Phospholipid subcellular localization and dynamics

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Membrane biology seeks to understand how lipids and proteins within bilayers assemble into large structures such as organelles and the plasma membranes. Historically, lipids were thought to merely provide structural support for bilayer formation and membrane protein function. Research has now revealed that phospholipid metabolism regulates nearly all cellular processes. Sophisticated techniques helped identify >10,000 lipid species suggesting that lipids support many biological processes. Here, we highlight the synthesis of the most abundant glycerophospholipid classes and their distribution in organelles. We review vesicular and nonvesicular transport pathways shuttling lipids between organelles and discuss lipid regulators of membrane trafficking and second messengers in eukaryotic cells.

Lipid components of eukaryotic membranes

Glycerophospholipids (GPL),[5] sphingolipids, and sterols are the three major classes of lipids found in eukaryotic membranes. This review will focus primarily on the GPLs, including the biophysical nature of these molecules, biosynthetic pathways and the role of lipases in vesicular transport pathways, and the generation of critical signaling molecules. Additionally, we will highlight the distribution of GPLs between various organelles, their transbilayer distribution, and the role of nonvesicular transport pathways to shuttle lipids between organelles.

Glycerophospholipids

Phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylserine (PtdSer), and phosphatidylinositol (PtdIns) make up the majority of the glycerol backbone-containing phospholipids and are prominent components of most organellar membranes (Fig. 1) (1). Because of their amphiphilic nature, GPLs are energetically favored to self-assemble to form a continuous bilayer with the headgroups facing outward and the hydrophobic tail lining the interior (2). PtdCho is the most abundant phospholipid in the majority of organelles, ranging from 41 to 57 mol % of the total GPL (Fig. 1). The cylindrical shape of PtdCho allows it to spontaneously organize into planar bilayers, and its propensity to contain at least one unsaturated fatty acyl chain means that the bilayers possess significant fluidity at 37 °C (3). PtdEtn is the second most abundant phospholipid in eukaryotic membranes, which comprises 17–38 mol % of total phospholipid (Fig. 1). PtdEtn differs from PtdCho by the absence of the three methyl groups compared with the choline moiety (4). As a result, PtdEtn has a smaller headgroup that results in a more conical shape compared with PtdCho. Additionally, PtdEtn can arrange as a hexagonal phase, unlike most other GPLs (5). The addition of the nonbilayer forming PtdEtn to PtdCho facilitates the generation of spontaneous curvature that in the context of the cell is vital for membrane bending and tubulation that is necessary to support the fission and fusion steps in vesicular transport (6). In mammalian cells, PtdEtn and PtdCho also serve as substrates for the production of PtdSer via enzyme-mediated base-exchange reactions (7).

PtdSer is, by comparison, a relatively minor component of eukaryotic membranes (comprising 1–6 mol % of total phospholipids) (Fig. 1), and it plays a crucial role in providing a negative surface charge to membranes due to the acidic nature of its headgroup (8). PtdSer is enriched in the inner leaflet of the plasma membrane despite the fact that it is synthesized in the ER and consumed in the mitochondria (4). A significant fraction of the newly synthesized PtdSer can be transported from the ER to the mitochondria (28). There it serves as a substrate to produce a mitochondrial pool of PtdEtn that is essential for mitochondrial function (29, 30). The mechanism by which Ptd-

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[5] The abbreviations used are: GPL, glycerophospholipid; PIP, phosphoinositide; CPT, choline phosphotransferase; CEPT, choline/ethanolamine phosphotransferase; ER, endoplasmic reticulum; DAG, diacylglycerol; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; PtdGly, phosphatidylglycerol; PA, phosphatidic acid; CL, cardiolipin; PM, plasma membrane; TMEM, transmembrane protein; LTP, lipid-transport protein; OSBP, oxysterol-binding protein; ORP, OSBP-related protein; PITP, PtdIns-transfer protein; E-Syts, ER-tethered extended synaptojanin; MCS, membrane contact site; PLA, phospholipase A; AA, arachidonic acid; PAF, platelet-activating factor; GPCR, G-protein–coupled receptor; PLC, phospholipase C; IP_3, inositol 1,4,5-triphosphate; SM, sphingomyelin; CTP, cytidine triphosphate; TGN, *trans*-Golgi network; LPA, lysophosphatidic acid; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PLD, phospholipase D; PIP, phosphoinositide; PtdIns(3,4,5)P_3, phosphatidylinositol 3,4,5-trisphosphate.

