have been found with binding specificities for phosphatidylcholine, phosphatidylinositol/phosphatidylcholine, phosphatidylinositol/sphingomyelin and glycolipids. They all stimulate lipid exchange *in vitro*. However, *in vivo* the dual-specificity proteins might function as membrane-bound lipid sensors that have regulatory functions in vesicle flow and lipid metabolism[29,30]. Exchange of phosphatidylcholine, but not phosphatidylethanolamine, between cellular membranes is rapid and might be mediated by transfer proteins. These proteins are not required for phosphatidylcholine secretion into bile or lung surfactant[31]. Finally, proteins might

Box 3 | Topology of membrane lipids



Cellular membranes contain unique sets of proteins, as indicated in the figure by different symbols. The differences in lipid composition are more gradual. From the endoplasmic reticulum (ER) (60 mol% phosphatidylcholine; 25 mol% phosphatidylethanolamine and 10 mol% phosphoinositol), the lipid composition gradually changes along the stack of Golgi cisternae to that of plasma membranes (25 mol% phosphatidylcholine; 15 mol% phosphatidylethanolamine; 30–40 mol% cholesterol; 10 mol% sphingolipids and 5 mol% phosphatidylserine)[40,79]. In addition, the symmetric transbilayer lipid distribution in the ER changes into the asymmetric arrangement in the plasma membrane with sphingolipids in the outer, and most phosphatidylserine and phosphatidylethanolamine in the cytosolic leaflet. The 'sidedness' of cholesterol is unknown. The specialized apical membrane of epithelial cells is covered by glycosphingolipids and/or sphingomyelin, and contains little phosphatidylcholine. The basolateral membrane is similar to the plasma membrane of non-polarized cells[80]. The main differences reside in the outer leaflet, where diffusion is prevented by tight junctions[81]. The lipid composition of endosomes (E) and lysosomes (L) is similar to that of the plasma membrane, but they contain lysobisphosphatidic acid (LBPA) in their internal vesicles[74]. Peroxisomes (P) and mitochondria (M) have ER-like compositions, but the inner mitochondrial membrane contains the unique phospholipid cardiolipin. The transbilayer lipid distribution in these organelles is unknown.

Box 4 | Vesicular transport pathways



The organelles along the exocytic and endocytic transport routes are connected by carrier vesicles that bud from one organelle and fuse with the next. Vesicular transport can be visualized as a set of recycling pathways, one between the endoplasmic reticulum (ER) and *cis* Golgi (G), and one between endosomes (E) and the plasma membrane. The latter has a bidirectional connection with the *trans*-Golgi network (TGN). Vesicles move up and down the Golgi stack. Each arrow might represent more than one pathway mediated by different coats and, therefore, regulated independently. Examples are COPI- and COPII-mediated pathways out of the ER, AP-1- and AP-3-mediated pathways from the TGN to the endosomal system, and AP-2/clathrin-, caveolin- and non-clathrin/non-caveolin-mediated pathways from the plasma membrane. Caveosomes (C) do not seem to be connected to endosomes (E) and lysosomes (L)[82]. Not depicted here are the vesicular connections from the TGN to the basolateral plasma membrane and back through basolateral endosomes (BOX 3), and the bidirectional transcytotic pathway that connects the apical and basolateral surfaces through a specialized endosomal compartment[83]. Equivalents of these pathways also exist in non-epithelial cells[84].

DOMAIN
An area in a membrane with a concentration of proteins and/or lipids that is different from its immediate environment. The term 'domain' carries no information about its size relative to the total membrane area, whereas terms such as 'microdomain' or 'raft' suggest that that they cover far less than half of the surface.

