Synthesis and Biosynthetic Trafficking of Membrane Lipids

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Eukaryotic cells can synthesize thousands of different lipid molecules that are incorporated into their membranes. This involves the activity of hundreds of enzymes with the task of creating lipid diversity. In addition, there are several, typically redundant, mechanisms to transport lipids from their site of synthesis to other cellular membranes. Biosynthetic lipid transport helps to ensure that each cellular compartment will have its characteristic lipid composition that supports the functions of the associated proteins. In this article, we provide an overview of the biosynthesis of the major lipid constituents of cell membranes, that is, glycerophospholipids, sphingolipids, and sterols, and discuss the mechanisms by which these newly synthesized lipids are delivered to their target membranes.

The endoplasmic reticulum (ER) is the main site for lipid synthesis. Intracellular lipid trafficking is necessary to maintain most other organelle membranes as they lack the capability to synthesize lipids de novo (van Meer et al. 2008). In their target locations, lipids may be present as structural compounds or they may undergo further biosynthetic modifications to generate different lipid species, some of which may be transported further to new destinations. Because of their hydrophobic nature, most lipids cannot be effectively transferred by free diffusion from one compartment to another and must therefore rely on active mechanisms to facilitate intercompartmental transport. In principle, three basic mechanisms can be depicted. A major form of trafficking is membrane transport that involves the budding of vesicles or tubules from a donor membrane and subsequent fusion with an acceptor membrane (Fig. 1A). The acceptor membrane may contain enzymes that modify the inserted lipids, helping to generate a lipid composition that differs from the donor membrane. In addition, cells use cytosolic carrier proteins for transferring lipids between compartments (Fig. 1B). The hydrophobic lipid binding pockets of these proteins are selective, allowing only one—or more often, a few closely related—lipid species to bind. Carrier proteins may also contain peptide determinants that target to the donor and acceptor membranes, providing compartmental specificity for transfer. Yet another possibility is the bringing of donor and acceptor
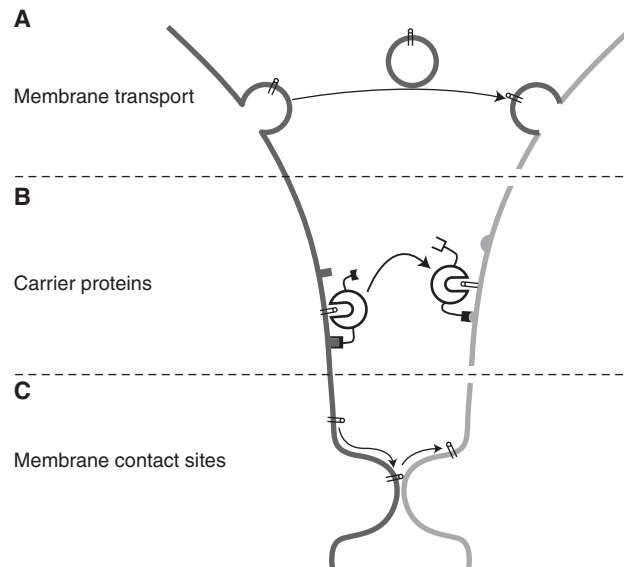
Figure 1. Mechanisms of intermembrane lipid transport. (*A*) Membrane transport moves lipids together with proteins in vesicular and tubular carriers that bud off from a donor membrane, and are transported along cytoskeletal tracks to the acceptor membrane, in which they fuse to deliver their cargo. (*B*) Cytosolic carrier proteins transfer lipids in hydrophobic pockets that show selectivity toward one or a few lipid types. Carrier proteins often contain domains that bind to the donor and/or acceptor membranes. (*C*) Lipid exchange may also occur between membranes that are in very close proximity. Transfer via such membrane contact sites may be facilitated by carrier proteins (combination of models B and C).

membranes into close proximity and transfer of lipids via membrane contact sites (Fig. 1C). In vivo, combinations of these three mechanisms are likely to operate in parallel, but their dissection is not simple.

It was postulated already in the late 1960s that lipid exchange can be facilitated by intracellular lipid transfer proteins (Wirtz and Zilversmit 1969). Following the initial observations, several proteins with lipid transfer activity have been identified and cloned. Based on their lipid binding specificity they are broadly divided into three classes, namely glycerophospholipid, sphingolipid, and sterol transfer proteins (Lev 2010). Proteins that accelerate the exchange of lipids between donor and acceptor membranes in vitro have traditionally been regarded as lipid transfer proteins. However, the in vitro lipid transfer may be a consequence of a general binding activity of a lipid sensing or chaperone domain of the protein, and is not necessarily indicative of a direct physiological role in lipid transfer. Consequently, a long-standing problem

has been the unequivocal identification of proteins that are physiologically relevant mediators of intermembrane lipid transfer. Most of the proteins with lipid transfer activity in vitro have been shown to affect lipid metabolism in vivo but apart from a few exceptions, it is at present unknown whether these effects are because of actual lipid transfer activity (Wirtz et al. 2006, D'Angelo et al. 2008).

In addition to targeting lipids to specific cellular compartments, the cell upholds a differential composition of lipids over the membrane bilayer leaflets. The ER membrane leaflets are, however, considered similar in lipid composition because of a high degree of lipid "flip-flopping" between leaflets (Kol et al. 2004; Holthuis and Levine 2005). Because of this, the decision whether a lipid will have its head group oriented toward the cytosol or the extracellular environment is largely made in post-ER membranes. Here, cells actively transport lipids to enrich specific lipid types in the cytosolic leaflet, and others in the exoplasmic leaflet.

The compartmentalization of lipids is involved in regulating cellular functions. For instance, in polarized cells membrane proteins are differentially transported to the apical versus basal plasma membrane. This protein targeting is partially dependent on lipid sorting in the Golgi complex (Simons and Ikonen 1997; Weisz and Rodriguez-Boulan 2009). Similarly, localized sphingolipid metabolism is associated with membrane budding and the formation of exosomes in multivesicular bodies (Trajkovic et al. 2008). Lipids do not only modulate the localization and function of constitutively membrane associated proteins but may also recruit soluble proteins to membranes, by serving as temporally and spatially regulated tags for lipid binding cytosolic proteins.

