Non-vesicular lipid transport by lipid-transfer proteins and beyond

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Abstract | The movement of lipids within and between intracellular membranes is mediated by different lipid transport mechanisms and is crucial for maintaining the identities of different cellular organelles. Non-vesicular lipid transport has a crucial role in intracellular lipid trafficking and distribution, but its underlying mechanisms remain unclear. Lipid-transfer proteins (LTPs), which regulate diverse lipid-mediated cellular processes and accelerate vectorial transport of lipid monomers between membranes *in vitro*, could potentially mediate non-vesicular intracellular lipid trafficking. Understanding the mechanisms by which lipids are transported and distributed between cellular membranes, and elucidating the role of LTPs in intracellular lipid transport and homeostasis, are currently subjects of intensive study.

Vesicular transport
An active process in which materials move into or out of the cell enclosed in vesicles. This process is mediated by a sequence of events involving the budding of the vesicles from a donor membrane and their subsequent fusion with an acceptor membrane.

Peroxisome
An organelle present in most eukaryotic cells that is involved in the oxidation of fatty acids and the production and destruction of hydrogen peroxide.

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Eukaryotic cells are organized into separate membrane-bound compartments or organelles, each with a specialized function and unique protein and lipid composition[1]. This compartmentalization ensures efficient segregation of diverse metabolic processes mediated by distinct sets of enzymes, regulatory and structural proteins. The distribution of proteins among cellular organelles is often mediated by specific protein-targeting motifs and can be regulated by chemical modifications and/or conformational changes. By contrast, lipids lack any intrinsic motifs that mediate their distinct intracellular distribution; nevertheless, different cellular membranes vary in their lipid composition[2,3]. For example, the plasma membrane, which exhibits a transverse lipid compositional asymmetry, is enriched in sphingolipids and sterols, whereas the endoplasmic reticulum (ER), which displays a symmetrical transbilayer lipid distribution, contains low levels of both lipids[4,5].

Eukaryotic cells contain more than 1,000 chemically distinct lipid species[6], which can be divided into three major classes: glycerophospholipids, sphingolipids and sterols[2]. It is not fully understood how these lipids are delivered to their target destinations. Increasing lines of evidence, however, suggest that intracellular lipid trafficking is mediated by both vesicular transport and non-vesicular transport mechanisms[7]. Vesicular transport plays a major part in protein transport along the exocytic and endocytic pathways, and requires metabolic energy and an intact cytoskeleton. Given that lipids are the basic constituents of transport vesicles, large amounts of lipids must be transported between organelles by vesicular transport. Nevertheless, lipid transport has been detected under conditions in which vesicular transport was blocked by either ATP depletion, reduction in temperature or treatment with specific pharmacological drugs (such as brefeldin A and colchicine)[8,9]. Lipid transport has also been seen between organelles that are not connected to the vesicular transport machinery (for example, mitochondria and peroxisomes)[10,11]. These observations suggest that non-vesicular transport mechanisms have an important role in intracellular lipid trafficking. Furthermore, current studies suggest that intracellular lipid trafficking is greatly facilitated at membrane contact sites (MCSs)[12–14]. These sites are defined as small cytosolic gaps (10–20 nm) between the membranes of the ER and virtually all cellular organelles and enable the transport of Ca^{2+}, metabolites and lipids by a non-vesicular transport mechanism[11,15].

Non-vesicular lipid transport could occur, in principle, by spontaneous lipid desorption of a lipid monomer from a bilayer and its free diffusion through the cytosol, but this process is slow and not sufficient to support substantial transport of most lipids[16,17]. Lipid-transfer proteins (LTPs) can greatly facilitate lipid transport between membranes *in vitro*[18,19]. LTPs were initially discovered as soluble factors that accelerate the exchange or net transfer of different lipid species between membranes *in vitro*[20]. Since then, many LTPs have been isolated, cloned and crystallized. LTPs have been identified in all eukaryotes, in plants and in bacteria, and have been subdivided into different protein families according to

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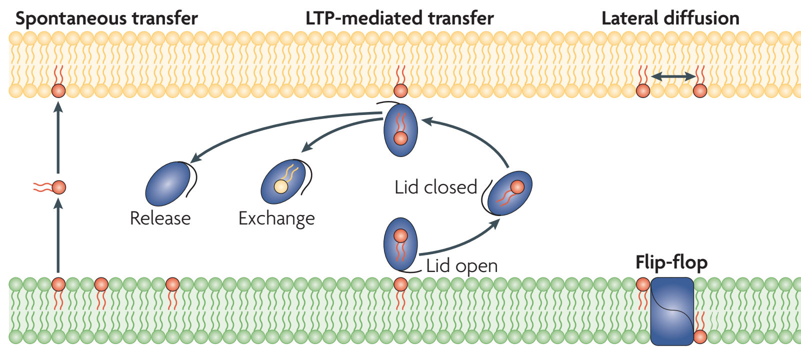

Figure 1 | **Modes of non-vesicular lipid transport.** There are three mechanisms involved in non-vesicular lipid transport: monomeric lipid exchange, lateral diffusion and transbilayer flip-flop. Monomeric lipid exchange can be spontaneous or mediated by lipid-transfer proteins (LTPs). LTPs can transfer a lipid to the acceptor membrane or exchange it with a lipid of the acceptor membrane. Lateral diffusion occurs in the lateral plane of the membrane (at 0.1–1 μm per second)[10]. Transbilayer flip-flop can be either spontaneous (not shown) or mediated by proteins such as flippases and translocases.

Lipid desorption
The release of a lipid molecule from a lipid bilayer to the surrounding aqueous phase. This process involves both the disruption of lipid–lipid interactions in the bilayer and the formation of a cavity in the aqueous phase that accommodates the diffusing lipid molecule.

Vectorial lipid transport
A directional transport of lipids that is driven by a concentration gradient.

Lipid droplet
An organelle that stores neutral lipids and has a crucial role in lipid metabolism.

Flippase
A membrane protein that catalyses the transport of lipids across the membrane bilayer in an ATP-dependent manner. Flippases commonly transport lipids towards the cytoplasm, whereas floppases transport lipids from the cytofacial surface to the opposite side of the membrane.

Membrane curvature
The bending of the membrane, which can be influenced by the relative distribution of cone-like and inverted cone-like lipids (for example, diacylglcerol and phosphatidic acid, and lysophospholipids, respectively) between the inner or outer leaflets of the bilayer.

their sequence and structure similarity[21]. It seems that most LTPs can bind lipid monomers in a hydrophobic pocket and transfer hydrophobic lipids through an aqueous phase.

Although LTPs have been extensively studied over the past 30 years, their modes of action in intact cells have not been fully explored. Increasing lines of evidence suggest that LTPs do not mediate a simple vectorial lipid transport from one membrane to another. Instead, they facilitate lipid transport between membranes according to their membrane environment. LTPs can, therefore, locally modulate the lipid composition of membranes and consequently regulate various cellular processes, including vesicular trafficking, signal transduction and lipid metabolism[22–24]. This Review discusses the latest advances in our understanding of non-vesicular lipid trafficking, the mechanisms by which LTPs act *in vitro* and their diverse modes of action in intact cells.

Non-vesicular transport of lipids

Lipid movement in and between biological membranes is mediated by three mechanisms: monomeric lipid exchange, lateral diffusion and transbilayer flip-flop[4,6] (FIG. 1).

Transport of lipids between membranes is mainly mediated by monomeric lipid exchange, in which a lipid molecule is transported through an aqueous phase from the outer leaflet of a donor membrane to the outer leaflet of an acceptor membrane. This process does not require metabolic energy and can be either spontaneous or mediated by LTPs.

Lateral diffusion is the process by which lipids move in the lateral plane of the membrane bilayer. Although it mainly occurs within membranes, it can mediate lipid transport between membranes that are connected by membrane bridges. Membrane continuities have been seen between the plasma membrane and lipid droplets, the ER and the outer mitochondrial membrane, and lipid droplets and the outer mitochondrial membrane, among others[6,25].