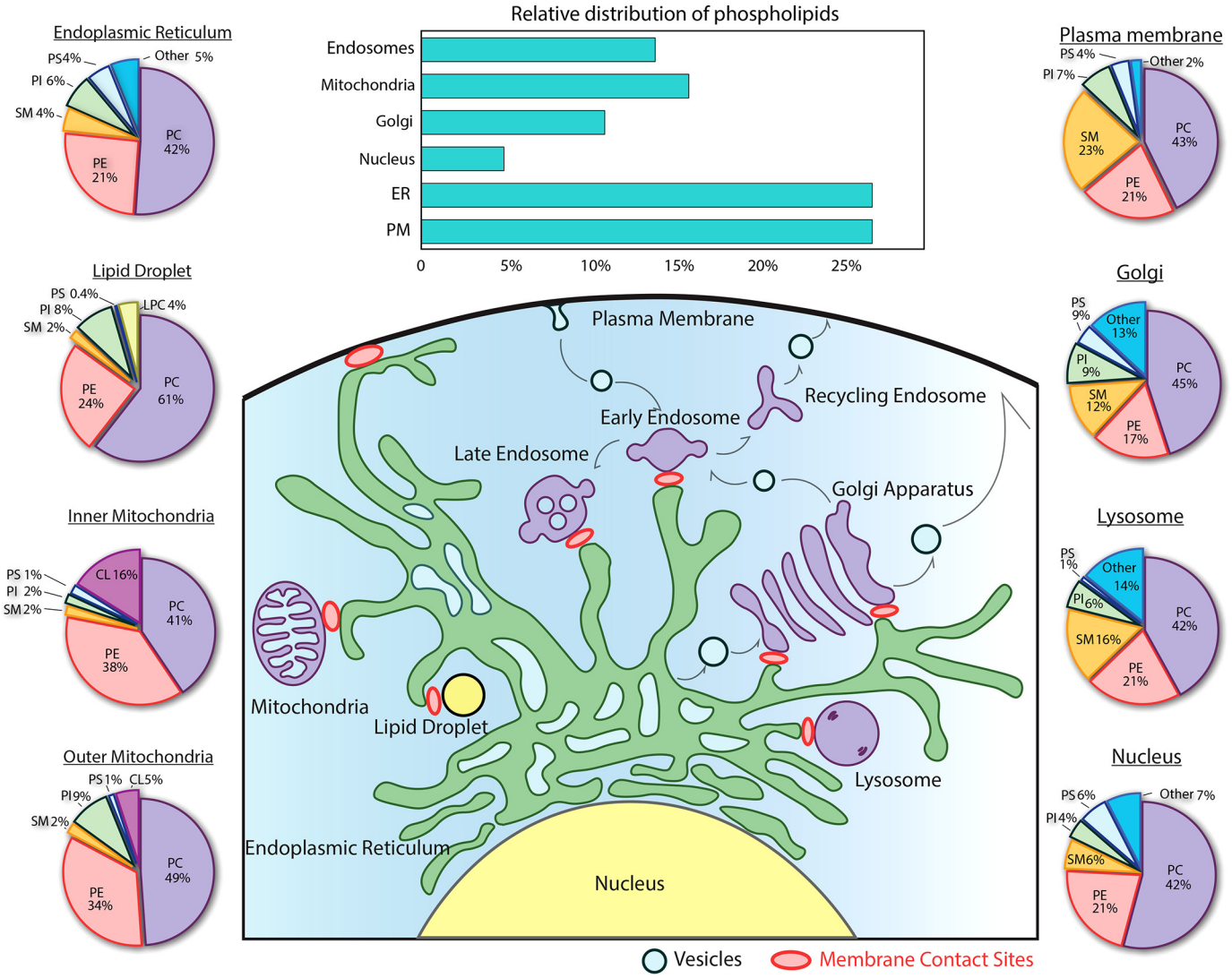

Figure 1. Glycerophospholipid composition of organelles. The *bar graph* highlights the subcellular distribution of glycerophospholipids (GPLs) between the different organelles in baby hamster kidney cells (128). The *pie charts* display the relative abundance of each class of GPL in organelles based on composite data from rat hepatocytes (1) and for lipid droplets from murine hepatocytes (129). Except for the mitochondrial PtdGly (*PG*) and CL, the other GPLs are present in all organelle membranes but display heterogeneity. PtdCho (*PC*) is the most abundant, comprising 45–55 mol % of the GPL in the cell. PtdEtn (*PE*) is the second most abundant GPL and is mainly enriched in inner membranes of mitochondria (~35–40 mol %), although it is less prominent in other organelles (~17–25 mol %). PtdSer (*PS*) is a precursor for the mitochondrial PE, and as a result of repaid consumption, its abundance is very low in the inner mitochondrial membranes (1 mol %). PS is also a minor component of lysosomes (~1 mol %), the endoplasmic reticulum (~4 mol %), nucleus (~6 mol %), Golgi (~4 mol %), and plasma membrane (~4%). Post-Golgi apparatus organelles are enriched in sphingomyelin (*SM*) with it being most abundant (~23%) in the PM. The designation *Other* includes essential precursors and signaling lipids such as PA, DAG, and lysolipids. The *schematic* highlights the vesicular and nonvesicular pathways responsible for the intracellular trafficking of lipids. The endoplasmic reticulum (*ER*) is the principal site of synthesis for most lipid species. The extensively branched reticular network of the ER facilitates the establishment of MCS with other organelles, including the Golgi apparatus, mitochondria, endosomes, lysosomes, lipid droplets, and plasma membrane. These MCS bring donor and acceptor membranes in proximity (~30 nm) where the exchange of GPLs and cholesterol can occur. Most organelles are interconnected via the vesicular transport pathways. GPLs are essential for the formation of vesicles that transport transmembrane and lumenal proteins throughout the cell. Thus, a by-product of vesicular transport is the movement of GPLs and other lipids.

Ser is accumulated in the PM and enriched in the inner leaflet of the PM is discussed below.

Similarly, PtdIns is also negatively charged, comprising 2–9% of total phospholipids of organelle membranes (Fig. 1). However, its arguably more important feature is the fact that the inositol ring can be phosphorylated on the 3-, 4-, and 5-OH groups to produce phosphoinositides (discussed below). Furthermore, PtdIns is also used to covalently link peripheral proteins to the outer leaflet of the plasma membrane. Collectively, these proteins are referred to as glycosylphosphatidylinositol-linked proteins (9). Thus, in addition to being critical for the structure of biological membranes, GPLs also play critical roles in protein targeting and signaling.

Other GPLs, such as phosphatidic acid (PA), phosphatidylglycerol (PtdGly), and cardiolipin (CL), typically constitute a relatively minor portion of the total cellular GPLs. PA comprises a relatively minor 1 mol % of total phospholipids of organelle membranes but is a critical intermediate in biosynthetic pathways and as a signaling molecule (Fig. 2). PtdGly is predominantly found in the mitochondria where it is an intermediate in the biosynthesis of CL. However, PtdGly synthase activity has been found in the ER (10), although there is the