BIOSYNTHETIC TRAFFICKING OF GLYCEROPHOSPHOLIPIDS

Glycerophospholipids represent the most abundant class of lipids in mammalian cells. The synthesis of glycerophospholipids takes place in the ER and is initiated by acylation of glycerol 3-phosphate (or acylation and subsequent reduction of dihydroxyacetone-phosphate) to form phosphatidic acid (Fig. 2), from which all other glycerophospholipids are formed by the addition of a polar head group. The most common head group in mammalian cells is choline and the respective phospholipid class, phosphatidylcholines, makes up approximately half of the total cellular phospholipids. Phosphatidylethanolamines, -serines and -inositols are other common mammalian glycerophospholipid classes. Each of them consists of tens or hundreds of different molecular species varying in their alkyl chain composition (van Meer et al. 2008). Accordingly, a single cell may contain thousands of different phospholipid molecules. The functional significance of this complexity is only starting to be unraveled.

Phosphatidylcholine

Phosphatidylcholine (PC) is formed by the transfer of a phosphocholine group from CDP-choline to a diacylglycerol molecule.

This reaction can take place both in the ER and the Golgi apparatus. In the ER, the final step of PC synthesis is mediated by a choline/ethanolamine phosphotransferase of dual specificity, whereas a choline specific phosphotransferase catalyzes this reaction in the Golgi (Henneberry and McMaster 1999; Henneberry et al. 2000, 2002). In the ER, PC and other lipids move readily over the bilayer, but in the Golgi, such movement is more restricted (Buton et al. 1996; van Meer et al. 2008). Because PC is the major lipid in most organelle membranes, it must be carried by membrane transport. However, as PC is efficiently transferred from the ER to the plasma membrane when protein secretion is inhibited, nonvesicular mechanisms also play an important role in this transfer (Fig. 5) (Kaplan and Simoni 1985). There are several possible mechanisms of nonvesicular transport of (phospho)lipids, including spontaneous or protein-mediated diffusion and membrane contact-site dependent translocation (Fig. 1), but the relative contributions of these are not known (Voelker 2009). Potentially important phospholipid transfer proteins include StARD2, StARD7, and StARD10, which can bind a PC molecule in a hydrophobic pocket of their steroidogenic acute regulatory protein related lipid transfer (START) domain and mediate intermembrane transfer of PC in vitro. StARD2 and StARD7 bind PC only, whereas StARD10 binds phosphatidylethanolamine (PE) as well (Olayioye et al. 2005; Wirtz 2006; Kanno et al. 2007; Horibata and Sugimoto 2010; Kang et al. 2010). However, it is unclear if these proteins play a relevant role in phospholipid trafficking in vivo.

Phosphatidylethanolamine

Phosphatidylethanolamine (PE) is the second most abundant phospholipid in mammalian cells. PE is synthesized either by transfer of phosphoethanolamine from CDP-ethanolamine to diacylglycerol or by decarboxylation of phosphatidylserine (PS) (Vance 2008). The former pathway takes place in the ER and the nuclear envelope and is catalyzed either by ethanolamine phosphotransferase or choline/ethanolamine
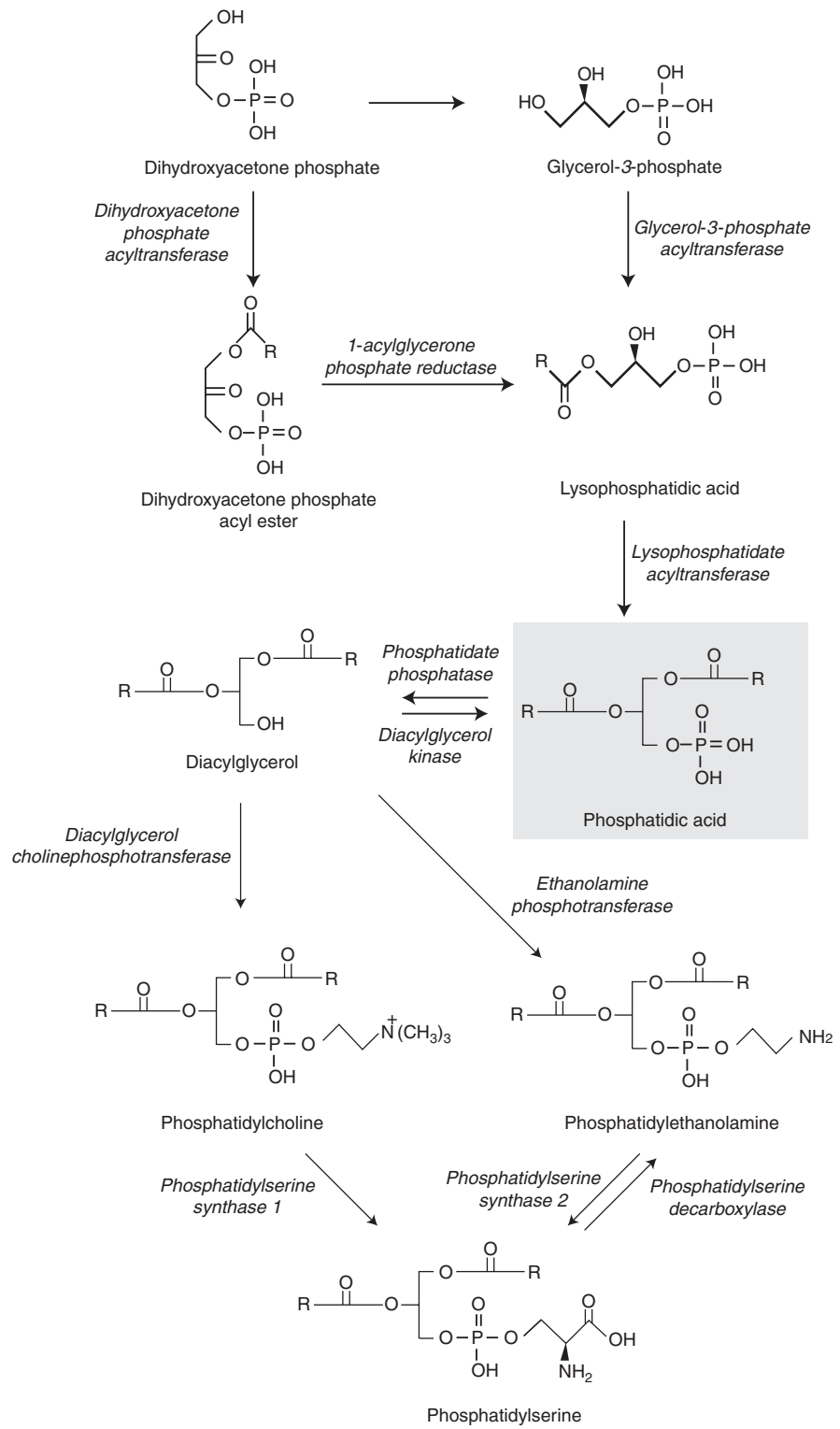
Figure 2. *See facing page for legend.*