Transbilayer flip-flop is the process by which lipids are moved between the two leaflets of the membrane bilayer either spontaneously or with the assistance of proteins such as flippases and translocases[2]. In some cases, protein-assisted flip-flop requires metabolic energy. Although transbilayer flip-flop is not directly involved in lipid transport between different organelles, it may indirectly influence inter-organelle lipid transport mediated by monomeric lipid exchange or vesicular transport. The flip-flop of lipids from the inner to the outer leaflet would enable monomeric lipid exchange. Furthermore, flip-flop of lipids with either a small or a non-polar headgroup, such as phosphatidic acid or diacylglycerol, could markedly affect membrane curvature, and consequently vesicle budding, vesicle fission and vesicle fusion[2,26]. Thus, the interplay between vesicular and non-vesicular lipid transport is crucial for the establishment and maintenance of intracellular lipid distribution.

Monomeric lipid exchange

Spontaneous movement of lipids between membranes. Although most cellular lipids are highly insoluble in water, numerous studies have shown that lipid monomers can spontaneously move between membranes[27–31]. For most classes of lipids, this process is extremely slow (FIG. 2a,b), with half-times on the order of days. The rate of spontaneous lipid exchange usually correlates with their aqueous-phase solubility[29,32]. Lyso-phosphatidylcholine (Lyso-PtdCho), for example, which has only one acyl chain, exchanges more rapidly than PtdCho. Cholesterol, which is more soluble than PtdCho, exchanges much faster between synthetic vesicles with a similar lipid composition. It was estimated that six molecules of cholesterol and one molecule of PtdCho are transferred between such vesicles in 2 minutes[28]. The lipid exchange rate is also influenced by membrane curvature, and indeed cholesterol exchanges more rapidly from small donor vesicles with high membrane curvature than vesicles with low membrane curvature[33]. However, the interaction of cholesterol with phospholipids in the bilayer can form condensed complexes, which markedly attenuate its membrane desorption[34].

Based on kinetic measurements, three types of spontaneous lipid exchange mechanisms have been proposed[35]. The first mechanism is aqueous diffusion, which occurs at low membrane concentrations and is determined by the concentration of donor, but not acceptor membranes[30]. In this case, a lipid desorbs out of one bilayer, enters the aqueous phase, diffuses across it and inserts into a second bilayer (FIG. 2c). This is a first-order process, in which lipid desorption from the bilayer is the rate-limiting step[27,29,36].

The second possible mechanism occurs at high membrane concentrations as a result of lipid–membrane collision[16] and is dependent on the concentration of both donor and acceptor membranes (FIG. 2d). This is a second-order process, in which the rates of lipid desorption from donor membrane and its collision with the acceptor membrane are the first- and second-order rate constants, respectively.

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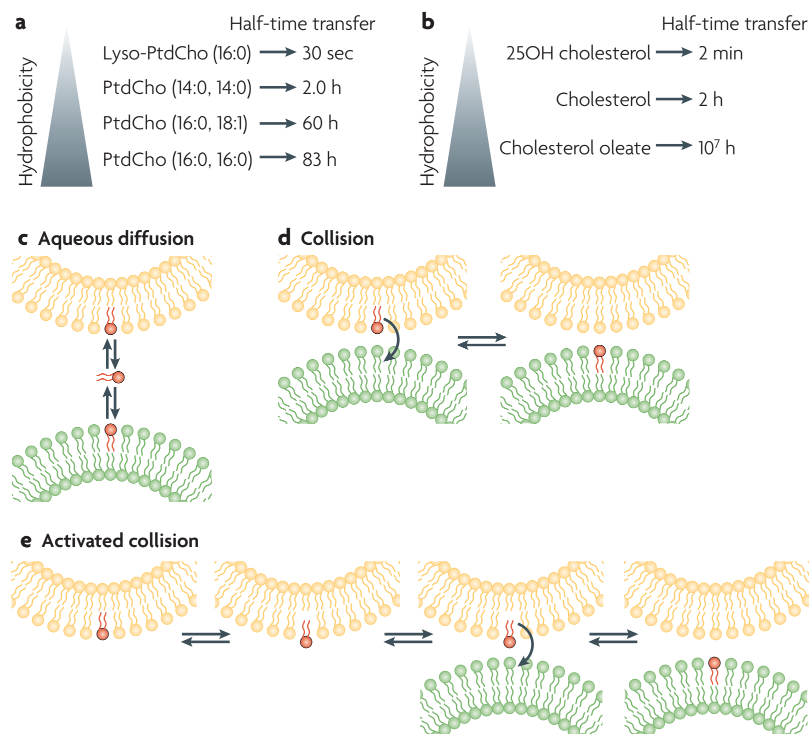

Figure 2 | **Spontaneous lipid exchange between membranes. a**, **b** | Rates of exchange of various phosphatidylcholine (PtdCho; **a**) or sterol (**b**) species correlate with their aqueous-phase solubility[29,30,110]. **a** | Lyso-PtdCho, which has only one acyl chain, exchanges most rapidly. Because most PtdCho in cells has acyl chains with 16 or more carbons, spontaneous PtdCho exchange between membranes is probably not physiologically relevant. The ratios in brackets refer to the length of the fatty acid chain at sn1 and sn2 positions of the phospholipids (14:0, myristic acid; 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid). The structures of the lipids can be found in Supplementary information S3 (figure). **b** | 25-hydroxylcholesterol (25OH) is more hydrophilic than cholesterol and therefore exchanges more rapidly, whereas cholesteryl oleate is more hydrophobic than cholesterol and exchanges more slowly. **c–e** | Mechanisms of spontaneous lipid exchange. **c** | During aqueous diffusion, a lipid desorbs out of one bilayer and diffuses across an aqueous phase to insert into a different bilayer. **d** | Lipids can also transfer during the collision of two membranes. **e** | Activated collision can occur when a lipid partially extends from the bilayer, increasing the probability of transfer.

Vesicle fission
The pinching-off of a vesicle from a membrane bilayer.

Vesicle fusion
The merging of a vesicle with a membrane bilayer.

Condensed complex
A complex formed between cholesterol and saturated phospholipids with long fatty acid chains or with sphingomyelin.

First-order process
A reaction with a rate that is proportional to the concentration of only one reactant. Other reactants can be present but have no influence on the reaction rate.

The third mechanism is activated collision (FIG. 2e). In this case, a lipid partially extends from the bilayer (and is referred to as activated), increasing the probability of its transfer to a second membrane during collision[37] by decreasing the energy required for transfer. Lipid activation can be stochastic, as thermal motion probably drives lipids partially in and out of membranes. The activated collision model is consistent with studies showing that hydration forces between liposomes prevent them from getting closer than about 20 Å. This suggests that lipids can only be exchanged during membrane collision if they protrude from the bilayer.

Collisional lipid transfer is a concentration-dependent process (see Supplementary information S1 (table) for an example of the effect of concentration on transfer); it could, therefore, occur in intact cells owing to a high concentration (~40 mM) of cellular membranes[16,38].

***LTPs as accelerators of lipid transport* in vitro.** The slow rate of spontaneous lipid exchange between membranes can be accelerated by LTPs, possibly by increasing the rate of lipid desorption from membranes[39]. This could be accomplished by decreasing the energy barrier for the lipid monomer–membrane equilibrium reaction, thereby facilitating the dissociation rate of lipid monomers from membranes[40]. Thus, LTPs may act as both lipid carriers and catalysts for monomeric exchange[41].

LTPs carry a lipid monomer in their hydrophobic pocket and show specificity for one or more lipid types. Nonspecific LTP (NSLTP; also known as SCP2), for example, transfers all common diacyl phospholipids, glycolipids and cholesterol[19,38], whereas ceramide-transfer protein (CERT) displays high specificity towards natural ceramide with C_{14}–C_{20} but not longer acyl chains[42], thus showing specificity to both the lipid headgroup and the backbone.