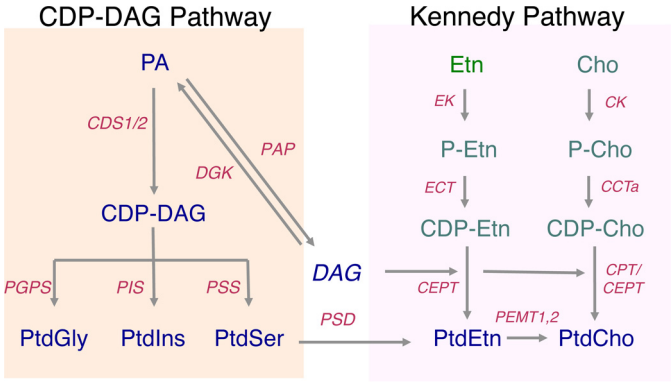

Figure 2. CDP-DAG and Kennedy pathways. The CDP-DAG pathway begins with the consumption of phosphatidate (*PA*) on the cytosolic leaflet of the endoplasmic reticulum (*ER*) by either CDP-DAG synthase (*CDS*) 1 or 2 to produce cytidine diphosphate diacylglycerol (*CDP-DAG*). The CDP-DAG is then used as a substrate by the phosphatidylinositol synthase enzyme (*PIS*) to catalyze the production of phosphatidylinositol (*PtdIns*) from CDP-DAG. CDP-DAG is also used in the mitochondria by the phosphatidylglycerophosphate synthase (*PGPS*) to produce phosphatidylglycerol (PtdGly)-phosphate, which is, in turn, dephosphorylated to produce PtdGly. Phosphatidylserine (*PtdSer*) synthesis is catalyzed by PtdSer synthase 1 (*PSS1*). A significant fraction of the newly synthesized PtdSer is transported from the ER to the mitochondria. There it serves as a substrate for the enzyme PtdSer decarboxylase (*PSD*) to produce a mitochondrial pool of phosphatidylethanolamine (*PtdEtn*). In the Kennedy pathway, choline (*Cho*) and ethanolamine (*Etn*) are first activated for phosphorylation by choline kinase (*CK*) and ethanolamine kinase (*ETNK*), respectively. Next, the phosphobase serves as substrates for the rate-limiting step of the pathway catalyzed by CTP:phosphocholine cytidyltransferase (*CCT*) and CTP:phosphoethanolamine cytidyltransferase (*ECT*), respectively, yielding CDP-Cho and CDP-Etn. The final step of the pathway is catalyzed by two homologous proteins, the phosphatidylcholine (PtdCho)-specific CPT and the PtdCho/PtdEtn producing CEPT. Finally, PtdEtn can be converted to PtdCho by three successive methylation reactions catalyzed by PtdEtn methyltransferases, PEMT1 and -2, in the liver of mammals. *PAP*, phosphatidate phosphatase.

possibility that this is due to the contamination of mitochondrial membranes during the cellular fractionation. CL is also primarily restricted to the mitochondria where it mainly supports functions into the mitochondria, including protein translocation, and respiratory chain function (11–13).

Sphingolipids and sterols

Sphingolipids, which consist of a serine backbone as opposed to the glycerol backbone, are critical components of the exofacial leaflet of the plasma membrane with sphingomyelin (SM) comprising 23 mol % of the total phospholipid (Fig. 1). Sphingolipids display an extensive range of headgroup size; they can possess merely a hydroxyl group, as in the case of ceramide, an intermediate-sized phosphocholine headgroup as seen in the highly abundant sphingomyelin, or the more complex sugar-modified headgroups collectively referred to as glycosphingolipids (14).

The third class of lipid molecules essential for eukaryotes is classified as steroid alcohols or simply sterols. Cholesterol is the predominant sterol found in all mammalian cells. Cholesterol acts to modulate the membrane fluidity and permeability via interacting with neighboring lipids, such as PtdCho and SM (15, 16). Because of the importance of cholesterol in cell biology and the development of atherosclerosis, many aspects of its synthesis, transport, and functions have been extensively studied.

An overview of glycerophospholipid biosynthetic pathways

The ER is the predominant site of GPL biosynthesis, including PtdCho, PtdEtn, PtdIns, and PtdSer (13), whereas the mitochondria produce two other GPLs, PtdGly and CL (14). The condensation of the glycerol backbone and two acyl chains represents the initial steps in GPL synthesis. Typically, glycerol 3-phosphate, a key intermediate in the glycolysis pathway, is dually acylated to form PA that in turn can be dephosphorylated to form diacylglycerol (DAG) (15–17). Together, DAG and PA are essential substrates for the further production of GPLs by the CDP-DAG and Kennedy pathways (Fig. 2).

The CDP-DAG pathway begins with the consumption of PA and cytidine triphosphate (CTP) on the cytosolic leaflet of the ER to produce CDP-DAG and pyrophosphate (17). The CDP-DAG is then used by a variety of enzymes such as PtdIns synthase together with *myo*-inositol to produce PtdIns (Fig. 3) (18). A recent study identified highly mobile ER-derived PtdIns synthase containing vesicles in the cytoplasm as a potential site of PtdIns synthesis (Fig. 3) (19). These small vesicles were termed PIPEROsomes for "PtdIns Producing ER-derived Organelle." The importance and regulation of the PIPEROsome are not understood, but it is hypothesized that it represents an intermediate between the ER and PM to supply the PM with PtdIns. Whether this is through direct fusion, hemifusion, or requires a PtdIns transfer protein is still unknown. Within the plasma membrane, PtdIns serves as a substrate for kinases and the generation of phosphorylated forms of PtdIns, in particular, PtdIns(4,5)P_2, a lipid that regulates a multitude of cellular processes. See below for more on the PI cycle.

The next pathway responsible for synthesizing much of the PtdCho and PtdEtn is named after Dr. Eugene Kennedy who described the pathway (Fig. 2) (20–22). The final step of the pathway is catalyzed by two homologous proteins, the PtdCho-specific choline phosphotransferase (CPT) and the PtdCho/PtdEtn-producing choline/ethanolamine phosphotransferase (CEPT). Experimental evidence suggests that CEPT resides in the ER, whereas CPT is enriched in the Golgi apparatus. The presence of CPT in the Golgi is important as it will consume DAG and thereby temper vesicular transport emanating from the Golgi (25). The Kennedy pathway both produces PtdEtn and PtdCho, whereas PtdEtn can be converted to PtdCho in yeast and in the liver of mammals via the concerted actions of two PtdEtn methyltransferases that triply methylate PtdEtn (26, 27).