COPI VESICLES
Coated vesicles involved in transport through the Golgi and probably in retrograde transport from the Golgi to the endoplasmic reticulum. The COPI coatomer is made of seven subunits (α-, β-, β'-, γ-, δ-, ε- and ζ-COP).

reduce the activation energy needed to desorb the lipid into the aqueous phase. For example, the steroidogenic acute regulatory protein (STAR) has been implicated in cholesterol transport from the outer to the inner mitochondrial membrane, while the STAR homologue MLN64, a similar cholesterol-binding membrane protein localized to late endosomes, might stimulate cholesterol release from the cytosolic surface of endosomes[32]. In all cases, lipids exchange between the cytosolic surfaces. This explains, for example, why sphingolipids synthesized on the lumenal surface of the Golgi do not reach mitochondria and peroxisomes[33]. Furthermore, directionality is determined by the relative affinity of the lipid for the various membranes. Cholesterol, which exchanges and translocates 10–30 times faster than phospholipids[34], primarily concentrates where the sphingolipids and disaturated phospholipids are situated[35], probably because of its high affinity for these lipids. However, the maintenance of the steep cholesterol gradient in the secretory pathway might also require an active sorting process (see below).

Lipid segregation during vesicular transport. Although organelles along the secretory and endocytic pathways are connected by bidirectional vesicular transport (BOX 4), they maintain distinct membrane compositions. This shows that vesicular transport is not random. Membrane proteins are preferentially incorporated into one set of budding transport vesicles and excluded from others. So, proteins destined for different organelles are laterally segregated. The same is true for lipids. The main difference between ER and plasma membrane is an enrichment of sphingolipids in the latter, which can be maintained only by preferential transport of the sphingolipids from their site of synthesis in the Golgi to the plasma membrane and not to the ER. Because sphingomyelin and the complex glycosphingolipids are synthesized in the lumen and have no access to monomeric transport[7,36], the only way they can be transported preferentially towards the plasma membrane is by incorporation into anterograde transport vesicles and exclusion from retrograde vesicles. This implies a lateral segregation of the sphingolipids from other membrane lipids, particularly phosphatidylcholine, in the lumenal leaflet of a Golgi cisterna[37]. A solid physico-chemical basis for this behaviour has been uncovered (FIG. 3): sphingolipids experience stronger van der Waals interactions and hydrogen bonding than phosphatidylcholine. Because a sphingolipid-rich DOMAIN (or liquid-ordered domain) is more rigid than the rest of the membrane (FIG. 3), it is probably excluded from retrograde transport vesicles. Consistent with this, COPI-coated transport vesicles derived from the Golgi contain reduced levels of sphingomyelin[38].

How do cells maintain a low cholesterol concentration in the ER? We speculate that the intracellular distribution of cholesterol is essentially governed by its high affinity for sphingolipids. Cholesterol could be continuously depleted from ER and Golgi indirectly, by anterograde sorting of sphingolipids[37]. Newly synthesized sphingolipids in the *trans* Golgi and *trans*-Golgi network (TGN) might attract cholesterol from both the closely apposed ER[28] and from the cytosol. The cholesterol then contributes to (or induces) the sphingolipid/phosphatidylcholine phase separation (FIG. 3). Cholesterol also has a high affinity for disaturated phosphatidylserine[39], suggesting that the cytosolic leaflet of the plasma membrane, which is enriched in disaturated phosphatidylserine and phosphatidylcholine[40], also contains a high cholesterol concentration. So, disaturated glycerophospholipids and cholesterol might be sorted towards the plasma membrane by forming lateral domains on the cytosolic surface of the Golgi.

The sphingolipid–phosphatidylcholine segregation is probably the basic feature of lipid sorting in the Golgi. However, sorting in the distal Golgi is more complex. Several vesicular transport pathways originate in the *trans* Golgi and the TGN. Except for glycerolipids, which are transported in the retrograde direction, little is known about how lipids partition into the anterograde