According to their lipid-binding specificity and transfer capability, LTPs can be grouped into three main classes: phospholipid-, sterol- and sphingolipid-transfer proteins (FIG. 3a). In mammals, PtdCho-transfer protein (PCTP), phosphatidylinositol (PtdIns)-transfer protein (PITP) and NSLTP are the three main classes of phospholipid-transfer proteins. Steroidogenic acute regulatory protein (STAR) is an example of a cholesterol-transfer protein[43], and oxysterol-binding protein (OSBP) and OSBP-related proteins (ORPs) are considered to be either sterol-sensing and/or sterol-transfer proteins. Examples of sphingolipid-transfer proteins include CERT and FAPP2 (also known as PLEKHA8)[44].

LTPs can contain a single structural lipid-transfer domain (LTD) or can have additional structural domains with varying functions (FIG. 3a). Based on the structure of the LTD, several LTP families have been defined, including SEC14, PITP, STAR-related lipid transfer (START), glycolipid-transfer protein (GLTP), SCP2, OSBP and ORP[21].

Mechanisms of LTP action. LTPs have been extensively studied using different *in vitro* lipid-transfer assays (see Supplementary information S2 (box)). Together with molecular, biophysical and crystallographic approaches, these studies have shed light on their mechanisms of action.

The crystal structures of many LTPs have been resolved both in the presence and absence (known as the apo form) of their lipid ligands. In general, the structures are dominated by β-sheet motifs, such as β-barrels, β-cups and β-grooves. Several α-helices (2–4) are closely packed with central β-sheet motifs and together form a hydrophobic binding tunnel (FIG. 3b–d). The shape and size of the tunnel dictate the specificity for a cognate lipid ligand. A network of hydrophobic interactions and hydrogen bonds stabilize the lipid binding and affect the binding affinity. Typically, a 'lid' covers the hydrophobic tunnel and acts as a gate for lipid uptake and release[45,46]. The interaction of an LTP with membranes is thought to induce conformational changes, leading to displacement of the lid from the lipid-binding cavity and, consequently, tunnel opening.

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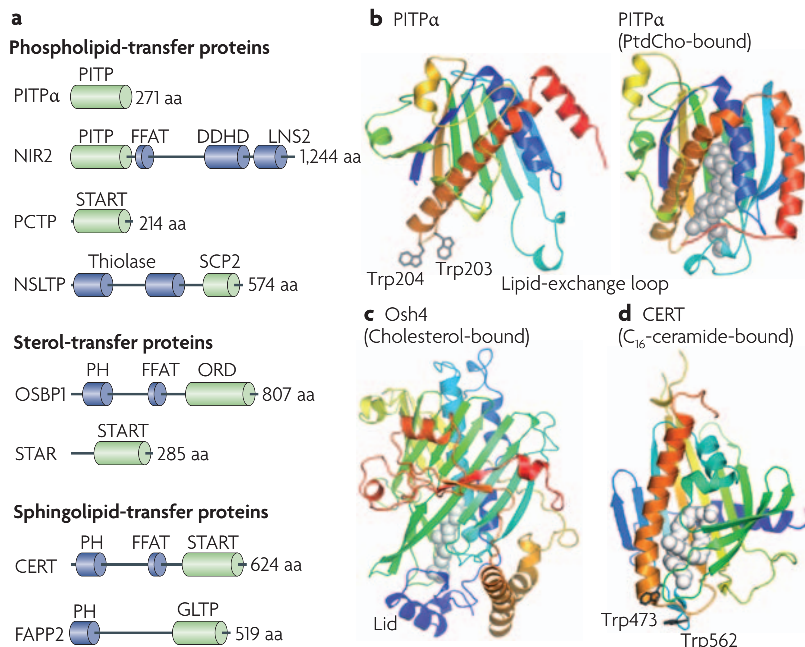

Figure 3 | **Domain organization and three-dimensional structure of LTPs.** **a** | The domain organization of representatives of the three main classes of human lipid-transfer proteins (LTPs). The lipid-transfer domains are shown in green. **b** | A ribbon diagram of the open (left panel; protein data bank (PDB) code 1KCM)[111] and closed phosphatidylcholine (PtdCho)-bound (right panel; PDB code 1T27)[112] conformation of phosphatidylinositol (PtdIns) transfer protein-α (PITPα). The PtdCho- and PtdIns-binding site is formed by the concave surface of a central eight-stranded β-sheet and two α-helices. The lipid-exchange loop and the carboxy-terminal tail act as a lid. Trp203 and Trp204 are crucial for membrane association and lipid-transfer activity[57,58]. **c** | The oxysterol-related domain (ORD) of oxysterol-binding protein (OSBP) homologue 4 (Osh4) consists of a β-barrel (17 strands) and three α-helices (PDB code 1ZHX). A short amino-terminal region forms a lid over the tunnel. The 3-hydroxyl of cholesterol is buried at the bottom of the tunnel[46]. **d** | A ribbon representation of the steroidogenic acute regulatory protein (STAR)-related lipid transfer (START) domain of ceramide transfer protein (CERT) in complex with C_{16}-ceramide (PDB code 2E3P). The overall structure contains two α-helices at the N and C termini, separated by nine β-strands and two shorter α-helices. Trp473 and Trp562 are crucial for membrane association[59]. PCTP, PtdCho-transfer protein; PH, pleckstrin homology; NSLTP, nonspecific LTP (also known as SCP2).

Second-order process
A reaction with a rate that is proportional to the square concentration of a single reactant or to the concentration of two reactants.

Thermal motion
The random motion of lipid molecules in the bilayer that is due to temperature.

Hydration force
The repulsive force acting between apposing lipid bilayers in aqueous solution.

Liposome
An artificial microscopic vesicle consisting of an aqueous core surrounded by a lipid bilayer.

Thus, LTPs may exist in two distinct conformations: a 'closed' conformation, which reflects a transport-competent conformation in which one lipid molecule is enclosed in the tunnel; and an 'open', membrane-bound conformation[45,46] (FIG. 3b). In the closed conformation, the lid is often stabilized by hydrogen bonds and hydrophobic interactions with the rest of the protein. Polar interactions between the lid and membrane phospholipids could facilitate the opening of the tunnel and stabilize membrane anchoring. Indeed, truncation of the lid in various LTPs markedly affects their ability to interact with membranes and abolishes their lipid-transfer activity[47,48]. Hence, the transport of lipids by LTPs could be mediated by sequential events involving the interaction with a donor membrane and the opening of the lipid-binding tunnel, extraction of a lipid from the bilayer, dissociation from the donor membrane and diffusion through the aqueous phase in a closed conformation. The transport is terminated by interaction with an acceptor membrane, tunnel opening and lipid desorption.

This simple mechanism raises two important questions. How does an LTP associate with and dissociate from the donor and acceptor membranes? What are the mechanisms that dictate the direction, specificity and efficiency of lipid transport events?

The lipid composition and biophysical properties of the membranes (curvature and membrane fluidity) directly affect the kinetic parameters and rate constants of the transfer reactions[19]. High transfer activities are generally seen with more fluid or highly curved membranes. Under such conditions, the interactions between the lipid and the bilayer are much weaker and, therefore, the absorption of the lipid to the LTP, the most energy-consuming step, is more efficient[49]. Furthermore, membrane curvature can also influence the dissociation rate of the LTP from the membrane. PCTP, for example, dissociates 100-fold faster from highly curved vesicles than liposomes and so transfers PtdCho between such vesicles more efficiently than between liposomes[50].

In contrast to the membrane-association steps, which mostly depend on physical parameters such as membrane charge, curvature and size[38,41], the lipid absorption step mainly depends on the lipid type and its surface concentration. Indeed, LTPs can mediate a net lipid transfer between donor and acceptor membranes down the lipid concentration gradient[51]. PITPs, for example, which exhibit a dual specificity for both PtdIns and PtdCho, can transfer PtdIns to membranes with low PtdIns content in return for PtdCho, and can thereby mediate a net PtdIns transfer according to the PtdIns concentration gradient[52]. Furthermore, PCTP, which generally mediates a PtdCho-exchange reaction, can also mediate a net transport of PtdCho to acceptor membranes that lack PtdCho[53]. Thus, the lipid composition of the donor and acceptor membranes has a crucial role in dictating the direction of the lipid transport event and the rate of lipid desorption from the bilayer[38,54,55]. However, the association and dissociation of LTPs with and from the membranes also influence the efficiency of transport. GLTP, for example, which is positively charged at neutral pH, strongly interacts with negatively charged donor membranes. This strong electrostatic interaction slows its dissociation from a donor surface and apparently diminishes GLTP-mediated transfer[56].