Glycerophospholipid topology and topogenesis

As constituents of an amphiphilic bilayer, GPLs display lateral diffusion and rotation about their longitudinal axis (23, 24). However, the spontaneous transversal diffusion of the polar headgroups through the hydrophobic core of the bilayer is restricted due to the high-energy barrier (25). Estimates suggest that within a bilayer GPLs laterally diffuse $\sim 10^9$ faster than they spontaneously translocate (or flip-flop) between leaflets, which occurs on the order of hours per molecules (Fig. 4) (25, 26). These observations suggest that in the absence of any facilitated movement the transbilayer distribution of lipids should remain

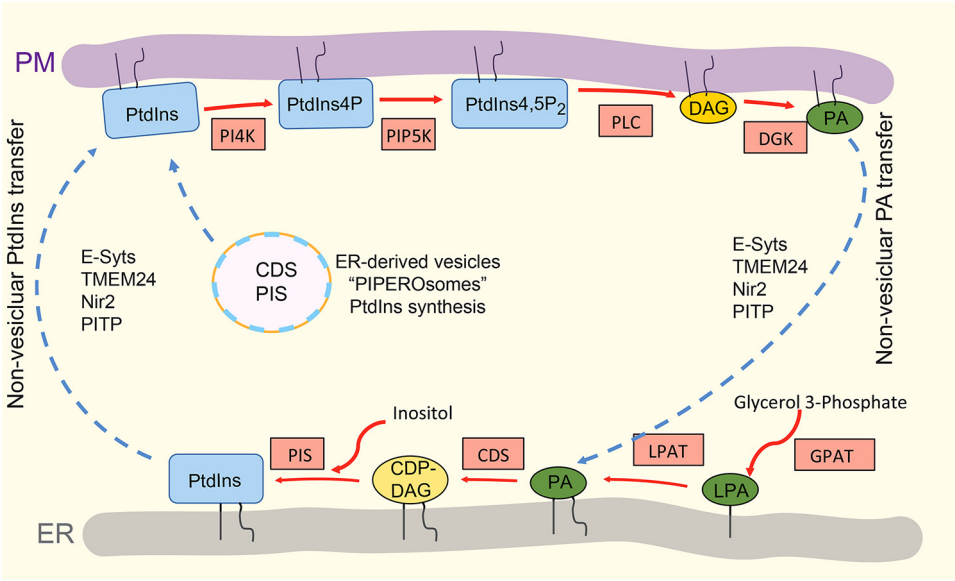

Figure 3. Phosphatidylinositol cycle. The synthesis of PtdIns occurs in the ER or possibly in ER-derived vesicles termed PIPEROsomes. First, glycerol 3-phosphate (*G3P*) is dually acylated by the actions of acyltransferases glycerol-3-phosphate *O*-acyltransferase (*GPAT*) and 1-acylglycerol-3-phosphate *O*-acyltransferase (*LPAT*), which forms lysophosphatidic acid (LPA) and produces phosphatidic acid (*PA*), respectively. Next, PA in the ER or the PIPEROsomes is converted to CDP-diacylglycerol (*CDP-DAG*) by the enzyme CDP-DAG synthases (*CDS*). Phosphatidylinositol synthase (*PIS*) in the ER of PIPEROsome catalyzes the coupling of CDP-DAG to *myo*-inositol to form PtdIns. Once synthesized in the ER or the ER-derived vesicles, PtdIns is delivered to the PM by the secretory pathway (not depicted) or by the actions of either nonselective (*TMEM24, E-Syts*) or PtdIns (*Nir2, PITP*). The PtdIns serves as a substrate for generating the plasmalemmal phosphoinositides. $PI4,5P_2$ is vital to facilitate many of the plasmalemmal transactions such as signaling in response to growth factors, exocytosis, endocytosis, and the polymerization of cortical actin. The activation of PLC isoforms converts $PI4,5P_2$ into DAG, which can then be converted back to PA by one of 10 DAG kinases. To prevent the accumulation of PA and to replenish the plasmalemmal PtdIns pool, PA is transferred back to the ER via nonvesicular lipid transport proteins. *Red arrows* represent metabolic reactions; *blue arrows* represent intracellular transport process; enzymes. *DGK,* diacylglycerol kinase.

virtually indelible once a membrane or vesicle is formed. At the cellular level for GPLs to transverse a bilayer, proteins must lower the energy barrier of the movement.

GPLs, with perhaps the exception of PtdSer (27, 28), are symmetrically distributed between both leaflets of the bilayer in the ER. Progression through the secretory pathway is accompanied by the appearance of asymmetry in the *trans*-Golgi and is apparent in the PM with PtdEtn and PtdSer being enriched in the cytosolic leaflet, and PtdCho and sphingolipids present mainly in the exofacial leaflet (29–31). The majority of the active sites responsible for the synthesis of sphingomyelin and other sphingolipids are found on the lumenal side of the Golgi (32–34), and thus, their asymmetry can be explained by a lack of flip-flop in post-Golgi membranes. The topological distribution of the GPLs is controlled by the actions of proteins referred to as scramblases, floppases, and flippases (see Fig. 4) (35). Overall, the marked asymmetry of the PM generates two monolayers that are chemically distinct. The abundance of the anionic PtdSer in the cytosolic leaflet contributes to a negative surface charge that accommodates ionic interactions of polybasic proteins with the inner layer of the PM (36). This negative surface charge can also influence the activity of integral membrane proteins by interacting with N- or C-terminal tails and cytosolic loops of these proteins. The importance of asymmetry of the PM is evident, and remarkably little is known about the topological distribution of GPLs in other organelles such as endosomes and lysosomes (37). As the cellular functions of flippases are examined in greater detail, we suspect that the role of asymmetry or at least phospholipid flipping will become more apparent. Indeed, the loss of the flippase Tat-1 in *Caenorhabditis elegans* is associated with a loss of PtdSer asymmetry in endosomal compartments and defective sorting and recycling pathways (38).