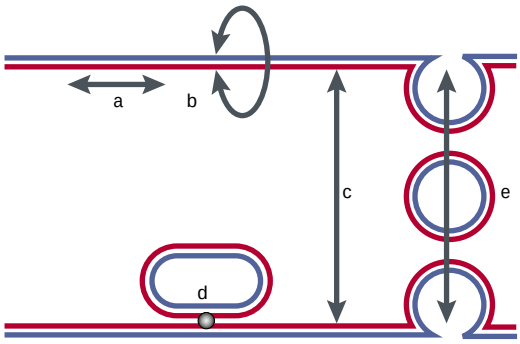


Figure 1 | **Mechanisms of lipid transport across and between cellular membranes.** Membrane lipids diffuse laterally in the membrane (**a**), and they can translocate between the two leaflets of the bilayer (**b**). Monomers can diffuse into the cytosol and equilibrate with the cytosolic surface of another organelle (**c**), sometimes at membrane contact sites (**d**). Last, lipids can be included in carrier vesicles, in which case the transbilayer orientation of the membrane lipids is maintained during fission and fusion (**e**). Unidirectional transport of a certain lipid is achieved when the net flux, the difference between forward and backward transport, is unidirectional. To maintain differences in lipid distribution, nonspecific transport by one mechanism (for example, monomeric transfer down a concentration gradient) must be counterbalanced by specific transport by a different mechanism (for example, unidirectional vesicular transport).

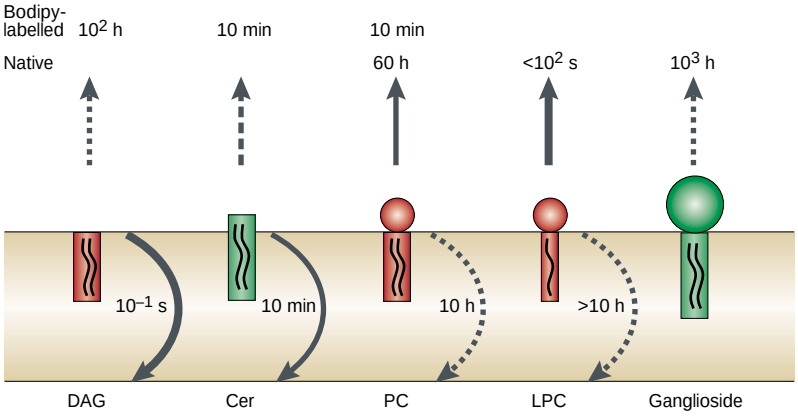


Figure 2 | **Rates of spontaneous movement across the bilayer and diffusion into the aqueous phase**[34, 85–87]**.** As the polar headgroup becomes larger or more polar, or the hydrophobic moiety becomes smaller, the lipid flips less readily but leaves the bilayer more easily. When the hydrophobic tail becomes more hydrophobic, transbilayer translocation becomes easier whereas the tendency to leave the membrane is reduced. Concomitant increases in the size and/or hydrophobicity of the hydrophobic moiety, and the size and polarity of the headgroup lower both rates (for example, in the case of ganglioside). Both rates are also reduced when lipids are closely packed in liquid-ordered domains. ABC transporters could combine translocation with extrusion: they may pick up amphipathic substrates in one bilayer leaflet and hydrolyse one ATP to extrude the molecule into the aqueous phase on the opposite side[88]. This is, however, unlikely for phospholipids, sphingolipids and cholesterol for which the change in free energy between the monomer in aqueous solution and the membrane form (70 kJ mol^{-1} for phosphatidylcholines) is far more than the energy released from ATP (30 kJ mol^{-1})[34]. Therefore, ABC transporters must move lipids onto bile acid micelles or HDL by direct contact without a monomeric intermediate. Alternatively, they could move the lipid into the opposite bilayer leaflet and would not be involved in the subsequent desorption step. (Cer, ceramide; DAG, diacylglycerol; LPC, lysophosphatidylcholine; PC, phosphatidylcholine.)

pathways to (apical and basolateral) cell surfaces, secretory granules, endosomes and other specialized compartments such as melanosomes. A similar complexity is observed in the endosomal system. Although lipid analogues do not behave exactly like natural lipids[41,42], experiments with fluorescent probes indicate that lipid sorting in the endocytic pathway might follow similar principles as in the Golgi. For example, sorting takes place in early endosomes, as more fluid probes were shown to be targeted towards recycling endosomes whereas less fluid lipids proceeded to late endosomes[43]. In another study, recycling endosomes were enriched in markers for liquid-ordered domains such as sphingomyelin[44]. Specificity was also found for transport from endosomes to the TGN[45]. Late endosomes present an additional complexity as they sort a subset of proteins and lipids, including the phosphoglycerolipid lysobisphosphatidic acid (LBPA)[46], into vesicles that bud towards the endosomal lumen. Moreover, sphingolipids, cholesterol and proteins can exit the late endosomal lumen, but this process is not well understood. It involves LBPA and several cholesterol-binding proteins, and is blocked by many conditions that affect sphingolipid hydrolysis[46].