Currently, the mechanisms responsible for the targeting of LTPs to specific donor or acceptor membranes are not clear. Nevertheless, studies over the past few years suggest that many LTPs interact with membranes through specific protein motifs. Membrane-binding sites containing a Trp residue, which has a high propensity for membrane interaction, have been identified in several LTPs, including PITPs[57,58] (FIG. 3b), CERT[59] (FIG. 3d) and GLTPs[56]. These Trp residues are crucial for membrane binding and therefore for lipid transport activity *in vitro*[57–59], but were proposed to mediate nonspecific binding with membranes[60]. Distinct binding sites may contribute to the specificity of LTP–membrane interactions. These binding sites could be found either on the LTD or in other structural domains of large LTPs. For example, the LTD of OSBP homologue 4 (Osh4; also known as Kes1), a yeast

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ORP, contains at least two membrane-binding surfaces that bind phosphoinositides, one near the mouth of the sterol-binding pocket and another at a distal site that binds PtdIns-4,5-bisphosphate (PtdIns(4,5)P_2)[61]. It has recently been shown that the transport of sterol by Osh4 is enhanced when PtdIns(4,5)P_2 is present on the acceptor membrane. PtdIns(4,5)P_2 may, therefore, enhance the directional transport of sterol from PtdIns(4,5)P_2-poor to PtdIns(4,5)P_2-rich membranes. Likewise, PtdIns-4-phosphate (PtdIns4P), which binds the pleckstrin homology domain (PH domain) of OSBP[62], stimulates *in vitro* transport of sterol by OSBP when present in the donor membranes[63]. These examples show how the transport of specific lipid monomers by LTPs can be influenced by the relative distribution of other lipid species in the acceptor and donor membranes, which can bind to specific lipid-binding motifs on the LTPs. Thus, membrane-binding determinants on the LTPs, which may interact with specific membrane lipids and/or proteins, could affect the membrane specificity and influence the directionality of the lipid transport events.

Transport efficiency can be greatly enhanced by close proximity between the donor and the acceptor membranes, as has been recently shown for Osh4-mediated sterol transport *in vitro*[61]. This close apposition could reduce the diffusion distance between donor and acceptor membranes or even enable simultaneous interaction with the two membranes[8]. Such close proximity between different membrane compartments can be conferred by MCSs and has been identified in intact cells[64].

Lipid transport at MCSs

MCSs are currently recognized as dynamic structures that facilitate non-vesicular lipid transport and are involved in the regulation of cellular lipid and Ca^{2+} homeostasis[11,64,65]. MCSs have been identified in all eukaryotes by morphological and biochemical studies, and some have been physically isolated. These include the mitochondrion-associated membrane (MAM) fraction, which contains unique regions of ER membranes attached to the outer mitochondrial membrane, and the plasma membrane-associated membrane (PAM) fraction, which contains many types of intracellular membrane (mainly from the ER and mitochondria) that co-isolate with the plasma membrane. In addition to MAM and PAM, MCSs have been identified between the ER membranes and those of the Golgi apparatus, vacuoles, peroxisomes, lipid droplets, late endosomes and lysosomes[11,64].

Despite their heterogeneity (TABLE 1), different MCSs share several common features: they are typically enriched in proteins involved in lipid biosynthesis and trafficking, they are formed and/or stabilized by the tethering of apposing membranes through protein–protein or protein–lipid interactions and they are dynamic structures that can be formed and/or stabilized in response to different physiological conditions[15,66]. Although the molecular mechanisms underlying MCS formation and/or stabilization have not been fully characterized, current efforts have led to the identification of tethering complexes and bridging proteins that are involved in MCS formation and/or stabilization[66–75] (TABLE 1). Such tethers can affect the lipid composition of their tethered membranes and/or Ca^{2+} transport between the membranes. For example, the ER–mitochondrion encounter structure (ERMES) complex, which acts as an ER–mitochondrion tether in yeast, affects the lipid composition of the mitochondrial membrane[67]. Mitofusin 2, which has been implicated in ER–mitochondrion tethering in mammals, is required for Ca^{2+} uptake into mitochondria[68] (FIG. 4a).

Lipid transport at MCSs can occur by both the spontaneous and LTP-mediated routes. The production of phosphatidylethanolamine (PtdEtn) from phosphatidylserine (PtdSer) at the MAM fraction is thought to be mediated by spontaneous lipid transport[15,66] (FIG. 4a,b). In mammalian cells, the formation of PtdSer occurs through the exchange of L-Ser with either the choline moiety of PtdCho or the ethanolamine moiety of PtdEtn, and is catalysed by two different enzymes, PtdSer synthase 1 (PSS1) and PSS2, respectively[76] (FIG. 4c,d). These enzymes are highly enriched in the MAM fraction. The newly formed PtdSer at the MAM is transported into the mitochondrial intermembrane space, where it undergoes decarboxylation by PtdSer decarboxylase to produce PtdEtn. When the mitochondrial PtdSer decarboxylase 1 is inhibited, for example by hydroxylamine, accumulation of PtdSer in the MAM fraction is observed, indicating that transport of PtdSer from ER to mitochondria occurs through the MAM compartment[77]. The decarboxylation of PtdSer in the mitochondria seems to account for most PtdEtn synthesis in mammalian cells. PtdEtn is then exported from mitochondria back to the ER. Hence, the transport of PtdSer from the ER to mitochondria and PtdEtn back from mitochondria to the ER represents bidirectional non-vesicular lipid transport and demonstrates the importance of MCSs in regulating cellular lipid biosynthesis and distribution.

Several LTPs have been localized to MCSs and implicated in diverse functions (TABLE 1). OSBP-related protein 1L (ORP1L), for example, was implicated in the formation of ER–late endosome MCSs under low cholesterol conditions[75]. Osh1, which is recruited to nucleus–vacuole junctions (NVJs) by direct interaction with the outer nuclear membrane protein Nvj1, may regulate the lipid composition of NVJs in yeast[70]. It thus seems that many LTPs function at MCSs.

Perhaps the most convincing evidence of LTP-mediated lipid transport at MCSs has emerged from studies of CERT-mediated ceramide transport at ER–Golgi MCSs[12,78]. In mammalian cells, ceramide is synthesized in the ER and primarily transported by CERT to the Golgi complex, where it is converted into sphingomyelin by sphingomyelin synthase (SMS). It was proposed that CERT efficiently transports ceramide at ER–Golgi MCSs owing to its dual membrane targeting determinants: the PH domain, which binds PtdIns4P and mediates its interaction with the Golgi complex, and an FFAT motif (two Phe residues in an acidic tract)[79], which interacts with the ER membrane proteins of the VAMP-associated protein (VAP) family (VAPA and VAPB)[80], thereby mediating interaction with the ER[14].

Membrane fluidity
The viscosity of the lipid bilayer, which is determined by the length and saturation of the fatty-acid side chains of phospholipids and the content of cholesterol and sphingolipids..

Phosphoinositide
The phosphorylated form of PtdIns. The inositol ring of PtdIns can be phosphorylated in three different positions (3, 4 and 5), yielding seven distinct phosphoinositides. Phosphoinositides play a key part in signal transduction and membrane trafficking.

PH domain
A protein domain of ~100 amino acids that is present in numerous proteins and in many cases binds phosphoinositides with high affinity and specificity.

FFAT motif
A short sequence motif, containing the EFFDAxE consensus sequence, that has been identified in 17 eukaryotic proteins, most of which are involved in lipid transfer, sensing or binding.