Scramblase-mediated expansion of the ER

The ER resident enzymes responsible for the synthesis of GPLs are oriented in such a manner that their active sites are exclusively facing the cytosol (39). Immediately, one should recognize that having an utterly asymmetric synthesis of GPLs would pose a problem for the cell if not dealt with rapidly: having lipid synthesis confined to one leaflet would lead to the rapid expansion of the cytosolic leaflet while the luminal leaflet remained constant. It is suggested that a phospholipid transporter known as scramblase allows for the rapid flip-flop of GPLs between leaflets of the bilayer. Collectively, scramblases facilitate the bidirectional translocation of GPLs in an energy-independent manner (40). In the case of the ER with on-going lipid synthesis, scramblase activity supports not only symmetry between the two leaflets but also to couple growth of the two leaflets (41). The protein(s) responsible for scramblase activity in the ER had remained elusive for many years, but recent results suggest that select G-protein–coupled receptors (GPCRs) might provide this activity. Two GPCRs, the apoprotein opsin and holo-rhodopsin, are proposed to equilibrate GPLs across the photoreceptor disc membranes in the retina (42, 43). Additionally, it was demonstrated that the β2-adrenergic and the adenosine A2A receptors could scramble lipids *in vitro* (42). Furthermore, molecular dynamics simulations of using opsin suggest that the headgroup of the phospholipids pass through a hydrophilic cavity created by transmembrane helices 6 and 7 (44). To date, the notion that select GPCRs could act as scramblases is based on *in vitro* reconstitution experi-

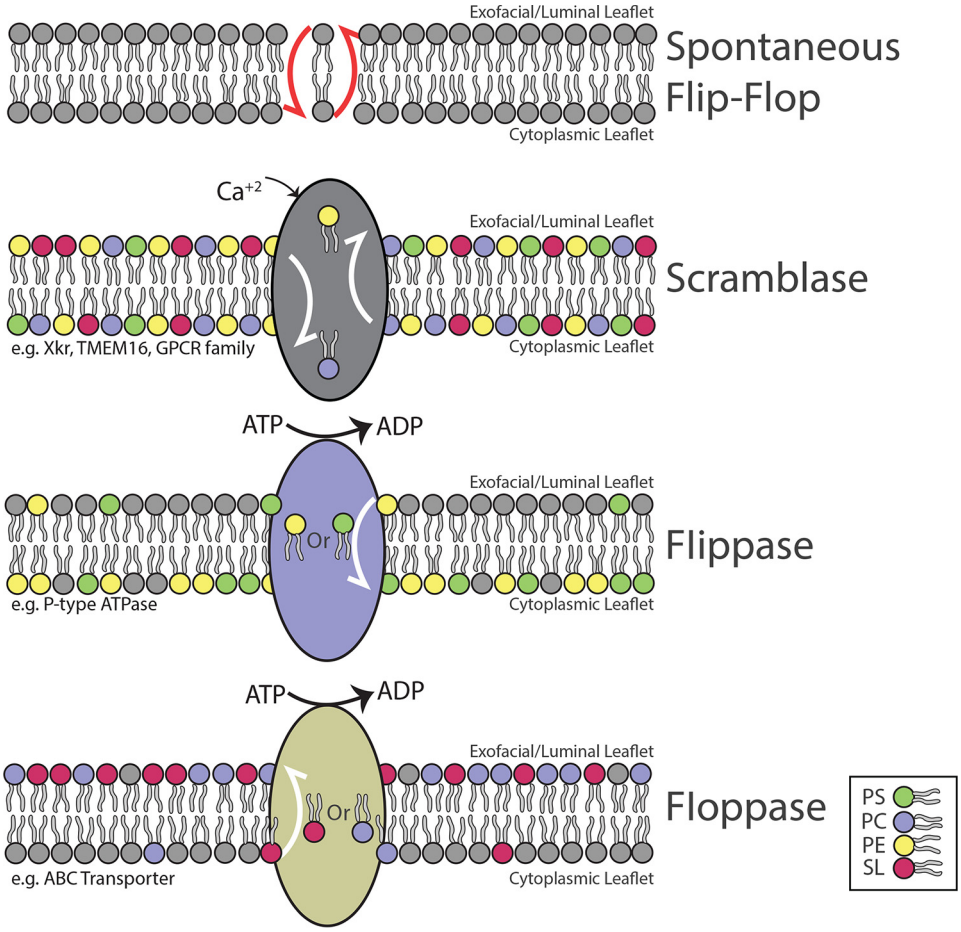

Figure 4. Phospholipid translocases in lipid bilayers. The spontaneous flip-flop of GPLs between leaflets of a bilayer is energetically unfavorable. Scramblase is the term used to describe a variety of proteins (*i.e.* TMEM16F, Xlr4, select GPCRs) that can in an energy-independent manner mediate the bidirectional transfer of GPLs between leaflets thereby collapsing the symmetry of the PM. Conversely, Flippase (inward movement) and Floppase (outward movement) are energy-dependent proteins that couple the consumption of ATP with the movement of lipids across the bilayer.

ments and has not yet been examined in living cells. The obvious question that arises from these studies is as follows: why do GPCRs act as scramblases in the ER but have no apparent activity in the PM? One possibility is that the local lipid environment influences the ability of the GPCRs to scramble phospholipids. For instance, when compared with the PM, the ER contains more unsaturated acyl chains, less cholesterol, and little sphingolipids. Additional biophysical differences exist as the ER is not as thick as the PM (45), and although the cytosolic leaflet of the PM is negatively charged, the ER is near neutral (36). Undoubtedly, this area needs further investigations to help clarify the role of GPCRs as scramblases and to determine whether a specific subset of GPCRs provide scramblase activity to the ER, during transit to other subcellular compartments, or whether another unknown ER-resident scramblase exists.