phosphotransferase (Henneberry and McMaster 1999; Henneberry et al. 2002; Horibata and Hirabayashi 2007). The decarboxylation reaction, on the other hand, takes place in the inner mitochondrial membrane and much, if not all, of the mitochondrial PE derives from this reaction (Zborowski et al. 1983; Shiao et al. 1995; Vance 2008). Elimination of the PS decarboxylase in mice is lethal, probably because (1) PE is essential for the proper structure and function of mitochondria and (2) the PE formed in the endoplasmic reticulum via the CDP-ethanolamine pathway cannot be efficiently imported into mitochondria (Steenbergen et al. 2005). Notably, not all of the PE formed by decarboxylation of PS remains in mitochondria, but is transported to the ER and other organelles. The mechanism(s) of PE transport out of mitochondria remain to be established, but could be similar to that of PS transport from the ER to mitochondria (Achleitner et al. 1995, 1999) (see below). In the plasma membrane, most of the PE is found in the inner leaflet and this asymmetrical distribution is maintained by P-type ATPases (Riekhof and Voelker 2009).

Phosphatidylserine

In mammalian cells, PS is synthesized by exchanging the polar head group of a PC or a PE molecule for serine. The PS synthases 1 and 2 responsible for this reaction are highly enriched in mitochondria-associated ER domains (Fig. 5) (Stone and Vance 2000). From there, phosphatidylserine translocates to other organelles, such as the plasma membrane as well as to mitochondria where it is decarboxylated to PE. In the plasma membrane, PS is abundant but resides almost exclusively in the cytoplasmic leaflet. Such asymmetrical distribution of PS is maintained by an ATP-driven PS translocation (catalyzed by a yet unidentified protein) from the outer to the inner leaflet (Daleke 2007; Smriti et al. 2007). PS interacts with and regulates several important enzymes, including protein kinase C in the cytoplasmic leaflet of the plasma membrane (Nishizuka 1992). In addition, loss of the transbilayer asymmetry of PS is one of the hallmarks of apoptosis (Leventis and Grinstein 2010).

Interestingly, the transport of PS from the ER to mitochondria is a relatively slow process with a half-time of several hours (Voelker 1985; Heikinheimo and Somerharju 1998). The mechanism of this transfer process has not been resolved. It has been proposed that the close apposition of mitochondria and the ER membranes is important for the transfer, and the transfer may be mediated by specific proteins located at the ER/mitochondria contact sites (Vance 2008). However, the relatively slow transfer of PS in general as well as faster transfer of the less hydrophobic PS species from ER to mitochondria implies that spontaneous diffusion of PS monomers via the cytoplasm may also contribute to this process (Heikinheimo and Somerharju 2002). PS may move by lateral diffusion via membrane contact sites from the outer to the inner mitochondrial membrane where PS decarboxylase resides (Jasinska et al. 1993).

BIOSYNTHETIC SPHINGOLIPID TRAFFICKING

Numerous types of sphingolipid species exist in eukaryotic cells and most of them are enriched in the plasma membrane. Sphingomyelin (SM) and glycosphingolipids (GSLs) are important structural lipids that, together with cholesterol,

Figure 2. An overview of the biosynthetic pathways of major glycerophospholipids. De novo synthesis of the glycerophospholipids begins in the ER by a series of reduction and acylation reactions leading to the formation of phosphatidic acid. Dephosphorylation of phosphatidic acid yields diacylglycerol, which can be turned into phosphatidylcholine or phosphatidylethanolamine by the addition of a phosphocholine or a phosphoethanolamine head group, respectively. Phosphatidylserine is formed by exchanging the head group from either phosphatidylcholine or phosphatidylethanolamine for serine. Mitochondrial phosphatidylethanolamine is synthesized on location by decarboxylation of phosphatidylserine.

are thought to participate in the formation of membrane domains which can affect the distribution and function of membrane proteins (Coskun and Simons 2010). On the other hand, some less abundant sphingolipids, such as sphingosine, sphingosine-1-phosphate, and ceramide, are important modulators of cell signaling (Hannun and Obeid 2008). The enzymes that catalyze the different steps in the synthesis of the more complex sphingolipids (e.g., SM and GSLs) differ in their subcellular location and membrane sidedness. Thus, the synthesis of complex sphingolipids requires both interorganelle and transbilayer lipid movement.

The initial steps of sphingolipid synthesis take place on the cytosolic face of the ER (Fig. 3). First, 3-dehydrosphinganine is formed by condensation of serine and palmitoyl-CoA by the enzyme serine palmitoyl transferase (SPT). The 3-dehydrosphinganine is then reduced to sphinganine by 3-dehydrosphinganine reductase (Futerman and Riezman 2005; Sabourdy et al. 2008). Sphinganine can be N-acylated by one of the several ceramide synthases to form dihydroceramide (Lahiri and Futerman 2007) with an acyl chain of varying length and degree of unsaturation. This provides diversity in the membrane behavior of the sphingolipids eventually formed (Ewers et al. 2010; Koivusalo et al. 2007). Dihydroceramide is then converted to ceramide by a desaturase residing in the ER. Ceramide is the first lipid in the de novo synthesis pathway that contains a sphingosine backbone, and can therefore be considered a central compound in sphingolipid metabolism.

