Lipids in biological membranes are organized non-randomly across the bilayer. In eukaryotic cells, the cytoplasmic surface is enriched in anionic and primary amine-containing phospholipids (phosphoinositides (PIP_n)), phospha-tidic acid, phosphatidylethanolamine (PE), phosphatidylserine (PS), whereas the extracytoplasmic leaflet and the topologically equivalent luminal surface of internal organelles are enriched in choline-containing phospholipids (phosphatidylcholine (PC) and sphingomyelin) and glycosphingolipids. In prokaryotic cells, PE is enriched in the cytoplasmic leaflet of the plasma membrane, phosphatidylglycerol (PG) is localized predominantly to the outer leaflet, and cardiolipin (CL) has a symmetric transbilayer distribution.

The origin of lipid asymmetry stems primarily from vectorial biosynthesis. Most eukaryotic glycerophospholipids are synthesized on the cytoplasmic face of the ER, whereas sphingolipids are either synthesized or modified on the luminal surface of the ER and Golgi. To maintain bilayer balance, some newly synthesized lipids must traverse to the side of the membrane opposite that of the site of synthesis. Once synthesized, lipids must also move to other membranes within the cell, which occurs primarily by a vesicular process.

Lipids form the basic structure of the outer coat of Gram-negative bacteria and serve as carriers of oligosaccharide residues destined for attachment to lipids and proteins. The assembly of these units requires the collection of material from both sides of the membrane and the delivery of the final product to the correct location, processes that require the transbilayer movement of lipids with bulky or hydrophilic headgroups.

Once generated, transverse phospholipid asymmetry is preserved by the combination of slow transbilayer movement of lipids (3) and the presence of selective lipid transporters. The thermodynamic barrier for transbilayer movement must be overcome for the regulated and rapid exchange of complex lipids across the membrane bilayer. The task of moving a large polar group, attached to a hydrophobic tail, across the membrane requires transporters with the capability of interacting with amphipathic compounds. These transporters must provide a sizeable hydrophilic pathway for the lipid headgroup to pass through the membrane yet must also be able to accommodate the hydrophobic character of the lipid. Some of the proteins that have been assigned lipid transport activity are relatively nonspecific. Others exhibit a high degree of specificity. Interestingly, the generation and the dissipation of the transbilayer lipid gradient rely on the activity of a relatively small number of lipid transporter families that move lipids and their precursors from one side of the membrane to the other.

Mark Bretscher first coined the term “flippase” to refer to lipid transporters that serve to equilibrate newly synthesized lipid across biogenic membranes such as the ER (4). Although the definition of flippases has come to include all types of lipid transporters, transbilayer lipid transporters can be classified based on their substrate specificity, direction of transport, and requirement for ATP. Transporters that move lipids to the cytoplasmic face of the membrane are commonly called “flippases,” whereas those that transport lipids from the cytofacial surface to the opposite side of the membrane are called “flopases” (Fig. 1). The degree of substrate selectivity for lipids varies, but both classes of these transporters typically require an input of energy (usually in the form of ATP), allowing them to pump lipids against a concentration gradient. In contrast, “scramblases” transport lipids in both directions, are driven by a pre-existing transbilayer lipid gradient, and in the plasma membrane are Ca\(^{2+}\)-activated. Scramblases play an important role in the controlled randomization of lipid distribution that occurs during apoptosis or during cell activation, and by externalizing PS, scramblases signal the engulfment of dead or dying cells by phagocytes (5–7).

Biogenic membrane flippases are energy-independent and belong to the class of transporters first envisioned by Bretscher. These are distinct from the ATP-dependent flippases, although the term “flippase” is commonly used to refer to both classes of transporters, and are associated with lipid biosynthetic processes. These include eukaryotic transporters in the ER and Golgi (glycolipids), as well as prokaryotic transporters.