Scramblase activation and the disruption of plasmalemmal asymmetry

Constitutively active scramblases support the growth of the ER. However, the activity of plasmalemmal scramblases is often less apparent. Two classic examples of scramblase activation are present in mammalian cells. The first example is the exposure of PtdSer on the surface of platelets in response to activation that is required for blood coagulation (46, 47). The second example is the exposure of PtdSer by apoptotic cells with the exposed PtdSer serving as an "eat-me" signal for neighboring cells and macrophages (48, 49). The protein identity of these scramblase activities remained elusive for many years. However, recent seminal findings have identified the proteins responsible for these activities. The importance of scramblase activity in platelets is exemplified in Scott syndrome, a rare bleeding disorder where PtdSer fails to translocate to the extracellular leaflet (50, 51). This syndrome is due to inactivating mutations in the protein referred to as transmembrane protein (TMEM) 16 family of proteins F (TMEM16F) (52). The TMEM16 family of proteins has been characterized as calcium-gated chloride channels, but a growing body of literature suggests that TMEM16F and possibly TMEM16C, -D, -F, -G, and -J have evolved the ability to scramble phospholipids in response to elevated cytosolic calcium (53, 54). The manifestation of Scott syndrome appears to be due to the predominance of TMEM16F in platelets. The cellular roles for this family of scramblases in other cell types are less clear. However, TMEM16E has recently been shown to be necessary for the motility of mouse sperm (55).

Platelets from Scott syndrome patients still expose PtdSer in response to apoptotic signals (56). Additionally, apoptotic

platelets can expose PtdSer in the absence of calcium, consistent with the notion that another type of scramblases is present in the PM (56, 57). This second class of scramblase responsible for PtdSer exposure during apoptotic cell death was identified as XK family protein 8 (Xkr-8) (58). Additional studies using Xkr8-deficient murine cells and human cancer cell lines with low levels of Xkr-8 demonstrated that these cells fail to expose PtdSer during apoptosis (58). Importantly, in all cases, the impaired exposure of PtdSer resulted in the inefficient clearance by phagocytic cells. Expression of two related proteins Xkr-4 and -9 also rescued apoptotic scrambling in Xkr8-deficient cells (59) demonstrating that, similar to the TMEM16 family, multiple members of the Xk family possess scramblase activity. PtdSer exposure is a critical step in the execution of apoptotic cell death and clearance by phagocytes. Instead of being stimulated by cytosolic calcium, Xkr-8 is activated following proteolysis by active caspase-3 (58). This regulation ensures that PtdSer is only exposed after a sufficient level of caspase-3 activation has been reached. The exposure of PtdSer on the cell surface is further potentiated by the caspase-3–mediated cleavage of ATP11C, a PtdSer flippase (60).

Flippases and floppases, generators of membrane asymmetry

The asymmetric distribution of GPLs in the PM is a hallmark of eukaryotes. Flippases are aminophospholipid translocases that consume ATP to move PtdSer and PtdEtn from the lumenal and exofacial leaflets to the cytosolic leaflet (61). A flippase activity had initially been purified from red blood cells, chromaffin granules, and synaptic vesicles with the gene encoding the chromaffin granule resident flippase being cloned in the mid-1990s (62). Sequence comparisons determined that this gene encoded an unrecognized P-type ATPase with a homolog in yeast named Drs2. The original study found that *drs2* cells were deficient in the flipping of fluorescently-tagged NBD-PtdSer across the PM, and later studies suggest that the Drs2 functions primarily in the Golgi (63) and that its homologs, Dnf1 and Dnf2, provide the plasmalemmal flippase activity (64). P4-ATPases are absent in prokaryotes but are present as multiple members in eukaryotes. For example, the human genome contains 14 putative flippases and the yeast *Saccharomyces cerevisiae* genome encodes five. Several of the P4-ATPases form heterodimers with the noncatalytic β-subunit Cdc50 that contains two transmembrane domains and a glycosylated ectodomain (65). Although Cdc50 is noncatalytic, association with P4-ATPase is vital for export from the ER and flippase activity (66).

Conceptually, aminophospholipid flipping and the generation of membrane asymmetry are straightforward. Perhaps a less obvious by-product of flipping is the generation of membrane curvature. Studies in yeast have revealed that flippases–and likely the generation of membrane curvature– can support the formation of secretory vesicles or endocytic carriers (64, 67). In mammalian cells, the first appearance of bilayer asymmetry is found in the *trans*-Golgi cisternae (TGN) (27), supporting the notion that flippase activity could be supporting vesiculation of these membranes. Flippases have also been shown to support endocytic recycling (68), although in this compartment the identity of the substrate is less clear. Overall, mutations in the mammalian flippases have been described to be associated with a wide range of pathophysiological conditions impacting nearly every system in the body (69). This is likely due in part to the difference in the tissue distribution of the various flippases. However, it is unclear whether the defects arise from a disruption in asymmetry or impairment in vesicular transport pathway(s).

In contrast, floppases mediate the translocation of lipids in the opposite direction: from cytosolic leaflet to exofacial leaflet (70, 71). Known members of the floppase family include the ATP-binding cassette (ABC) transporter superfamily of transporters that were initially identified as multidrug-resistant pumps (71). In contrast to the somewhat promiscuous drug-effluxing ABC transporters, the lipid floppases ABCA1, ABCB1, ABCG1, ABCB4, and ABCC4 have evolved more specialized function to transport sterols, PtdCho, SM, and PtdSer. For instance, ABCA1 and ABCG1 are known to be critical for the ability of macrophage to efflux cholesterol to high-density lipoprotein (72). However, in hepatocytes ABCG4 catalyzes the movement of PtdCho to the exofacial leaflet of the canalicular membrane to allow excretion of PtdCho into the bile (73).