Ceramide can be further metabolized to other sphingolipids by the addition of a polar head group or by removal or exchange of the acyl chain in the recycling pathway. Most of the newly synthesized ceramide is used in the Golgi for the synthesis of SM and glycosphingolipids (Tafesse et al. 2006). In oligodendrocytes ceramide can be glycosylated in the ER to form galactosylceramide which is an important constituent of the myelin sheath (Raff et al. 1978; Stoffel and Bosio 1997; Sprong et al. 2003). In mammalian cells, the sphingoid backbone is almost invariably composed of 18 carbon units (C_{18}). Diversity—and alterations in the

membrane behavior of the sphingolipid—is achieved by linking acyl chains with varying length and degree of saturation to the sphingosine backbone. In addition to its impact on membrane fluidity, the variations in the acyl chain moiety has functional implications, such as affecting, for example, endocytosis of virus particles (Ewers et al. 2010) and the organization of myelin sheaths (Ben-David and Futerman 2010).

Sphingomyelin

The synthesis of SM, the major sphingolipid in the plasma membrane, takes place in the Golgi and requires transfer of the ceramide precursor from the ER. Ceramide can be transferred from the ER either in transport vesicles or by soluble transfer proteins. In mammalian cells the latter route dominates and is mediated by the ceramide transport protein (CERT) that takes ceramide from the cytosolic leaflet of the ER and transfers it to the *trans*-Golgi cisternae where it is converted to SM by sphingomyelin synthase 1 (SMS1) (Hanada et al. 2003, 2009; Yamaji et al. 2008). CERT is a cytosolic protein with four domains that are needed for transfer of ceramide from the ER to the Golgi. The amino-terminal pleckstrin homology (PH) domain specifically binds to phosphatidyl inositol-4-phosphate, a phospholipid that is abundant in Golgi membranes. The PH domain is crucial for targeting of CERT to the Golgi apparatus, because mutations in the PH domain can impair binding of CERT to the Golgi, thus inhibiting the synthesis of SM (Hanada et al. 2003). The START domain in the carboxyl terminus of CERT is responsible for extracting ceramide from the cytosolic leaflet of the ER. The START domain of CERT has a high specificity for ceramide as it does not transfer other sphingolipids or cholesterol in vitro (Kumagai et al. 2005). Molecular modeling studies have shown that the START domain binds one ceramide in its hydrophobic pocket, suggesting that CERT carries a single ceramide at a time from the ER to the Golgi (Kudo et al. 2008, 2010). CERT also contains a FFAT motif that mediates binding to the ER-resident protein
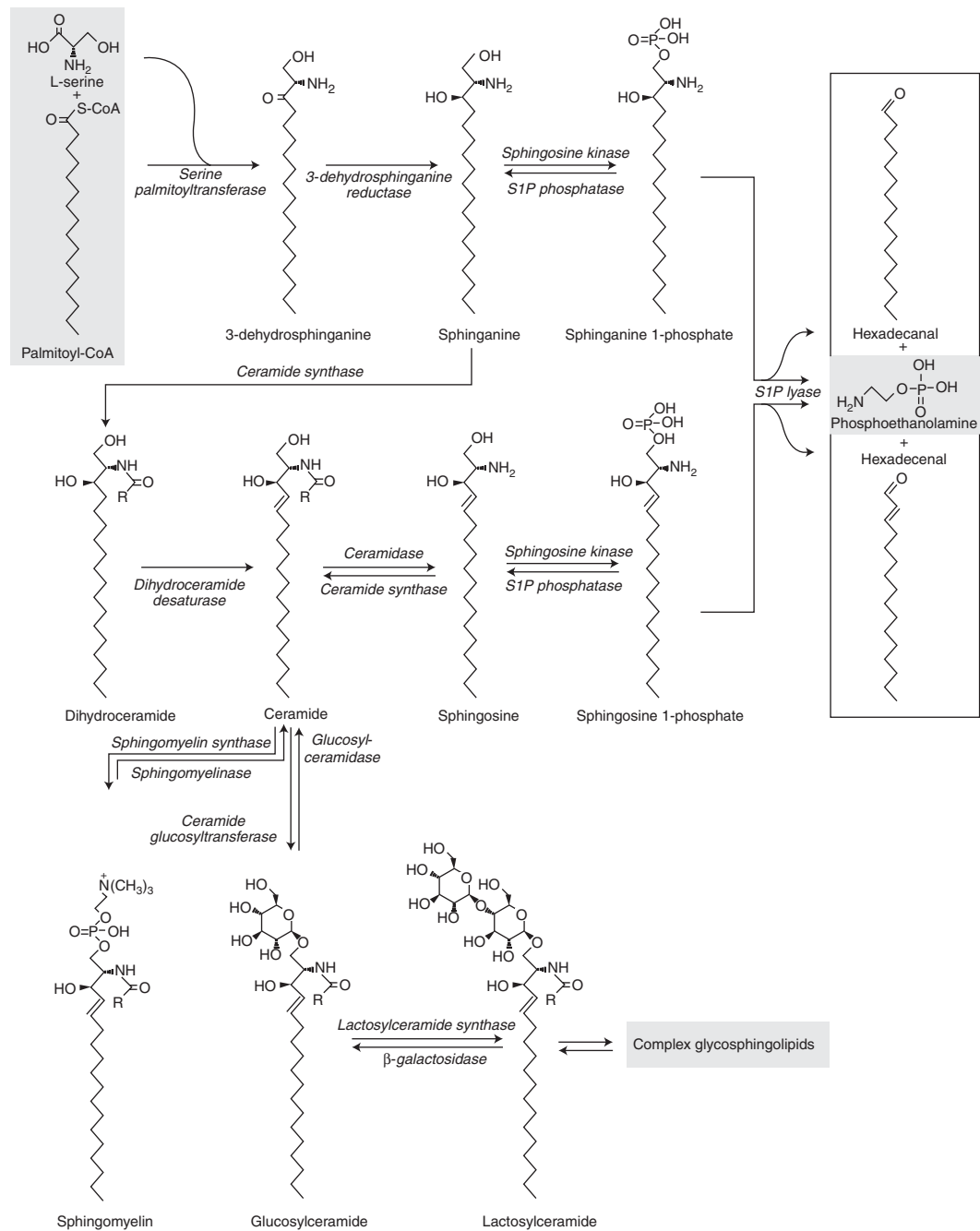
Figure 3. Overview of the biosynthetic pathways of sphingolipids. The sphingoid backbone is formed by the condensation of serine and palmitoyl-CoA. Three further synthetic steps are needed to produce ceramide, which the first compound with a bona fide sphingosine backbone. After its synthesis in the ER, ceramide can be metabolized into sphingomyelin or glycosphingolipids in the lumenal leaflet of the Golgi. The postceramide metabolic steps are reversible and ceramide can also be formed by the sequential degradation of more complex sphingolipids. Deacylation of ceramide yields sphingosine that can be phosphorylated to sphingosine-1-phosphate. The irreversible degradation of the sphingoid backbone is catalyzed by a lyase that acts on either sphingosine-1-phosphate or sphinganine-1-phosphate.