A number of excellent recent reviews have summarized in depth the role of ABC transporters (8, 9), P_4-ATPases (10, 11), and biogenic flippases (12) in lipid-mediated transport (see also Refs. 13 and 14). The goal of the present review is to summarize the current state of knowledge of each of these families of putative lipid transporters, with emphasis on the biogenic and the unidirectional ATP-dependent transporters.

**Biogenic Lipid Transporters**

Lipid biosynthetic pathways rely on the communication of substrates and products between the leaflets of biogenic membranes, such as the ER, Golgi, and prokaryotic plasma membrane. The substrates are diverse and include glycerophospholipids and their precursors, glycolipids, and dolichol-linked sugars.
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**Eukaryotic Glycerophospholipid Transporters**—One of the first flippase activities to be identified was the ER flippase. Using a novel reconstitution assay based on the density shift of flippase-containing liposomes due to transport of brominated lipids, Backer and Dawidowicz reported the activity of a rat liver microsomal PC flippase (15). Bell and co-workers demonstrated the expression of a protease and N-ethylmaleimide-sensitive ER flippase activity in liver microsomes that was specific for short chain PC (dibutyryl PC) and its metabolites (16, 17). Using spin-labeled and fluorescent lipids, ER transport was shown to be fast \( t_{1/2} \leq 1 \text{ min} \) and non-selective for glycerophospholipids (18). Transport activity has been reconstituted from detergent extracts of ER microsomes, and recent evidence indicates that the transport of fluorescent analogs of PI in the ER is independent of inositol and glycerol stereochemistry (19). An implication of these data is that transport of PC and PI in biogenic membranes may occur through the same nonspecific transporter.

**Prokaryotic Transporters**—The synthesis of phospholipids in prokaryotic membranes occurs on the cytoplasmic face of the plasma membrane, generating the same issues of bilayer balance encountered by phospholipid synthesis in the ER. In 1977, Rothman and Kennedy demonstrated that newly synthesized PE in *Bacillus megaterium* is equilibrated with the outer monolayer pool of this lipid much faster than spontaneous diffusion, indicating that transbilayer PE movement was facilitated by membrane proteins (20). Subsequent studies have shown that fluorescent analogs of PI in the ER is independent of inositol and glycerol stereochemistry (19). An implication of these data is that transport of PC and PI in biogenic membranes may occur through the same nonspecific transporter.

**Glycolipid Transporters**—The movement of sugar-linked lipids across the membrane is essential for protein glycosylation and the synthesis of cell surface protein membrane anchors. Phosphatidylinositol forms the root of the PI-glycans, which tether some cell surface proteins to the membrane. Using a reconstitution method similar to the one described for the ER flippase, the transport of fluorescent (7-nitrobenz-2-oxa-1,3-diazole-4-yl (NBD)) analogs of PI-glycan precursors (NBD-GlcN-P) have been demonstrated in proteoliposomes containing an extract of ER proteins (24).

The synthesis of glycosphingolipids involves the assembly of the monohexosylphingolipids GalCer and GlcCer on the cytoplasmic face of the Golgi. These lipids are transported to the luminal surface where additional monosaccharides are added to create more elaborate glycolipids, which then become trapped in the Golgi lumen. The transport of monohexosylphingolipids (GlcCer and GalCer) across ER and cis-Golgi membranes has been shown to be fast, ATP-independent, bidirectional, and requires transporters specific for dolichol phosphomonoamine, glucosylceramide and, in isolated vesicles, is bidirectional. At least two classes of proposed flippases are involved in Golgi plasma membrane-endosome trafficking: Drs2p, Dnf3p (ATP8A1 homologs), and Neo1p (ATP9 homolog). The plasma membrane contains putative phospholipid flippases (Dnf1p, Dnf2p (ATP8B1 homologs)), the aminophospholipid transporter (APLT), ATP-dependent flippases (ABC1A, ABC1B, ABC1C, ABC1G (Pdr5p, Crd1p, MsbA are not shown)), and the Ca\(^{2+}\)-activated scramblase. Not shown, additional flippases are also present in specialized membranes such as the bile canalicular membrane (ABCB11, ABC22, ABC3), peroxisomes (ABCD family), and retinal disk membrane (ABCA4). PAF, platelet-activating factor; SM, sphingomyelin; PL, phospholipid.