Physical and biochemical role of lipids

Proteins and energy are essential to maintain the heterogeneous lipid distribution in the cell (BOX 3) through local metabolism and selective transport. At the same time, lateral differences in lipid composition are used to sort proteins, whereas alterations in the levels of signalling lipids are used to regulate various aspects of vesicular protein transport; and transbilayer translocation and local changes in the biophysical properties of lipids are used to bud vesicles.

Sphingolipid domains sort proteins. A membrane that contains mostly sphingomyelin, with or without cholesterol, is thicker than one composed of phosphatidylcholine and cholesterol, which is in turn thicker than a membrane of phosphatidylcholine alone. This implies that sphingolipid–cholesterol domains are thicker than the surrounding membrane (FIG. 3). Cells probably use this feature to sort membrane proteins that are destined for the plasma membrane from Golgi proteins by the length of their transmembrane domains (FIG. 4). For example, the transmembrane domains of plasma membrane proteins are 20 residues long, whereas those of Golgi proteins are only 15 residues long[47]. Discrete increases in membrane thickness would allow the sorting of various populations of membrane protein. An attractive alternative is that the membrane gradually thickens along the *cis–trans* axis of the Golgi[48] and that membrane proteins partition to the Golgi cisterna that fits the length of their transmembrane domain.

Sphingolipid–cholesterol domains (defined by their resistance to detergent extraction) are recognized by GPI-anchored proteins on the outer leaflet of the plasma membrane and by palmitoylated transmembrane proteins. The domains on the cytosolic side are populated by peripheral proteins carrying myristoyl (C14:0)