VAP and thus allows CERT to target to the ER. Next to the PH domain there is a serine repeat motif that regulates the activity of CERT. Hyper-phosphorylation of the serines in this motif impairs binding of CERT to the ER and Golgi membranes, thus inhibiting ceramide transfer from the ER to the Golgi (Yamaji et al. 2008; Hanada et al. 2009; Voelker 2009).

Glycosphingolipids

The synthesis of glycosphingolipids from ceramide takes place mainly in the Golgi apparatus. Similarly to SM, efficient de novo synthesis of glycosphingolipids is dependent on CERT (Hanada et al. 2003; Halter et al. 2007). In contrast, the synthesis of glyco-sphingolipids from sphingosine is largely independent of CERT (D'Angelo et al. 2007; Giussani et al. 2008), and thus probably relies on vesicular trafficking of ceramide from the ER to the Golgi. The synthesis of most glyco-sphingolipids begins by the addition of a glucosyl moiety to a ceramide on the cytosolic side of Golgi membranes. Glucosylceramide then needs to be translocated to the lumenal side of the Golgi to be converted to more complex glycosphingolipids. This process requires the involvement of the four-phosphate-adaptor protein 2 (FAPP2). FAPP2 contains a carboxy-terminal domain that is homologous to the gly-colipid transfer protein (GLTP) domain and binds glucosyl ceramide with high specificity. The amino-terminal region of FAPP2 contains a PH-domain that binds phosphatidyl inosi-tol-4-phosphate and ARF1, which tether the protein to the Golgi (D'Angelo et al. 2007; Halter et al. 2007; Mattjus 2009). Glucosylceramide is converted into lactosylceramide which can then be further glycosylated to more complex glycosphingolipids in the lumenal side of *trans*-Golgi membranes. Because of their large and very polar head groups, glycosphingolipids do not readily flip over the membrane and thus remain on the lumenal side. Most of the SM and GSLs synthesized move in transport vesicles from the *trans*-Golgi network to the plasma membrane where they end up in the extracellular leaflet (van Meer et al. 2008).

Sphingolipid recycling

Sphingolipids are actively metabolized and re-cycled via salvage pathways. Some of the meta-bolic steps can occur at the plasma membrane. For instance, SM can be degraded therein to ceramide by sphingomyelinases, and ceramide can be converted back to SM by the sphingo-myelin synthase 2 (SMS2) located at the plasma membrane (Milhas et al. 2010). However, most of the sphingolipid metabolism takes place on intracellular membranes. Some of the endocy-tosed sphingolipids are transported back to the plasma membrane, whereas others are targeted to lysosomes for degradation. Whether an endocytosed sphingolipid is degraded or recycled back to the plasma membrane is in part dependent on its acyl chain length, with longer chain sphingolipids preferentially targeted for degradation (Koivusalo et al. 2007).

Endocytosed sphingolipids reaching the late endosomal/lysosomal compartments are degraded to simpler sphingolipids in a stepwise manner. This process is accomplished by the concerted action of sphingolipid activator proteins (SAPs) and hydrolytic enzymes. SAPs bind to and lift sphingolipids up from the membrane and then hand over to a hydrolase. However, the acid sphingomyelinase contains an internal saposin-homology domain and can function without the assistance of saposins proper (Linke et al. 2001; Kolter and Sandhoff 2005). As endosomes mature into late endo-somes/lysosomes, their lipid composition and structure changes and multivesicular bodies are formed. The vesicles inside these bodies are enriched in an acidic lipid with an uncon-ventional stereochemistry, namely bis-(mono-acylglycero)-phosphate (BMP) (Kobayashi et al. 1999; Mobius et al. 2003). SM and the complex sphingolipids in the internal vesicles are oriented so that they face the lumen of the multivesicular bodies/lysosomes. They are thus exposed to the SAPs and acid hydrolases, which allows for their facile degradation (Kolter and Sandhoff 2010).

Eventually, GSLs and SM are degraded to their building blocks, (e.g., monosaccharides), fatty acids and sphingosine. Sphingosine can

then leave the lysosomes and is either degraded or reused for building new sphingolipids via salvage pathways (Kolter and Sandhoff 2005; Schulze et al. 2009). Sphingosine can reenter the salvage pathway by being acylated in the ER to form ceramide. The reutilization of sphingosine for the synthesis of GSLs is largely independent of CERT, suggesting that this pathway relies on vesicular trafficking of ceramide from the ER to the Golgi apparatus (D'Angelo et al. 2007; Giussani et al. 2008). Sphingosine destined for degradation must first be phosphorylated to sphingosine-1-phosphate, which is then hydrolyzed to phosphoethanolamine and hexadecenal by a sphingosine-1-phosphate lyase in the ER (Ikeda et al. 2004).