Inter-organellar transport of lipids

As highlighted in Fig. 1, organelles possess their lipid compositions to go along with unique proteomes. However, the PM and endosomes do not possess a significant capacity to synthesize lipids, especially GPLs. Instead, these organelles acquire their GPLs from the other organelles, especially the ER. Eukaryotic cells rely on the following two modes of transport to move lipids: vesicular and nonvesicular trafficking (see Fig. 1). Vesicular transport has long been studied and is essential for the formation of secretory vesicles, endocytosis, and the inter-organellar transport of luminal and integral membrane proteins. For instance, COPII vesicles assemble at ER exit sites and contain proteins destined for the Golgi apparatus, and at the TGN, vesicles destined for the PM or endosomes are formed. Vesicles, like organelles, are delineated by a membrane bilayer, and consequently, this mode of protein trafficking inevitably includes bulk lipid transport. The action of TGN resident flippases together with the segregation of cholesterol and sphingolipids supports the formation of these carriers (67, 74, 75). As shown in Fig. 1, most organelles participate in vesicular trafficking either by giving rise to or accepting vesicles. However, as the rates of cell growth and vesicular transport can vary significantly by cell type, the precise estimates of the bulk vesicular movement of lipids are lacking.

An alternative to the formation of transport vesicles is the use of soluble proteins to shuttle the hydrophobic lipids from one organelle to the other. The existence of this nonvesicular lipid transport was alluded to many years ago as lipid transport could still be observed under conditions where vesicular trafficking is inhibited (76–78). Membrane contact sites (MCS) have been implicated in this mode of lipid transport (79). These regions are ≈30 nm apart between two adjacent organelles and likely help to facilitate the inter-organellar exchange of lipids through the local enrichment of specific enzymes and proteins (80, 81). The ER typically creates MCS with other organelles, including Golgi, mitochondria, endosomes, peroxisomes, lipid droplets,

and PM. *In vitro* studies have demonstrated that spontaneous exchange of lipids between vesicles can occur, but this process is exceedingly slow and would likely have little importance within the cell (82, 83). Thus, merely bringing membranes close together would have little benefit. To deal with this challenge, cells have evolved soluble lipid-transport proteins (LTPs), which contain a hydrophobic binding pocket that can extract lipids and facilitate transport through an aqueous environment (84, 85). *In vitro*, these LTPs can extract lipid and then either reinsert them in the same "donor" liposome or transport the lipid through an aqueous buffer and deposit it in a "recipient" liposome. However, most LTPs have modest transfer rates *in vitro*, considering the vast quantities of cellular lipids such as cholesterol and PtdSer. This has raised the question whether these molecules function as simple transfer proteins or whether they have specialized context-dependent functions (86, 87).

The number of putative LTPs has grown immensely in the last 2 decades. In general, LTPs are found as multigene families with varying substrate specificities. For instance, sterol transfer is mediated by a subset of the oxysterol-binding protein (OSBP)-related proteins (ORPs) (88), and the steroidogenic acute regulatory protein (StAR)-related lipid-transfer (StART) domain proteins (89). Conversely, the transfer of PtdIns is mediated by PtdIns-transfer proteins (PITP) and Nir2 (19, 90). Recently, ORP5/8 and TMEM24 have been implicated in PtdSer and PtdIns transfer, respectively, at ER-PM MCS (Fig. 1) (91, 92). Because of their modular nature, several of the LTPs can interact with both target donor and acceptor membranes. For example, OSBP has a pleckstrin homology domain that interacts with the phosphoinositides, PtdIns4P, and active ADP-ribosylation factor (Arf)1 (93). Additionally, the presence of an FFAT (two phenylalanines in acidic tract) motif within OSBP allows binding to the ER resident integral membrane protein, vesicle-associated membrane protein-associated protein (VAP), thereby associating OSBP with ER membranes (94, 95). This dual targeting to two proximal membranes creates a narrow bridge that is thought to support the transfer of sterols from ER to Golgi. Alternatively, a few LTPs are integral proteins that contain transmembrane domains to allow them to be anchored to organelles, including the ER and late endosomes. This includes the late endosome-anchored cholesterol transfer protein STARD3, the ER tail-anchored PtdSer–PI4P exchange proteins ORP5 and ORP8, and the nonselective ER-tethered extended synaptojanins (E-Syts). For more information about LTPs and MCS sites, we refer readers to review articles 79, 85.

Roles of GPLs as second messengers and molecular beacons

Phospholipases and cell signaling

In addition to being a critical structural building block of membrane bilayers, GPLs also serve as a reservoir for lipid second messengers. Indeed, many of low abundant lipids, such as lyso-PtdCho (LPC), lyso-PA (LPA), PA, and DAG, can act as signaling molecules with a variety of targets. Examples of this include the ability of LPA to act as an autocrine or paracrine signal through activation of a specific -protein– coupled receptor (*e.g.* LPA) (96). Within the cell, DAG and PA can influence the recruitment and activation of cytosolic proteins (97, 98). Some of these lipid molecules are intermediates in the *de novo* biosynthetic pathway, whereas the actions of phospholipases can locally generate individual species.

Phospholipase is a general term used to describe an enzyme that hydrolyzes phospholipids. However, phospholipases constitute several families of enzymes with unique activities, substrate preferences, and regulation. As such, phospholipases are categorized into four major classes, termed A, B, C, and D, according to the type of reaction they catalyze (99). Phospholipase A (PLA) enzymes cleave either the *sn*-1 acyl chain (designated PLA_1) or the *sn*-2 acyl chain (PLA_2), whereas the phospholipase B enzymes cleave at both the *sn*-1 and *sn*-2 positions. The PLA_2 family is extensive and impacts numerous biological processes. The family of PLA_2 enzymes can be divided into five distinct categories, namely secreted PLA_2, cytosolic PLA_2, Ca^{2+}-independent PLA_2, platelet-activating factor acetylhydrolase, and lysosomal PLA_2s. The PLA_2 superfamily of enzymes varies in catalytic mechanism, function, localization, and structural features. As this is a Minireview with limited space, we refer readers to other reviews for greater detail on PLA_2 enzymes (100, 101). Typically, PLA_2 uses GPLs as a substrate to release polyunsaturated fatty acids, such as arachidonic acid (AA), from the *sn*-2 position. AA can be used by a variety of enzymes to produce compounds called eicosanoids, which include prostaglandins and leukotrienes (102). Eicosanoids act to participate in a wide range of physiological and pathological processes, including immune response, inflammation, sleep regulation, and pain perception, by activating specific GPCRs (103). The lysophospholipids can be used as a precursor for LPA and platelet-activating factor (PAF), which are lipid mediators. LPAs play a role in cell proliferation, survival, and migration (104); PAF is vital to the processes of inflammation (105). Many of these molecules serve as ligands for GPCRs and can serve as autocrine, paracrine, or endocrine signaling molecules.