**FIGURE 1. Cartoon depiction of the intracellular location and function of lipid transporters in a stylized non-polarized eukaryotic cell.** "Flippases" (red) catalyze the transport of lipids toward the cytoplasm and require ATP, "floppases" (blue) catalyze the ATP-dependent transport of lipids away from the cytoplasm, and "scramblases" (purple) catalyze the bi-directional, non-energy-dependent transport of lipids. Biogenic lipid transporters are shown in light blue. Protein names and putative endogenous substrates are indicated. The ER contains a non-selective biogenic lipid transporter for glycerophospholipids as well as transporters specific for dolichol phosphomonoamine, GlcN-PI, and Man\(_5\)GlcNAc\(_2\), pyrophosphoryl dolichol. Monohexosylceramide transporters are present in ER and cis-Golgi membranes and, in isolated vesicles, are bidirectional. At least two classes of proposed flippases are involved in Golgi plasma membrane-endosome trafficking: Drs2p, Dnf3p (ATP8A1 homologs), and Neo1p (ATP9 homolog). The plasma membrane contains putative phospholipid flippases (Dnf1p, Dnf2p (ATP8B1 homologs)), the aminophospholipid transporter (APLT), ATP-dependent flippases (ABC1A, ABC1B, ABC1C, ABC1G (Pdr5p, Crd1p, MsbA are not shown)), and the Ca\(^{2+}\)-activated scramblase. Not shown, additional flippases are also present in specialized membranes such as the bile canalicular membrane (ABCB11, ABC22, ABC3), peroxisomes (ABCD family), and retinal disk membrane (ABCA4). PAF, platelet-activating factor; SM, sphingomyelin; PL, phospholipid.
posed to be the Man\textsubscript{3}GlcNAC\textsubscript{2}P-P-dolichol transporter (26), whereas a rat liver Man-P-dolichol transporter has been partially purified and reconstituted using a short chain lipid analog (Man-P-Dol\textsubscript{10}) to measure transport activity (27). Homologs of Rft1 have been found in the genomes of other eukaryotes, including humans, indicating that this is likely to be a ubiquitous family of proteins in organisms that are capable of N-glycosylation (28). Sequence analysis of Rft1 does not reveal an ATP binding site, consistent with the ATP-independent transport of Man\textsubscript{3}GlcNAC\textsubscript{2}P-P-dolichol.

**ABC Transporters**

The ABC class of transporters catalyze the ATP-dependent transport of a variety of substrates, including amphiphatic compounds, xenobiotics, ions, and peptides (for recent reviews see Refs. 8 and 9). These proteins are widely distributed in prokaryotes and eukaryotes, and defects in human isoforms have been associated with a number of diseases. They have a common functional structure consisting of two hexahelical transmembrane domains and two nucleotide binding domains. This review will address those ABC transporters for which endogenous lipid transport activity has been measured or suspected (see supplemental Table 1).

The ABCA family has been implicated in lipid and sterol transport. ABCA1 is a ubiquitous plasma membrane protein that transports cholesterol to the extracellular surface of cells in extrahepatic tissues, where it is harvested by high density lipoprotein for circulation to the liver. ABCA1 may also catalyze PS efflux (29, 30) and assist in the binding of apoA-I to the surface of the cell, although ABCA1-mediated efflux of NBD-PE and NBD-PS does not require an apoA-I (31). A highly homologous family member, ABCA7, has a more limited tissue distribution, but it may also play a role in cholesterol and PS externalization (32, 33). Defects in ABCA3 have been shown to produce severe surfactant deficiency, implying that this protein is involved in lipid export into the lung alveolar space (34). ABCA4 is localized to retinal rod outer segment disk membranes and transports all-trans-N-retinylidene PE to the cytosolic side of the disk for recycling of the all-trans-retinol to the cis form (35).