BIOSYNTHETIC TRAFFICKING OF STEROLS

Cholesterol is a major structural lipid in mammalian cell membranes and is enriched in the plasma membrane as well as intracellular membranes that actively communicate with the plasma membrane, such as recycling endosomes and the *trans*-Golgi network (Ikonen 2008). Cholesterol has a rigid four-ring structure with 17 carbon atoms, to which two methyl groups and an iso-octyl side chain are attached (Fig. 4). All nucleated cells are capable of synthesizing cholesterol by using acetate as the sole carbon source. The rate-limiting enzyme in the pathway, hydroxymethylglutaryl CoA reductase, catalyzes the synthesis of mevalonate. Six enzyme reactions then convert mevalonate to squalene. The mevalonate pathway is also used for the synthesis of other molecules, such as isoprenoids, dolichol, and ubiquinone. The first enzyme in the mevalonate pathway committed to cholesterol synthesis is squalene oxidase. This reaction requires molecular oxygen and the product, lanosterol, is the first cyclic intermediate in the pathway. The steps post lanosterol involve ~20 enzymatic reactions and there are two alternative pathways depending on whether or not there is a double bond between sterol carbons 24 and 25; the Bloch pathway is followed when the double bond is present, whereas Kandutsch-Russell pathway is followed when it is absent (Fig. 4) (Bloch 1992).

Of the individual sterol biosynthetic steps, the rate-limiting enzyme, HMG CoA reductase (which is the target of the cholesterol lowering statin drugs), has been extensively characterized. HMG CoA reductase is an ER integral membrane protein and is stringently regulated both at the transcriptional and posttranslational levels (see Ye and DeBose-Boyd 2011). Based on enzyme activity measurements, also the later steps of sterol biosynthesis take place in the ER. Sterol synthesis by microsomal enzymes is highly efficient to the extent that organization via multienzyme complex(es) have been suggested (Gaylor and Delwiche 1973). However, several of the cholesterol biosynthetic enzymes are also found in other compartments, such as peroxisomes (presqualene steps [Kovacs et al. 2007]), nucleus (Wu et al. 2004), or nuclear envelope (Zwerger et al. 2010) (sterol 24-reductase and lamin B receptor, respectively), lipid droplets (NAD(P)H steroid dehydrogenase like protein) (Ohashi et al. 2003), and the Golgi complex (Cotman et al. 2004) (lanosterol 14α-demethylase). What functions this compartmentalization of the enzymes might serve, is not known.