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Table 1 | **Tethering of MCSs**

Organelles	Lipid-modifying or binding proteins	Tethering complexes	Comments	Refs
ER and mitochondria (MAM)	PSS1, PSS2, FACL4 and acyl-CoA synthetase	ERMES: Mmm1* and Mdm12 (ER), Mdm10 and Mdm34 (mitochondrion)	ERMES was identified by a synthetic biological screen in yeast as a molecular tether between the ER and mitochondria	67
		MFN: MFN2* (ER), MFN1* and MFN2* (mitochondrion)	MFN1 and MFN2 are dynamin-like GTPases in the OMM that mediate mitochondrial fusion; a fraction of MFN2 is also present in the ER membrane and tethers the ER membrane to mitochondria by interacting with MFN1 or MFN2 on the OMM	68
		IP3R* (ER), VDAC* and GRP75 (mitochondrion)	VDAC in the OMM is physically linked to the ER Ca^{2+}-release channel IP3R by interacting with GRP75, which enhances mitochondrial Ca^{2+} uptake	69
		PACS2: calnexin‡ (ER) and unknown (mitochondrion)	PACS2 is a cytosolic multifunctional sorting protein that mediates ER–mitochondrion communication by an unknown mechanism	66
ONM and vacuole (NVJ)	Tsc13 and Osh1	Nvj1* (ONM) and Vac8 (vacuole)	The autophagic process PMN, which degrades portions of the yeast nucleus in the hydrolytic vacuole lumen, occurs at NVJs; Tsc13 and the OSH family are required for efficient PMN	70
ER and plasma membrane (PAM)	Erg1, Erg6, Erg9, PtdSer- and PtdIns-synthase (yeast); and RDGB (fly)	PTP1B (ER) and insulin receptor* (plasma membrane)	The Tyr phosphatase PTP1B resides in the ER membrane and directly interacts with insulin receptors in the plasma membrane, thereby enhancing the formation of ER–plasma membrane junctions	71
		STIM1* (ER) and ORAI1* (plasma membrane)	The interaction between the ER Ca^{2+} sensor STIM1 and the ORAI1 subunit of the CRAC channel is involved in the formation and stabilization of ER–plasma membrane MCSs and capacitative Ca^{2+} influx	72
		IP3R* (ER), TRP* (plasma membrane) and homer	An interaction between TRP on the plasma membrane and IP3R in the ER is mediated by the cytoplasmic adaptor protein homer and regulates store-operated Ca^{2+} entry	73
	ORP3	VAPA* (ER) and ORP3 (plasma membrane)	ORP3 interacts with the plasma membrane mainly through its PH domain and with the ER through its FFAT motif that binds VAPA	74
ER and late endosome	ORP1L	VAPA* (ER) and ORP1L (late endosome)	ORP1L on late endosomes interacts with VAPA on the ER through its FFAT motif and thereby tethers the ER to late endosomes under low cholesterol levels	75
ER and *trans*-Golgi	OSBP, CERT and NIR2	VAPA* (ER) and OSBP (*trans*-Golgi)	25OH enhances the Golgi targeting of OSBP, which also interacts with the ER through its FFAT motif; OSBP may interact with both the Golgi and the ER in the presence of 25OH, and induces their tethering	12,82,84

CRAC, Ca^{2+}-release-activated Ca^{2+}; ERMES, endoplasmic reticulum (ER)–mitochondrion encounter structure; GRP75, 75 kDa glucose-regulated protein; IP3R, inositol 1,4,5-triphosphate receptor; MAM, mitochondrion-associated membrane; MCS, membrane contact site; Mdm, mitochondrial distribution and morphology protein; MFN, mitofusin; Mmm, mitochondrial morphology protein; NVJ, nucleus–vacuole junction; 25OH, 25-hydroxycholesterol; OMM, outer mitochondrial membrane; ONM, outer nuclear membrane; ORP1L, OSBP-related protein 1L; ORP3, OSBP-related protein 3; Osh1, oxysterol-binding protein homologue 1; PACS2, phosphofurin acidic cluster sorting protein 2; PAM, plasma membrane-associated membrane; PH, pleckstrin homology; PMN, piecemeal microautophagy of the nucleus; PSS, phosphatidylserine (PtdSer) synthase; PtdIns, phosphatidylinositol; PTP1B, protein Tyr phosphatase 1B; RDGB, retinal degeneration B; STIM1, stromal interaction molecule 1; Tsc13, temperature-sensitive CSG2 suppressor protein 13; TRP, transient receptor potential; Vac8, vacuolar protein 8; VAPA, VAMP-associated protein A; VDAC, voltage-dependent anion channel. *Transmembrane protein. ‡Unclear involvement.

Mutations in the CERT FFAT motif abrogate not only the VAP–CERT interaction, but also CERT-mediated ER-to-Golgi ceramide transport in intact cells[12,81]. Similarly, mutations in the CERT PH domain abolish its targeting to the Golgi, and, consequently, sphingomyelin synthesis. These observations suggest that the Golgi and ER targeting of CERT spatially restrict ceramide transport to MCSs, thereby ensuring efficient ER-to-Golgi ceramide transport.

OSBP also contains a PH domain and an FFAT motif that mediate its interaction with the Golgi and the ER membranes, respectively[82]. OSBP binds 25-hydroxycholesterol (25OH) through its carboxy-terminal sterol-binding domain and facilitates CERT-mediated ER-to-Golgi ceramide transport in the presence of 25OH[82]. The PtdIns-transfer domain-containing protein NIR2 (also known as PITPNM1) also has an FFAT motif, which mediates its interaction with the ER through VAP binding[83]. It seems that CERT, OSBP and NIR2, which are all localized to the Golgi, function coordinately at ER–Golgi MCSs to regulate ceramide transport. Treatment with 25OH enhances the Golgi targeting of these proteins and the recruitment of VAPs to the ER–Golgi MCSs, and could thereby facilitate

PtdIns-transfer domain
A protein domain that is present in PITPs and mediates the exchange of PtdIns for PtdCho, and vice versa. PITPs have a ~16-fold higher binding affinity for PtdIns than PtdCho.

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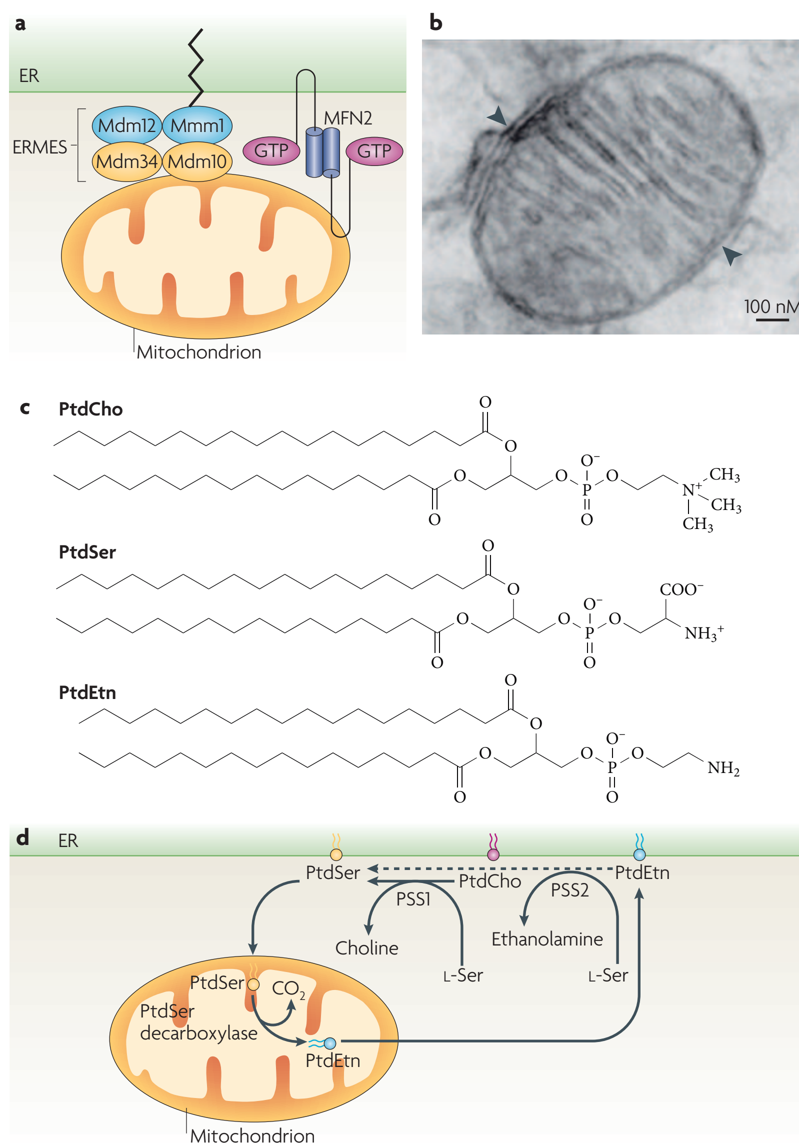