The ABCB family includes the multidrug resistance (MDR) or P-glycoprotein transporters. First discovered as xenobiotic transporters, proteins in this family have also been found to transport lipids. ABCB1 is localized to the apical surface of polarized cells and is capable of transporting a wide variety of lipids, including fluorescent (NBD) or short chain glycerophospholipids, sphingolipids, and platelet-activating factor (9, 36). In contrast, ABCB4 is a selective PC transporter (36) and may play a key role in the export of this lipid into the bile (37, 38).

The ABCG (MRP) family contains proteins that have a different structure and function than most ABC transporters. This family contains channels (cystic fibrosis transmembrane conductance regulator) and potassium channel regulators (sulfonamide receptors) as well as transporters of organic anions, glutathione conjugates, and lipids (8). ABCG1 is localized to the basolateral surface of polarized cells, and it has been shown to transport NBD-PC (39, 40), sphingolipids (41), and leukotriene C\textsubscript{4} (42). Members of the ABCG family have been shown to transport cytotoxic drugs and together with the ABCB and ABCC families are responsible for multidrug resistance (8). ABCG2 has been shown to transport NBD analogs of PS and PC but not PE (43).

Yeast express a number of ABC transporters, including homologs of human MDR and MRP proteins (44). The most well characterized yeast ABC lipid transporters are Yor1p and Pdr5p, which confer drug resistance and have been shown to be involved in the movement of NBD-PE across the plasma membrane (45). The transcription factors Pdr1p and Pdr3p have been shown to regulate NBD-PE and NBD-PC uptake by decreasing lipid efflux (45). Similar proteins have been shown to be involved in lipid transport in *Candida albicans*; Cdr1p is a homolog of Pdr5p and regulates PE distribution in the plasma membrane (46).

Prokaryotes have a rich array of ABC transporters, some of which have been linked to lipid transport (9, 47). Lipid A is a complex glycolipid that is an essential building block of the lipopolysaccharide outer membrane of Gram-negative bacteria. This lipid is transported to the periplasmic space by MsbA. The crystal structures of MsbA from three organisms (*E. coli*, *Vibrio cholerae*, and *Salmonella typhimurium*) have been determined (Ref. 48 and references therein), and these structures have provided insights into the possible transport mechanisms of the ABC lipid transporters.

**P\textsubscript{4}-ATPases**

The first evidence for a plasma membrane ATP-dependent flipase was reported in human erythrocytes by Seigneuret and Devaux (49). This and subsequent work by several laboratories have defined the biochemical properties of this family of transporters, including N-ethylmaleimide, vanadate, and Ca\textsuperscript{2+} sensitivity, as well as a high selectivity for PS (for a review, see Ref. 50). These studies established the criteria for the search for the erythrocyte (51) and other ATP-dependent flipases.

The discovery of PS flipase activity in bovine adrenal chromaffin granules led to the purification and cloning of the bovine ATPase II (52). This enzyme was found to be homologous to proteins in the P-type ATPase superfamily and most closely homologous to Drs2p, a yeast ATPase that had been associated with ribosomal assembly. A search of genomic data bases revealed that these enzymes were founding members of a novel class of P-type ATPases (P\textsubscript{4}-ATPases) that may be involved in amphipath transport. Over 100 members of the P\textsubscript{4}-ATPase subfamily have been identified, all of which are exclusively found in eukaryotic organisms, including approximately a dozen human isoforms (11, 53) (supplemental Table 2). Unique sequence characteristics divide the P\textsubscript{4}-ATPase family into approximately six subclasses (54). The study of P\textsubscript{4}-ATPase mutants has linked many of these enzymes to the maintenance of membrane structure, vesicle trafficking, and amphipath transport. At least two have been associated with human diseases (Ref. 11 and references therein).