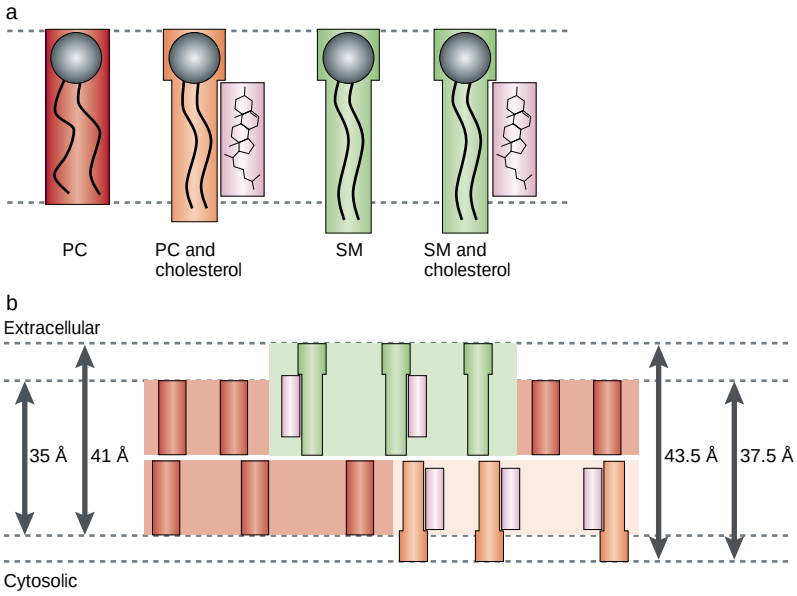


Figure 3 | **Lipid domains.** In a mixture of sphingolipids and glycerophospholipids, cholesterol can induce fluid–fluid immiscibility, resulting in a lateral segregation into two or more fluid phases. Cholesterol interacts with sphingolipids in the outer leaflet and with disaturated phosphatidylserine[39] in the cytoplasmic leaflet. The cholesterol-rich phases have a more ordered structure and have been termed 'liquid-ordered' (as opposed to liquid-disordered). Two domains of different composition may occur at micrometre distance[89], or the one domain may be juxtaposed to the other[90] or even form a circle around it. **a** | Cholesterol increases the length of the phosphatidylcholine (PC) but not the sphingomyelin (SM) molecule. Sphingomyelin with or without cholesterol forms bilayers with a thickness of 46–47 Å for C18:0 sphingomyelin (REF. 91) to 52–56 Å for C24:0 sphingomyelin (REF. 92). By contrast, the thickness of a C16:0/C18:1 phosphatidylcholine bilayer is 35 Å, and is expanded to 40 Å by cholesterol. The thickness of the hydrophobic core of the bilayer increased from 26 to 30 Å (REF. 93). **b** | Depending on whether the domains on both surfaces colocalize, four discrete bilayer thicknesses could be present in these membranes.

and palmitoyl (C16:0) chains, and they exclude prenylated proteins[49] (FIG. 4). Recently, we have shown that the glycosphingolipid glucosylceramide is required on the cytosolic surface for transport of membrane-spanning proteins from the Golgi to the melanosome (H.S., P.v.d.S. and G.v.M. unpublished data).

Cytosolic and lumenal domains might be kept together by proteins with a sufficiently long transmembrane domain (FIG. 4), but they could also be kept together purely on the basis of the properties of their constituent lipids[50]. Domain colocalization at the plasma membrane has been demonstrated for the acylated, cholesterol-binding protein caveolin on the cytosolic surface of CAVEOLAE and the sphingolipid GM1 on the opposite side[51], as well as by copatching of acylated proteins on the cytosolic side with GPI-proteins on the outside[52].

Exclusion of the liquid-ordered domains from retrograde transport could be due to a preferential location of these rigid domains in the flat parts of the cisternae with the liquid-disordered domain phospholipids populating the curved rims from where retrograde COPI vesicles bud. Alternatively, COPI coats could be specifically recruited onto the liquid-disordered parts of the membrane. The rigid domains would then end up in TGN remnants after removal of all retrograde material and move by default to the plasma membrane, without going through a budding step. To dock and fuse, the transport vesicles need the proper SNARES, and sorting of SNARES by membrane thickness has been shown[53]. SNARES on the target membrane have also been found to be dependent on cholesterol for clustering, thereby forming specialized fusion sites[54]. Although the physical details of lipid domains are not fully understood, it has become clear that they are important for cell signalling and vesicular transport.

Phosphoinositides in membrane budding and fusion. Originally, the signalling functions of phosphoinositides were thought to be carried out by the breakdown products of PtdIns(4,5)P_2 that are generated by stimulus-activated phosphoinositide-specific phospholipase C. Diacylglycerol (DAG) activates protein kinases C and inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3) opens Ca^{2+} channels in the ER (BOX 2). More recently, it was realized that cells use the phosphoinositides directly for regulatory functions. They form membrane-binding sites for soluble proteins and, as such, recruit cytosolic proteins to membranes, stabilize protein complexes on membranes, or activate membrane proteins. Phosphoinositides are involved in signalling, cytoskeleton–membrane interactions, and in membrane-vesicle budding and fusion[55]. Specificity of each phosphoinositide is based on its structure, its location and the timing of its synthesis, modification and hydrolysis. As the same phosphoinositide is found to complex with different proteins on distinct membranes, accessory binding sites for the proteins must be present on their target membrane. Phosphoinositides are products of, and substrates for, various kinases, phosphatases, and for phospholipases C and D, which might rapidly alter the levels of particular phosphoinositides in specific regions of the membrane. The regulated activity of these enzymes provides the basis for the efficient spatial and temporal regulation of vesicular transport.