Figure 4 | **Lipid transport at ER-mitochondrion MCSs. a** | Endoplasmic reticulum (ER)-mitochondrion tethers. The ER-mitochondrion encounter structure (ERMES) complex was identified in yeast as an ER–mitochondrion tether. It is composed of proteins residing in the ER (maintenance of mitochondrial morphology protein 1 (Mmm1) and mitochondrial distribution and morphology protein 12 (Mdm12)) and mitochondria (Mdm10 and Mdm34). ERMES-mutant strains suffer from mitochondrial phospholipid abnormalities owing to impaired phospholipid transport and biosynthesis[67]. ER–mitochondrion tethers can also affect Ca^{2+} transport. Mitofusin 2 (MFN2), a dynamin-like GTPase, is enriched in the mitochondrion-associated membrane (MAM) and was proposed to tether the ER to the mitochondria in mammalian cells by *trans* homotypic interaction with MFN2 or heterotypic interaction with MFN1 (not shown) on the mitochondrial surface[68]. In MFN2-deficient cells, both the number of ER–mitochondrion membrane contact sites (MCSs) and the uptake of Ca^{2+} into mitochondria were markedly reduced, showing a crucial role for MCSs in Ca^{2+} transport and signalling[68]. **b** | An electron microscopy image of MAM. Two ER membranes are seen closely associated with the mitochondrion and are indicated by arrowheads[25]. **c** | Structures of phosphatidylcholine (PtdCho), phosphatidylserine (PtdSer) and phosphatidylethanolamine (PtdEtn). **d** | In mammalian cells, PtdSer can be formed through the exchange of L-Ser for ethanolamine from PtdEtn or for choline from PtdCho. PtdSer is transported through the MAM and is converted to PtdEtn by decarboxylation in the mitochondrial intermembrane space. PtdEtn is subsequently exported back to the ER. PSS, PtdSer synthase. Image in part **b** reproduced, with permission, from REF. 25 © (1997) Elsevier.

non-vesicular lipid transport between the ER and Golgi. It was proposed that NIR2 is required for transport of PtdIns from the ER to the Golgi and that the subsequent production of PtdIns4P in the Golgi membrane enhances the recruitment of OSBP and CERT to the Golgi and consequently ER-to-Golgi ceramide transport[84]. Hence, NIR2 and CERT can mediate PtdIns and ceramide transport, respectively. It is not clear, however, whether OSBP mediates sterol transfer at ER–Golgi MCSs or exerts other functions, such as lipid sensing or MCS stabilization.

Together, these observations suggest that MCSs are ideal sites for non-vesicular lipid transport. Spontaneous lipid transport could be greatly facilitated by local membrane concentrations[16], whereas LTPs might be involved in various functions, including the formation and stabilization of MCSs, as well as lipid transport and/or sensing.

Functions of LTPs in cells

LTPs have been implicated in the regulation of several cellular processes. Their ability to transfer lipids between membranes *in vitro* suggests that they could also mediate lipid trafficking in intact cells. Although CERT is perhaps the most established example of an LTP that mediates intracellular lipid trafficking, several sterol transfer proteins have also been implicated in intracellular sterol trafficking[85]. ORP2 was proposed to function as an ER–plasma membrane sterol transporter, as its overexpression in mammalian cells enhanced efflux of newly synthesized cholesterol from the ER to extracellular cyclodextrin without perturbing the plasma membrane cholesterol content[86]. STAR has a crucial role in steroidogenesis by facilitating the delivery of cholesterol from the outer to the inner mitochondrial membrane, where the first step of steroid biosynthesis is catalysed[43]. It can transfer cholesterol between membranes *in vitro*, and robustly transfers cholesterol in steroidogenic cells. It was estimated that STAR can deliver ~400 molecules of cholesterol into mitochondria per minute[87], but its precise mechanism of action remains unclear. The OSBP homologues (Osh proteins) in budding yeast also mediate intracellular sterol transport, as a yeast strain deficient in all of the seven Osh proteins exhibits a severe reduction (~80%) in plasma membrane–ER sterol transfer[88]. However, Osh proteins may have an impact on sterol transport indirectly, by affecting actin dynamics[22] or modulating other cellular functions. Indeed, many LTPs have been implicated in the regulation of diverse cellular functions, such as signal transduction, vesicular transport and lipid transport and metabolism.

These diverse functions might be mediated by distinct modes of action. First, LTPs could facilitate vectorial lipid transfer in cells as they do *in vitro*. Second, they could act as lipid sensors that alter their interactions with partner proteins in response to binding lipids or membranes. For example, an LTP might activate a partner protein in response to lipid exchange with a membrane. Furthermore, LTPs might control the access of other lipid-binding proteins to lipids in the membrane,

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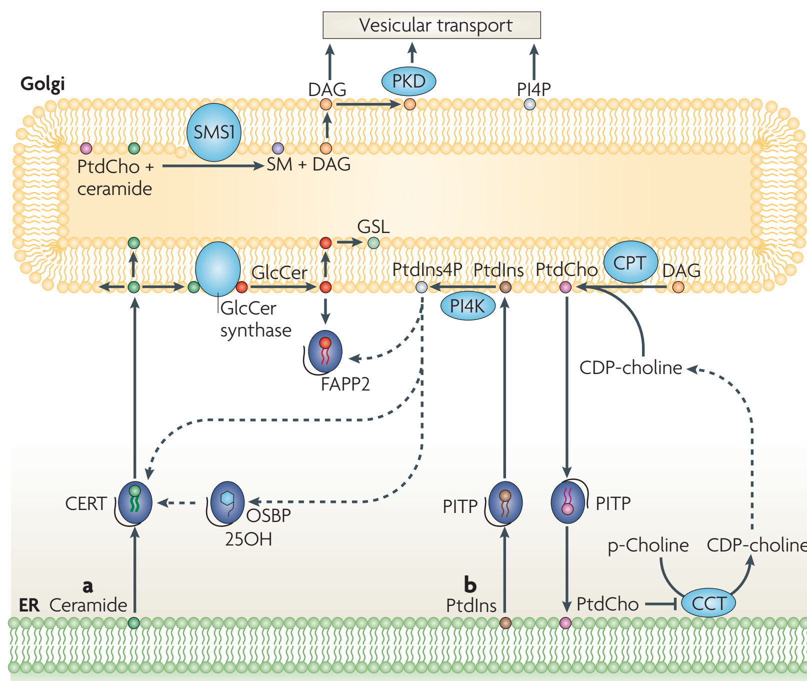