In addition to the breaking down of GPLs and the generation of signaling molecules, concerted deacylation-reacylation reactions also remodel the acyl chains of GPLs. This process, referred to as the Lands cycle, involves the generation of lysophospholipids by the actions of PLA enzymes together with specific LPAATs (106, 107). This cycle serves critical cellular roles, including the replacement of oxidized fatty acids and helping to provide diversity in acyl chain compositions (108). Additionally, the generation and consumption of lysophospholipids by the Lands cycle has essential implications in vesiculation and transport especially within the Golgi apparatus (107). The inverted conical shape of lysophospholipids supports vesiculation of membranes and vesicle fusion (109, 110). In the Golgi apparatus, the actions of at least four PLAs enzymes contribute to the structure of the Golgi cisternae as well as fusion and fission of transport carriers (107). The actions of these enzymes are counteracted by the actions of at least one enzyme, lysophosphatidic acid acyltransferase 3, as overexpression of this protein prevents Golgi tubulation while its genetic silencing promotes fragmentation (111). How these enzymes are coordinated and regulated is still not well-understood but again

highlights the importance of lipids as components of biological membranes.

Remodeling of CL has been described to occur in the mitochondria of yeast and mammals. Improper acyl chain remodeling of CL in humans results in an inherited cardiomyopathy termed Barth syndrome (112). Newly synthesized CL in yeast contains saturated acyl chains (113) that are replaced with oleic acid, whereas hepatocytes initially synthesize tetraoleoyl-CL that is remodeled to tetralinoleoyl-CL (114). In yeast, a cardiolipin-specific phospholipase A-like enzyme designated Cld1 (115) has been characterized that works upstream of Taz1, the homolog of the human enzyme Taffazzin, which possess monolyso-CL transacylation activity and is the causative agent in Barth syndrome (116, 117). To our knowledge, the functional human equivalent of Cld1 has not been identified. Regardless, the failure to adequately remodel the acyl chain composition of CL or possibly to prevent the accumulation of monolyso-CL is associated with a variety of mitochondrial defects, including protein import, oxidative phosphorylation, and fission and fusion.

The mammalian phosphoinositide-specific phospholipase C (PLC) enzymes are classified into six isotypes (β, γ, δ, ϵ, ζ, and η) and play an essential role in signal transduction (118). Upon isoform-specific activation, PLCs act to generate the secondary messengers, inositol 1,4,5-triphosphate (IP_3) and DAG, by hydrolyzing phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) (119). The released IP_3 can then bind and activate IP_3-gated calcium channels in the ER leading to increased cytosolic calcium. This increase in cytosolic calcium can stimulate secretion via SNARE-mediated exocytosis and further signaling by conventional protein kinase C isoforms. Importantly, the DAG generated by PLCs can activate both conventional and novel isoforms of protein kinase C (as well as other proteins) that further potentiate signals (120).

Phospholipase D (PLD) is the final type of phospholipase. PtdCho is the natural substrate of the mammalian PLD1 and PLD2 (121). The hydrolysis of the phosphodiester bond of PtdCho by PLD results in the production of free choline and phosphatidic acid. PLD1 is found in a variety of subcellular compartments, including the Golgi apparatus, endosomes, and lysosomes, whereas PLD2 is found primarily at the PM. The activation of these enzymes leads to the localized production of PA. The small headgroup of PA promotes membrane curvature, and PLD activation has been reported to be required for optimal clathrin-mediated endocytosis (122, 123). Additionally, as PA is negatively charged, it could also contribute to the activation or recruitment of proteins with polybasic regions such as Rac1. Finally, a variety of proteins have been described to bind PA in a "lock-and-key" manner, including mammalian target of rapamycin (mTOR), its downstream activation of S6 kinase, and Raf1 kinase (97).

Phosphoinositides

Among GPLs, the phosphorylated derivatives of PtdIns often referred to as "phosphoinositides" or "PIPs" are especially important in signal transduction and protein targeting. As described above, the metabolism of the seven PIP species is controlled by the actions of 19 phosphoinositide kinases and 28 phosphoinositide phosphatases, for information, please see the extensive review by Balla (124). Each of the seven PIPs displays its intracellular distribution with variable overall abundance. The PIPs display a highly-relative turnover rate compared with PtdIns, which likely helps control the magnitude of the signaling transduction or the coordination of vesicular trafficking. For instance, a small fraction of the plasmalemmal PtdIns(4,5)P_2 can be converted to phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3) by the actions of PI 3-kinase. In turn, PtdIns(3,4,5)P_3 leads to the recruitment and activation of Akt to promote cell survival and protein synthesis. PtdIns(3,4,5)P_3 is also rapidly dephosphorylated at the 3- or 5-hydroxyl positions thereby terminating and in some circumstances prolonging its signaling, respectively (125, 126). Beyond their role in signal transduction, individual PIP species support many cellular functions. This includes but is not limited to the following: (i) contributing to the establishment of organelle identity; (ii) recruiting small GTPase to support actin polymerization, organelle, and vesicular transport; and (iii) serving as a ligand for cytosolic proteins thereby recruiting or regulating their function. Collectively, phosphoinositides can mediate a large variety of effects, including survival, differentiation, proliferation, migration, endocytosis, and endosomal maturation (127).

Conclusion

Lipid metabolism and transport continue to be significant and intriguing areas of biochemistry and cell biology. To date, nearly all of the enzymes involved in the synthesis and catabolism of lipids have been identified. The next phase of lipid research will build on this wealth of knowledge generated by the research community and seek to investigate the more exquisite details of the biology of lipids and the proteins that bind and transport them. How control and integration of the lipidome are achieved and its relation to the broader metabolome will also be critical for understanding health and pathophysiology.

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