Although the ER is the main site of cholesterol synthesis, cholesterol concentration in the ER is low. This is because newly synthesized sterols are rapidly transported to other cellular membranes (Fig. 5). The fast transport of the intermediates may also explain the high efficiency of sterol biosynthesis despite the divergent localizations of the enzymes involved. Many of the newly synthesized postlanosterol intermediates can rapidly reach the plasma membrane. They can also be secreted out of cells to physiological (lipoprotein) or pharmacological (e.g., cyclodextrin) acceptors, or return to the ER for conversion into cholesterol (Johnson et al. 1995; Lusa et al. 2003). There is evidence that the efflux of individual precursor sterols from cells to extracellular acceptors varies, with the more polar zymosterol being more avidly effluxed than lathosterol and both exceeding that of newly synthesized cholesterol (Lange et al. 1991; Lusa et al. 2003). Indeed, circulating lathosterol levels can be used as a surrogate marker of cholesterol biosynthetic activity in humans (Gylling et al. 1989).
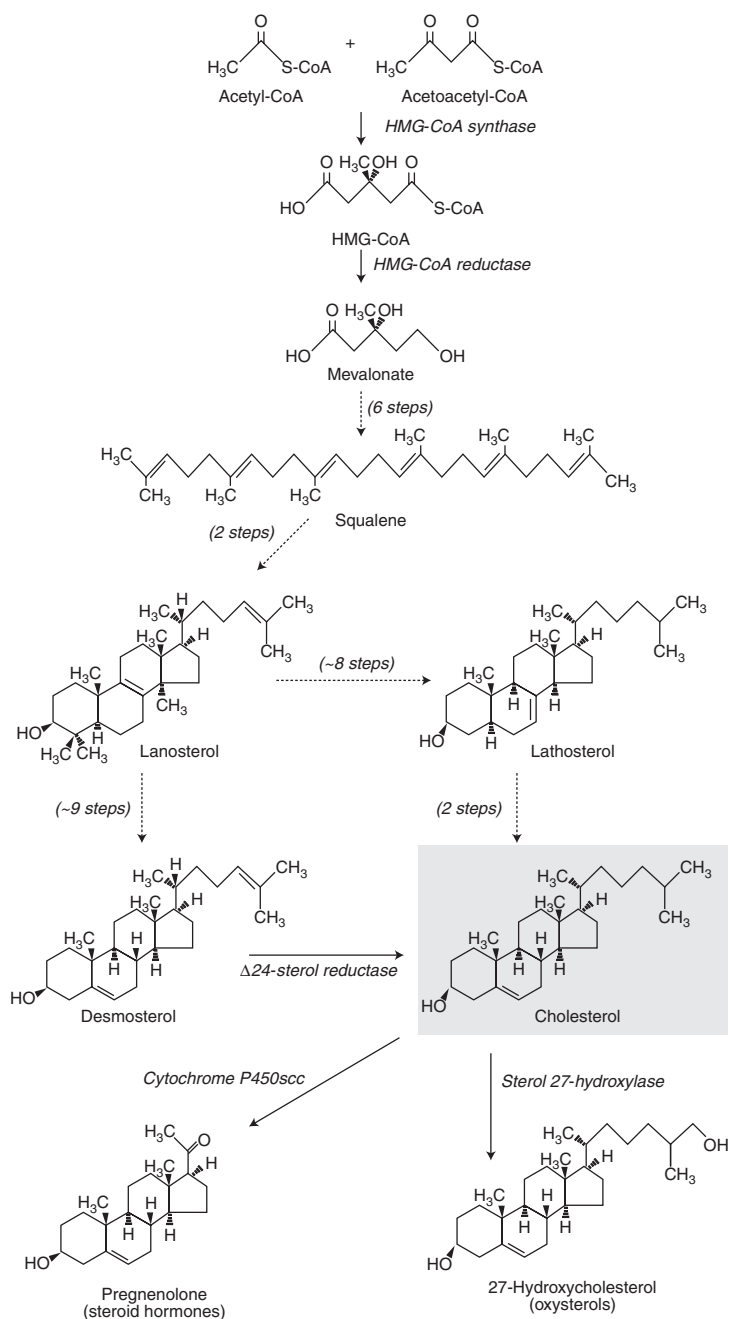
Figure 4. Overview of the biosynthetic pathways of sterols. The four-ring sterol backbone derives from reductive polymerizations of acetate to generate squalene, which is cyclized to form lanosterol, the first sterol in the pathway. The rate-limiting enzyme of cholesterol biosynthesis is HMG-CoA reductase. The postlanosterol pathway involves roughly 20 steps, with some of the enzymes capable of acting on multiple substrates. If the carbon-24 double bond is reduced early on, the pathway procedes via lathosterol (and 7-dehydrocholesterol, not shown) to cholesterol, whereas reduction of carbon-24 only in the last step results in the generation of desmosterol as the penultimate cholesterol cholesterol precursor. Cholesterol serves as a precursor for other bioactive sterols, such as steroid hormones and oxysterols.
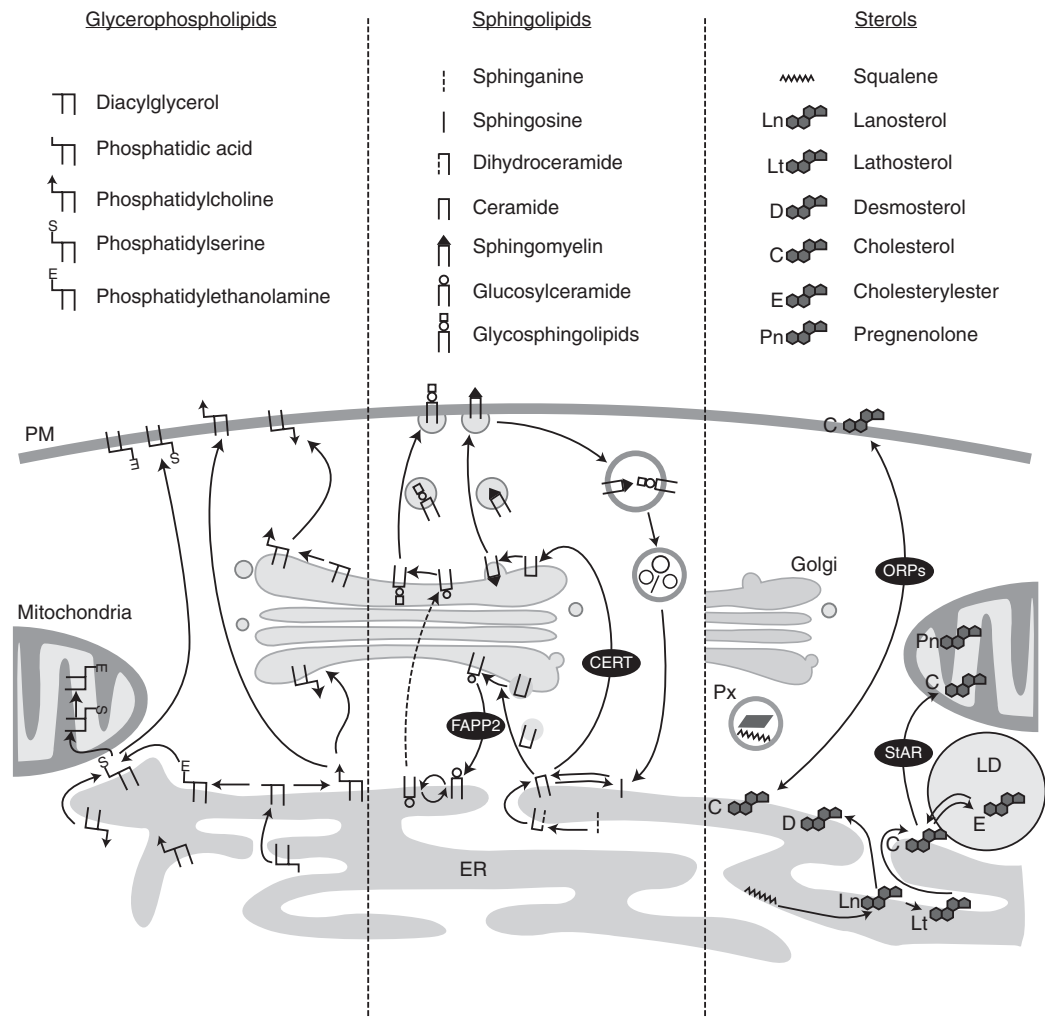
Figure 5. Biosynthetic trafficking of major membrane lipids. (*Left*) Glycerophospholipids are synthesized in the ER, with phosphatidylserine synthases enriched in mitochondria associated membrane fractions. Glycerophospholipids are transported from the ER both along exocytic membrane transport and by nonvesicular, yet poorly characterized mechanisms. (*Middle*) De novo sphingolipid synthesis is initiated in the ER. The ER to Golgi transport of ceramide for the assembly of more complex sphingolipids is mediated by CERT, and to a lesser extent by membrane transport. The post-Golgi transport of complex sphingolipids is dependent on membrane transport. Sphingosine that stems from lysosomally degraded complex sphingolipids can be acylated to form ceramide in the ER and recycled to sphingomyelin and glycosphingolipids. (*Right*) Cholesterol biosynthetic enzymes reside in the ER, with some presqualene enzymes also localized in peroxisomes (Px). Sterols are transported to the plasma membrane (PM) largely via Golgi bypass route(s) and the ORP proteins play a role in this process, as well as in the reverse transport from the plasma membrane to the ER. Sterols imported into mitochondria by StAR can be used for steroid hormone synthesis. Excess cholesterol can be esterified in the ER by acyl-CoA cholesterol acyltransferase and stored in lipid droplets (LD). Arrows indicate the direction of lipid transport. Please note that arrows do not necessarily reflect the transport distance as membranes move constantly and lipid transfer may be facilitated by close apposition of membranes. Carrier proteins are indicated by black circles.