PtdIns3P is found in endosomes (and in their internal membranes) in yeast[56] and animal cells[57]. In yeast, it is essential for sorting proteins to the vacuole[56]. In mammalian cells, it is involved in various aspects of endosomal transport. PtdIns3P is recognized by FYVE domains in a wide variety of proteins[58] and its synthesis by the 3′-kinase and hydrolysis in the endosome or vacuole have been characterized in detail[56]. However, the many mammalian phosphatidylinositol 3-kinase (PI3K) isoforms also phosphorylate other phosphoinositides[59]. PtdIns(3,4)P_2, PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 are recognized by pleckstrin homology (PH) domains in a range of proteins that function in plasma membrane to endosome transport and in signalling. They recruit, for example, proteins in the COPI-dependent transport pathways between Golgi and ER and within the Golgi (BOX 4), including ARF1 and its guanine nucleotide exchange factors, the first proteins required for COPI coat assembly[60]. ARF1 next recruits the coats in AP-1, AP-3, AP-4 and GGA-dependent transport pathways. Dynamin, which is involved in the final step of budding — vesicle fission — also contains a PH domain that is essential for its function.

CAVEOLA
Flask-shaped, cholesterol-rich invagination of the plasma membrane that might mediate the uptake of some extracellular materials, and is probably involved in cell signalling.

SNARES
(Soluble NSF attachment protein receptor, where NSF stands for *N*-ethyl-maleimide-sensitive fusion protein.) Proteins required for membrane fusion in exocytosis and other membrane transport events. When *trans*-SNARE complexes are formed between vesicle SNAREs and target-membrane SNAREs, they pull the two membranes close together, presumably causing them to fuse.

ARF1
Small GTPase responsible for recruiting different types of coat, leading to vesicle budding.

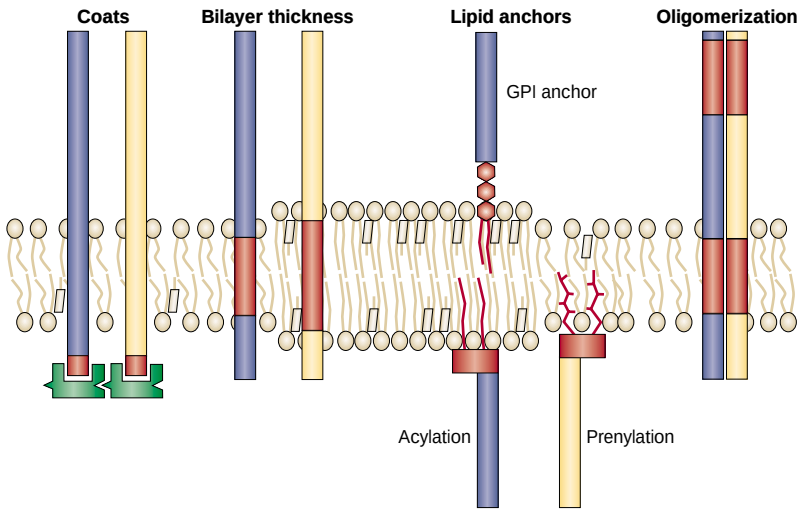


Figure 4 | **Lateral sorting of membrane proteins.** Proteins can be sorted using address labels in their cytosolic tails that interact with a protein coat, directing the resulting vesicle towards a specific target organelle. The interaction may be direct as in the case of COP coats, or indirect through adaptor proteins (APs), as in the case of clathrin coats. Proteins may also be sorted by the length of their hydrophobic domain, whereby the incorporation of lipid domains of a certain thickness into distinct transport carriers may be determined by physical properties of the membrane or through address labels on proteins that are present in each domain. Lipid-anchored proteins are sorted using the partitioning properties of their anchor: proteins anchored through a glycosylphosphatidylinositol (GPI) partition into liquid-ordered domains. Acylation of proteins with C14:0 and C16:0 tails is a signal for incorporation into the same part of the membrane that contains sphingolipids and cholesterol, indicating that the cytosolic surface of these sphingolipid–cholesterol domains might also have a special lipid composition. Prenylated proteins, which are anchored by farnesyl or geranylgeranyl tails, are excluded from the sphingolipid–cholesterol domains. Finally, proteins may be sorted through binding to another membrane protein. Oligomerization may be a physical determinant for the localization of proteins to the Golgi.