Figure 5 | **Networking of coordinated functions of LTPs in the Golgi complex of mammalian cells. a** | Ceramide is synthesized in the endoplasmic reticulum (ER) and transported by ceramide transfer protein (CERT) to the *trans*-Golgi complex at ER–Golgi membrane contact sites (MCSs)[14,78]. Ceramide is converted into sphingomyelin (SM) at the lumenal leaflet of the *trans*-Golgi by sphingomyelin synthase 1 (SMS1), which transfers phosphocholine from phosphatidylcholine (PtdCho) to ceramide, yielding diacylglycerol (DAG) as a by-product. Diacylglycerol affects membrane curvature and also stimulates several protein kinases, including protein kinase D (PKD), which in turn facilitates budding and fission of secretory vesicles[90]. The transfer of ceramide by CERT is facilitated by 25-hydroxycholesterol (25OH)-bound oxysterol-binding protein (OSBP). **b** | Phosphatidylinositol (PtdIns) is synthesized in the ER and can be transported to the Golgi complex by PtdIns-transfer proteins (PITPs), possibly through ER–Golgi MCSs[84]. PITPs exchange PtdIns with PtdCho, and thereby may transport PtdCho back from the Golgi to the ER, where PtdCho can inhibit CCT (cholinephosphotransferase (CPT)-phosphocholine cytidylyltransferase) and consequently the cytidine diphosphate (CDP)-choline pathway[103]. PtdIns is phosphorylated by Golgi-localized PtdIns-4-phosphate (PtdIns4) kinase (PI4K) to produce PtdIns4P, which facilitates the recruitment of several membrane-trafficking regulatory proteins, as well as CERT, OSBP and FAPP2 (also known as PLEKHA8) through their pleckstrin homology (PH) domains[14,44,62]. Glycosphingolipids (GSLs) are produced at the lumenal leaflet of the *trans*-Golgi from glucosylceramide (GlcCer), which is synthesized from ceramide by GlcCer synthase. GlcCer is transported by FAPP2, which is essential for glycosphingolipid (GSL) production[113,114]. However, the mechanism by which FAPP2 transfers GlcCer and consequently leads to the production of glycosphingolipids, as well as the localization of GlcCer synthase at the *cis*- or *trans*-Golgi, are currently subjects of controversy[113,114]. For simplicity, only the *trans*-Golgi is shown here.

either by presenting a lipid to a second protein or by preventing a lipid-binding protein from accessing a lipid in a membrane. Finally, an LTP might help to establish transient changes in the distribution of lipids in a membrane by extracting or delivering a lipid to a particular region of the membrane, or by affecting the lipid phase in the portion of a membrane to which it is bound. These modes of action are not mutually exclusive, and it seems possible that an LTP could employ more than one of them.

Steroidogenesis
The biosynthesis of steroid hormones.

LTPs can mediate intracellular lipid transport. The transport of ceramide from its synthesis site at the ER to the Golgi complex by CERT is an example of LTP-mediated vectorial lipid transport in mammalian cells. CERT was discovered as a protein that can restore sphingomyelin synthesis in LY-A cells, a mutant Chinese hamster ovary (CHO) cell line[81]. The reduced level of sphingomyelin in LY-A cells results from a defect in ceramide transport from the ER to the Golgi owing to a point mutation in CERT (Gly67Glu) that abrogates its PtdIns4P binding and consequently its Golgi targeting. This finding suggests that CERT does not mediate a simple vectorial transport of ceramide from the ER to the Golgi. Instead, it transfers ceramide on the basis of the PtdIns4P content of the Golgi membranes.

The transport of ceramide from the ER to the Golgi by CERT, most likely at ER–Golgi MCSs, not only provides a major route for intracellular ceramide trafficking, but also initiates a signal transduction cascade that eventually regulates Golgi-mediated membrane trafficking (FIG. 5). Ceramide is converted into sphingomyelin at the lumenal leaflet of the *trans*-Golgi by SMS1, which transfers phosphocholine from PtdCho to ceramide, yielding diacylglycerol as a by-product[89] (BOX 1). Diacylglycerol activates several Golgi-localized protein kinases, including protein kinase D (PKD), which in turn regulates vesicular fission and consequently protein transport from the Golgi to the plasma membrane[90]. In addition, PKD phosphorylates PtdIns 4-kinase IIIβ (PI4KIIIβ) and stimulates its lipid-kinase activity, thereby enhancing the production of PtdIns4P in the Golgi complex[91]. PtdIns4P can recruit numerous proteins to the Golgi complex; some, such as the adaptor protein 1 complex (AP1 complex)[92], are directly involved in the regulation of vesicular transport. Remarkably, it has been shown that CERT regulates PKD activation and is essential for protein transport from the Golgi complex. Furthermore, activated PKD phosphorylates CERT, leading to its dissociation from the Golgi and thereby attenuating ER-to-Golgi ceramide transport[93]. This phosphorylation could provide a feedback mechanism for regulating ceramide transport to the Golgi and, consequently, for the production of diacylglycerol by SMS. This example shows how lipid transfer mediated by an LTP can induce a network of lipid modifications in a specific membrane compartment, consequently regulating other cellular processes, such as membrane transport (FIG. 5).

Another example of an LTP that could mediate lipid transport in cells has emerged from studies of retinal degeneration B (RDGB) in *Drosophila melanogaster*. RDGB, a PtdIns-transfer domain-containing protein related to NIR2, is required for photoreceptor cell viability and light response[94,95]. Phototransduction in *D. melanogaster* is a light-induced G protein-coupled receptor cascade mediated by phospholipase C (PLC) activation, which in turn catalyses the hydrolysis of PtdIns(4,5)P_2 to generate inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3) and diacylglycerol. RDGB-mutant flies exhibit light-induced PtdIns(4,5)P_2 depletion[96], and rescue experiments suggest that the PtdIns-transfer domain of RDGB is crucial for all of RDGB's essential functions[97].

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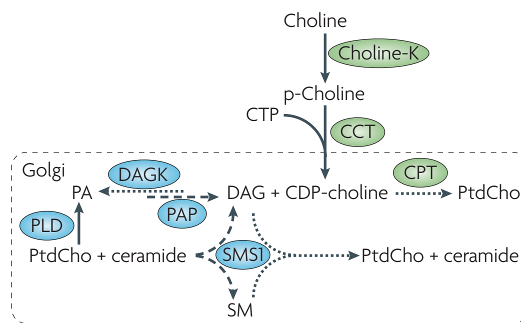
Box 1 | **Homeostasis of diacylglycerol in the Golgi**

The steady-state level of diacylglycerol (DAG) in the Golgi complex is determined by several metabolic pathways, which regulate its production (dashed arrows) and consumption (dotted arrows)[26,89,105]. Diacylglycerol is mainly produced by sphingomyelin (SM) synthase 1 (SMS1), which catalyses the transfer of phosphocholine from phosphatidylcholine (PtdCho) to ceramide, thereby producing diacylglycerol and sphingomyelin. Diacylglycerol is also produced by phosphatidic acid (PA) phosphatase (PAP), which dephosphorylates phosphatidic acid. Diacylglycerol consumption is predominantly mediated by the cytidine diphosphate (CDP)-choline pathway for PtdCho biosynthesis. This pathway begins in the endoplasmic reticulum, where choline is phosphorylated by choline kinase (choline-K) to phosphocholine (p-choline), which is then converted to CDP-choline by CCT (cholinephosphotransferase (CPT)–phosphocholine cytidylyltransferase); the rate-limiting enzyme of this pathway. CPT catalyses the production of PtdCho from diacylglycerol and CDP-choline. Inactivation of choline kinase, CCT or CPT in yeast can 'bypass' the requirement for Sec14 (REF. 101). The consumption of diacylglycerol is also regulated by diacylglycerol kinase (DAGK), which phosphorylates diacylglycerol to generate phosphatidic acid, or by SMS1, which converts diacylglycerol and sphingomyelin back to PtdCho and ceramide. Phosphatidic acid is produced from PtdCho by phospholipase D (PLD). The level of diacylglycerol in the Golgi is crucial for maintaining the Golgi structure and could directly affect Golgi-mediated transport events.



These observations suggest that RDGB is required for the regeneration of PtdIns(4,5)P_2 in response to light excitation. Accordingly, it was proposed that RDGB uses its PtdIns-transfer domain to transfer PtdIns from its synthesis site at the subrhabdomeric cisternae (part of the ER) to the rhabdomeric microvilli (part of the plasma membrane) in *D. melanogaster* photoreceptors. PtdIns is then phosphorylated to PtdIns(4,5)P_2, which promotes light-induced PtdIns(4,5)P_2 hydrolysis by PLC[98]. Indeed, inactivation of PLC in flies suppresses *rdgB* degeneration[94]. These RDGB-mediated PtdIns-transport events may also operate at MCSs (ER–plasma membrane), as RDGB is localized to the subrhabdomeric cisternae and is also associated with the base of the rhabdomeric microvilli[98]. Furthermore, RDGB contains an FFAT motif[79] and could possibly interact with ER-localized VAP proteins, which are found in many MCSs[12,75,80,82].