The techniques available to study the mechanisms of biosynthetic sterol trafficking from the ER to the more sterol-enriched membranes are indirect. Typically, cells are labeled with the radioactive precursors acetate or mevalonate, chased, and the incorporation of the radiolabel into cholesterol is measured, after subcellular fractionation, lipid extraction, and chromatographic separation of the radiolabeled lipophilic compounds. A simpler method for analyzing the plasma membrane arrival of sterols is rapid extraction of sterols to the efficient extracellular acceptor, methyl β cyclodextrin. This should be performed at reduced temperature (Heino et al. 2000), because at 37°C cyclodextrin is endocytosed and no longer assesses only the plasma membrane sterol pool (Rosenbaum et al. 2010).

Most of the newly synthesized cholesterol can reach the plasma membrane by route(s) that bypass the Golgi complex as assessed by pharmacological disassembly of the Golgi complex by using Brefeldin A treatment (Urbani and Simoni 1990; Heino et al. 2000) or by genetic perturbation of ER-Golgi membrane transport in yeast (Baumann et al. 2005). Interestingly, in yeast steroids and sterols can be acetylated by a sterol acetyltransferase bound to the ER membrane. This acetylation controls the export of steroids and sterols from cells via the secretory pathway, and acts as a lipid detoxification mechanism (Tiwari et al. 2007). Acetylation renders the lipid more hydrophobic, conferring higher affinity for membranes, and apparently therefore preferential delivery via membrane transport. Instead, mammals use a different strategy for sterol excretion: cholesterol is rendered increasingly hydrophilic via oxidation to generate oxysterols (Fig. 4) (followed by further oxidation to bile acids in the liver) (Russell 2003) to increase water solubility and excretion into the aqueous extracellular milieu.

There is increasing evidence for the role of soluble sterol carrier proteins in facilitating sterol trafficking between cellular membranes. Particularly two sterol-binding protein families, the START (steroidogenic acute regulatory protein related lipid transfer) and ORP (oxysterol-binding protein related protein) families are important in this process (Lavigne et al. 2010; Ridgway 2010). These proteins are encoded by 27 genes comprising almost 0.1% of the entire human genome (Ngo et al. 2010). The START and ORP proteins have verified sterol-binding domains (several of which have been crystallized) and adjacent regulatory and membrane-targeting motifs.

The steroidogenic acute regulatory protein (StAR) is the prototype of the START family proteins. StAR regulates the rate-limiting step in steroid hormone biosynthesis, that is, the delivery of cholesterol into the mitochondrial inner membrane (Manna et al. 2009). This membrane harbors the P450 side chain cleavage enzyme that catalyzes pregnenolone production from cholesterol. StAR is a labile phosphoprotein whose synthesis correlates tightly with steroid synthesis. Its amino-terminal mitochondrial leader sequence is cleaved during or after translocation into mitochondria, leaving intact the START domain that is responsible for inducing cholesterol transfer. However, the role of mitochondrial targeting and processing of StAR on steroidogenesisis is controversial, as the START domain can function at the outer mitochondrial membrane (Arakane et al. 1996), and internalization of StAR into mitochondria does not appear necessary for the sterol transfer (Bose et al. 2002).

In yeast, ORP homologs (Osh proteins) have been implicated in ER-plasma membrane sterol delivery. Moreover, recent data implicate Osh and ORP proteins in bidirectional delivery of sterols between the plasma membrane and the ER (Raychaudhuri et al. 2006; Jansen et al. 2011). Several of the ORP proteins carry a FFAT (phenylalanine in an acidic tract) motif that interacts with the ER protein VAMP associated membrane protein (VAP) (Loewen et al. 2003). This may display a regulatory function in sterol transfer analogously to CERT, in which this motif regulates ER interaction and ceramide transfer activity (Kawano et al. 2006). The plextrin homology (PH) domains present in several ORPs may provide additional binding specifities between donor and acceptor membranes. PIP interactions may also take place in the absence of a PH domain, as in Osh4, where

a membrane-binding surface of the protein can generate a phosphoinositide binding site (Schulz et al. 2009). In mammalian cells, ORP2 was shown to enhance biosynthetic sterol trafficking from the ER to the plasma membrane (Hynynen et al. 2005). It can also facilitate the delivery of sterols in the reverse direction, from the plasma membrane to the ER and lipid droplets (Jansen et al. 2011).

CONCLUDING REMARKS AND FUTURE PROSPECTS

The enzymes involved in the biosynthesis of glycerophospholipids, sphingolipids, and sterols have been characterized, and there is ample information on the regulation of the rate-limiting enzymes of the pathways. Instead, insight into the mechanisms of delivery of newly synthesized lipids to their target membranes in cells is less comprehensive.

Although the basic biophysical principles of lipid mobility have been extensively studied in model membranes, the unequivocal identification of physiologically relevant mediators of lipid transfer has not been easy. A major challenge is the redundancy of lipid trafficking systems in cells. This calls for manipulations that can target an entire protein family, for instance by pharmacologic compounds, or by genetic strategies in multicellular organisms, as has been pioneered in yeast. Moreover, analytical tools need to be further developed. Mass spectrometry based lipidomics has only recently opened the possibility to obtain comprehensive and precise structural information on individual lipid molecules. This technology should be extended and complemented with improved imaging systems to visualizing lipids and lipid–protein interactions in cells and tissues, to obtain improved spatial and temporal information on lipid delivery.

Finally, it is important to acknowledge that despite the redundancy in lipid biosynthetic trafficking systems, not all delivery systems are backed up in all cells. This is exemplified by human diseases because of defects in this process that manifest as complex tissue level malfunction. For instance, loss of function mutations in the mitochondrial cholesterol transporter StAR cause a severe impairment in the synthesis of all adrenal and gonadal steroid hormones, resulting in lipoid congenital adrenal hyperplasia (Lin et al. 1995), whereas mutations in the keratinocyte lipid transporter ABCA12 cause a skin disorder, harlequin ichtyosis, that results from the loss of long-chain ceramide esters in the skin and disruption of the epidermal permeability barrier (Zuo et al. 2008). Such examples with Mendelian inheritance patterns represent the severe end of the spectrum, the tip of the iceberg, in lipid synthesis and delivery problems in human disease. With improved analytical technologies, the number and variety of such disturbances is bound to increase.

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