These examples illustrate the widespread involvement of phosphoinositides in the formation of transport vesicles. In addition, PtdIns3P promotes homotypic fusion of early endosomes[61,62], whereas PtdIns(4,5)P_2 has been shown to be required in at least two stages during fusion between yeast vacuoles[63]. Participation of the same phosphoinositides in several signalling cascades might indicate that vesicle fission and fusion depend on the intricate signalling state of the cell.

Finally, the overall lipid composition of the Golgi might modulate vesicle transport. A membrane-bound phosphoinositol/phosphatidylcholine-binding protein, the phosphoinositol/phosphatidylcholine-transfer protein PITP, has been proposed to regulate the interface between lipid metabolism and Golgi secretory function through a Golgi DAG pool[29]. For an unknown reason, ether lipids also seem to be essential for normal vesicular transport[64].

Curvature in vesicle budding. Vesicle budding requires membrane bending and the generation of a lipid imbalance across the bilayer. At the level of the headgroups, the outer leaflet of a 60-nm diameter vesicle contains 1.5 times the number of lipid molecules of the inner leaflet. This might not pose a problem to the ER and possibly to *cis* Golgi membranes where lipids can freely cross the bilayer. In these flexible membranes, the assembly of the COP coats seems to be sufficient for budding. However, the situation is different in membranes in which lipids do not undergo free 'flip-flop', such as the plasma membrane, the TGN or endosomes. It has been proposed that the ATP-consuming aminophospholipid translocator, a 'flippase', drives vesicle budding from the plasma membrane by expanding the surface area of the cytoplasmic leaflet at the expense of the non-cytoplasmic bilayer leaflet[11]. Coats might therefore allow bending to be controlled and localized. Endocytosis is inhibited during mitosis, where, apart from the inactivation of essential proteins[65], the membrane tension increases. Release of the tension, which is regulated by cytoskeleton–plasma-membrane interactions through PtdIns(4,5)P_2 on the cytosolic surface, restored endocytosis. This provides yet another link between endocytosis and the phosphoinositide regulatory system (discussed in detail in REF. 66).

Apart from the positive curvature of the forming bud, an extreme, negative curvature must be generated at the site of membrane fission (FIG. 5). The cone shape of some lipids makes them ideally suited for fitting in the area of constriction. The budding of various types of transport vesicles involves the conversion of an inverted cone, lysophosphatidic acid (LPA), into a cone, phosphatidic acid, by the acyltransferase endophilin[67,68]. Phosphatidic acid is hydrolysed by a phosphohydrolase to DAG with an even more conical shape[69]. LPA formation involves the action of phospholipases D and A_2. Phospholipase D is activated during vesicle budding[70]. The activity of phospholipase A_2 seems to be required for tubulation of Golgi membranes[71], which might have a similar function in protein sorting as the movement of vesicles between different cisternae of the Golgi stack (vesicle percolation)[72,73].

In late endosomes, vesicles bud both towards the cytosol and towards the endosomal lumen[74]. The driving force behind lumenal budding and tubulation is unknown, but the event involves membrane sorting[75] and requires LBPA on the lumenal surface. Budding in the two directions might be temporally segregated: at one stage of endosomal maturation, budding towards the cytosol might be blocked, allowing budding in the opposite direction; at a later stage, cytoplasmic budding would resume. Lysosomal accumulation of lipids in some storage diseases might be due to obstruction of this last transition.