LTPs act as lipid sensors and/or lipid-presenting proteins. The function of LTPs as lipid sensors has been proposed by numerous studies. Perhaps the most established example is Sec14, the main PITP in *Saccharomyces cerevisiae*. Similarly to other PITPs, Sec14 facilitates the monomeric exchange of either PtdIns or PtdCho between membranes *in vitro*[49,99]. Sec14 is required for protein transport from a late Golgi compartment and is essential for yeast cell viability[100]. This requirement, however, can be bypassed by the inactivation of one of seven different genes[101,102]. Three of these genes encode structural enzymes of the cytidine diphosphate (CDP)-choline pathway for PtdCho biosynthesis, which consumes diacylglycerol to produce PtdCho[101] (BOX 1). In its PtdCho-bound form, Sec14 negatively regulates CCT (cholinephosphotransferase (CTP)–phosphocholine cytidylyltransferase), the rate-limiting enzyme of the CDP-choline pathway[103]. These observations, along with the low PtdIns and high PtdCho levels in the Golgi of *sec14*-mutant strains, suggest that Sec14 does not modulate Golgi secretory function through its PtdIns–PtdCho exchange activity, but instead through its direct effect on the CDP-choline pathway and therefore diacylglycerol consumption[103,104]. Thus, Sec14 was proposed to sense the PtdCho levels in yeast Golgi and to respond to increased PtdCho levels by inhibiting the activity of CCT. Hence, Sec14 is required to maintain a critical pool of diacylglycerol in the Golgi by regulating its consumption by the CDP-choline pathway, a process that is essential for Golgi-mediated trafficking. Remarkably, mammalian NIR2 uses a similar mechanism to regulate the levels of diacylglycerol in the Golgi complex[105]. In both cases, however, the PtdCho-exchange activity must be coupled to changes in phosphoinositides.

It was recently proposed that as PtdCho levels increase, Sec14 is activated for heterotypic PtdIns–PtdCho exchange, which in turn stimulates the production of PtdIns4P by PtdIns4-kinase (Pik1) in the yeast Golgi complex[106]. In heterotypic reactions that exchange PtdIns for PtdCho, an invading PtdCho may force PtdIns out of the Sec14 lipid-binding pocket, thereby generating a kinase-susceptible PtdIns intermediate; in this case, Sec14 acts as a lipid-presenting protein, as it presents PtdIns to the kinase[106]. Therefore, Sec14 may act both as a PtdCho sensor and as a PtdIns-presenting protein, which transmits PtdCho metabolic information to PtdInsP synthesis[106]. This example shows that a single LTP may have more than one mode of action in cells.

Members of the OSBP, ORP and OSH family may also function as sterol sensors[22]. ORP1L, for example, has been implicated in the sensing of cholesterol levels in late endosomes, and thereby in regulating their subcellular distribution. Specifically, by undergoing conformational changes in response to cholesterol content in late endosomes, ORP1L can mediate the assembly of a protein complex that may or may not be linked to the microtubule cytoskeleton. This causes late endosomes to cluster towards the microtubule minus end at high cholesterol levels and scatter at low cholesterol levels[75]. This example shows how an LTP can regulate protein–protein interactions according to its membrane environment. A related example is associated with the production of a protein complex between OSBP and two phosphatases, which dephosphorylate extracellular signal-regulated kinase (ERK)[107]. This complex disassembles at low cholesterol levels, losing its ability to dephosphorylate ERK, and consequently leads to an increase in phosphorylated ERK levels. It was, therefore, proposed that OSBP functions as a cholesterol sensor that regulates mitogenic signals in response to cellular levels of cholesterol through the assembly of a protein complex consisting of ERK phosphatases[107].

AP1 complex
A heterotetrameric complex with a role in protein sorting at the *trans*-Golgi network and endosomes. AP1 mediates the recruitment of clathrin to membranes and the recognition of sorting signals in the cytosolic tails of transmembrane cargo proteins.

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OSH proteins also respond to their membrane environment, but in this case they modulate the sterol content of membranes according to changes in phosphoinositide levels. It was shown that inhibition of PtdInsP biosynthesis markedly attenuates plasma membrane–ER sterol transport[88] and that OSH proteins bind phosphoinositides[61,108,109]. Consequently, OSH proteins may not mediate the simple vectorial transport of sterol from one membrane to another. Instead, they might dynamically modulate the sterol content of membranes that can recruit them; that is, membranes with specific lipid composition (for example, PtdInsP content) and/or biophysical properties (for example, curvature)[17]. This mode of action shows how an LTP uses its lipid-transfer activity to coordinate the intracellular distribution of different lipid species between different cellular membranes.

Concluding remarks

The mechanisms by which lipids are transported and distributed between and in cellular membranes have not been fully explored. The transport of several cellular lipids, such as sterols and ceramide, is mainly mediated by a non-vesicular transport mechanism. This mode of transport could potentially occur by spontaneous lipid exchange or by the action of LTPs. Spontaneous lipid exchange is a slow process and is therefore insufficient to support large lipid fluxes. However, it could be greatly facilitated by high membrane concentrations, suggesting that MCSs could be optimal sites for spontaneous lipid exchange. MCSs could also be ideal for the lipid transfer activity of LTPs, and indeed, many LTPs have been found in MCSs[12,70,74,75]. Whether MCSs represent the predominant sites of lipid transport in intact cells is unclear and difficult to show experimentally. It is also not known whether LTPs function exclusively at MCSs. Elucidating the mechanisms by which LTPs interact with specific membranes, identifying targeting determinants that could bind different membrane compartments and characterizing the subcellular localization of LTPs using ultrastructural analysis and live-cell imaging, could deepen our understanding of LTP function.

Regardless of their sites of action, it seems that LTPs do not mediate a simple vectorial lipid transport from one membrane to another. Instead, LTPs use their lipid-transfer activity to modulate the lipid content of membranes according to their local membrane environment. LTPs can, therefore, control lipid homeostasis and the diverse cellular processes associated with it, such as signal transduction, membrane trafficking and lipid metabolism, by capturing and responding to local membrane modifications. This ability enables a tight coordination of various cell responses that occur in the same or even different membranes. For example, the production of diacylglycerol in the Golgi is coordinately regulated by its consumption by the CDP-choline pathway. This pathway, however, is regulated by CCT, which resides on the ER (BOX 1). This coordination could be regulated by PITPs, such as NIR2, that may transfer PtdIns from the ER to the Golgi and PtdCho from the Golgi to the ER at the ER–Golgi MCSs (FIG. 5).

The ability of LTPs to coordinate and integrate different lipid-mediated cellular responses could be even more pronounced in large LTPs consisting of multiple structural domains. Although most have not been studied extensively, many large LTPs contain structural domains with cellular functions or enzymatic activities that are well established. Domains that regulate the activity of the Rho, Rac and/or Ras small GTPases have been identified in the Sec14 and START families[21]. These small GTPases are involved in the regulation of cytoskeletal remodelling and cell proliferation, both of which are tightly regulated by certain lipids. Thus, the spatial proximity between the LTD and these domains could provide a mechanism for coupling their corresponding functions, thereby enhancing the efficiency of their integrated physiological role. The coupling of lipid modifications to cytoskeletal remodelling events, for example, operates in diverse cellular processes such as membrane trafficking, cell migration and signal transduction. Thus, characterizing the functions of large LTPs and elucidating their mode of action and regulation is expected to shed light on some of the unsolved mysteries associated with this class of protein.

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Competing interests statement

The author declares no competing financial interests.

DATABASES

Protein Data Bank: http://www.pdb.org
1KCM | 1T27 | 1ZHX | 2E3P

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Sima Lev's homepage: http://www.weizmann.ac.il/mcb/Lev/

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