Lipid and protein transport

Over the past 25 years, we have learned a great deal about the lipid composition of membranes, lipid transmembrane asymmetry and, more recently, their heterogeneous lateral distribution. We also have a basic understanding of the molecular mechanisms by which cellular lipids are transported. New proteins are being identified, often during the study of metabolic disorders, and insights from the mode of action of related proteins shed new light on lipid behaviour in cells. Several fundamental questions, however, remain to be answered. We still have only sketchy information on the lipid composition of organellar membranes. In addition, our insight into the intracellular dynamics of lipids is limited. Finally,

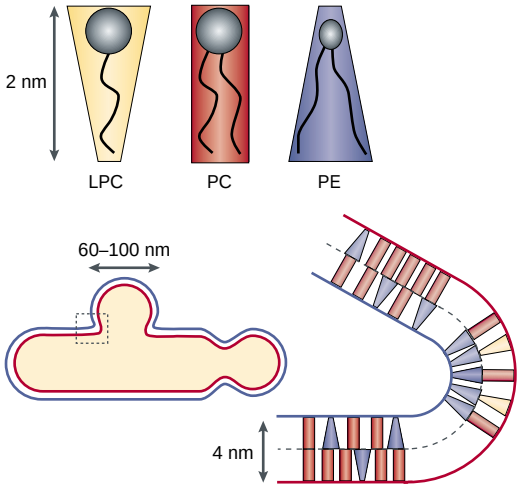


Figure 5 | **The molecular shape of lipids determines the physical properties of membranes.** The shape of a membrane lipid depends on the relative size of its polar headgroup and apolar tails[94]. In cases in which the headgroup and lipid backbone have similar cross-sectional areas, the molecule has a cylindrical shape (phosphatidylcholine (PC) and phosphatidylserine). Lipids with a small headgroup like phosphatidylethanolamine (PE) are cone-shaped. By contrast, when the hydrophobic part occupies a relatively smaller surface area, the molecule has the shape of an inverted cone (lysophosphatidylcholine (LPC) and, to some extent, sphingomyelin). This 'lipid polymorphism' might have a physiological role in the generation of curvature as during vesicle budding[67,68], and during membrane fusion[95]. The cytosolic surface of the plasma membrane contains 40% phosphatidylethanolamine, 60% phosphatidylserine plus phosphatidylcholine, and the lumenal leaflet 60% phosphatidylcholine, 30% sphingomyelin and 10% phosphatidylethanolamine. Phosphatidylethanolamine by itself adopts a hexagonal phase, and this tendency probably favours invagination of the membrane. Budding in the opposite direction, towards the lumen of endosomes may require lysobisphosphatidic acid, an inverted cone, on the lumenal surface[74] and phosphatidylinositol-3-phosphate[56]. Cholesterol is required for budding of the highly curved synaptic vesicles (40–50 nm diameter)[96]. Cholesterol plus sphingomyelin are also important for stabilizing membranes during fusion, and phosphatidylethanolamine greatly stimulates fusion efficiency[95,97].

we know little about lipid–protein interactions at the molecular level, let alone, lipid–lipid interactions in complex mixtures.

Addressing these questions at the molecular level will depend on the application of novel biophysical techniques (for example, to study the behaviour of single molecules in membranes). At the cellular level, the new developments in high sensitivity mass spectrometry allow the quantitative analysis of lipids, not only in cellular organelles but also in transport vesicles. These novel techniques will have to be combined with careful biochemical approaches, as was recently shown by the broad application of novel cholesterol acceptors in cholesterol transport studies. Comparable assays need to be developed for other membrane lipids.

Apart from the identification of novel lipid metabolic enzymes and transporters, genetic approaches reveal the fact that many of these belong to large families. The specific properties of individual family members and the effect of their combined actions on cellular lipid composition or dynamics will have to be assessed. It is a challenge to integrate these pieces of information into our view of how the cell works as a living entity.

Links

DATABASE LINKS scramblase | ABC transporter | ABCB4 | ABCB1 | Tangier disease | ABCA1 | sitosterolaemia | ABCG5 | ABCG8 | ABCA4 | ABCD1 | Niemann-Pick type C | NPC1 | NPC2 | STAR | MLN64 | caveolin | FYVE domains | PH domains | AP-1 | AP-3 | AP-4 | GGA | PITP | endophilin

ENCYCLOPEDIA OF LIFE SCIENCES Lipids | Membrane lipid biosynthesis | Cell membrane features

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We apologize that out of 30,000 PubMed papers on lipid and transport, we quote only 97.