The subclass I and III P\textsubscript{4}-ATPases include the bovine ATPase II, the yeast proteins Drs2p, Dnf1p, Dnf2p, and Dnf3p, and several human (ATP8A and ATP8B) enzymes. Recent studies have shown that Drs2p is enriched in the trans-Golgi compartment.
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but is present only in limited amounts in the plasma membrane (55). Mutational studies have shown that Drs2p (and a related homolog Dnf3p) support NBD-PS and NBD-PE transport in isolated trans-Golgi vesicles (56, 57) as well as implicate the involvement of these proteins in the formation of clathrin-coated vesicles (58). Drs2p and Dnf3p may play a role in membrane trafficking among the trans-Golgi, the plasma membrane, and endosomal compartments in addition to their potential role in maintaining transmembrane amino phospholipid asymmetry. Dnf1p and Dnf2p have been shown to mediate the uptake of fluorescent analogs of PC, PE, and PS in the plasma membrane (55). Deletion of any one of these enzymes is not lethal, indicating that they have overlapping functions; however, the quadruple mutant (Δdrs2Δdnf1Δdnf2Δdnf3) is not viable (10, 59). ATPase activity of the bovine chromaffin granule ATPase II is selectively activated by PS (60), and this specificity may be determined by key sequence elements; in four bovine brain isoforms of ATP8A1 the presence of a 15-amino acid sequence results in greater PS-stimulated, but not PE-stimulated, ATPase activity (61). In addition, the murine homolog of the bovine chromaffin granule ATPase II, Atp8a1, is selectively stimulated by PS (62), but not by PE or PC, and displays the same stereoochemical preferences for the sn-1,2-glycerol isomer of PS that the human erythrocyte flippase and PS-stimulated ATPase demonstrate (63).

Subclass II P4-ATPases members include the yeast protein Neo1p and the human ATP9 gene products. Although Drs2p is involved in late stages of the secretory pathway and Dnf1p/Dnf2p play a role in plasma membrane glycerophospholipid transport and early endosome formation, Neo1p is involved in endosome formation and retrograde vesicle trafficking from the Golgi to the ER and is essential for viability (59, 64).

Yeast P4-ATPases have also been shown to associate with an essential class of membrane proteins (Cdc50p, Lem3p/Ros3p, Crf1p) that contain two transmembrane segments and an extracellular, potentially glycosylated loop (10, 11). Drs2p and Neo1p interact with Cdc50p, whereas Lem3p (also known as Ros3p) interacts with Dnf1p (65). Disruption of Cdc50p recapitulates the effects of Δdrs2, whereas mutations to Lem3p mimic Δdnf1 (65), including the inhibition of the uptake of alkyl phosphocholine drugs and NBD-PC at the plasma membrane (66, 67). These interactions may represent the general paradigm in P-type ATPases of the association of the catalytic subunit with an unrelated, non-catalytic, accessory protein required for proper trafficking or regulation of activity (see supplemental Table 3). Several mouse (CDC50A, CDC50B, CDC50C) and human (TMEM30A, TMEMB, TMEMC) Cdc50p homologs have been discovered (68). Whether the Cdc50p family is necessary for catalytic activity or proper trafficking of the P4-ATPases is an important subject of current studies.

The putative roles of P4-ATPases in membrane trafficking and in lipid transport may be linked. Flippase-dependent PS transport across the human erythrocyte membrane generates a bilayer imbalance that results in membrane bending, bud formation, and endovesiculation (49, 69). Similar shape changes may occur in more complex cells and could drive endovesicle or trafficking vesicle formation. The collaboration of yeast P4-ATPases with guanine nucleotide exchange factors that regulate small molecular weight GTPases involved in binding vesicle coat proteins to the membrane may also be important (10). This effect is likely not because of direction associations alone; an ATPase defective mutant of Drs2p prevents vesicle formation (58).

Subclass V enzymes have been associated with a number of human diseases and pathologies. Angelman syndrome, which is characterized by neurologic abnormalities, including mental retardation, ataxia, and epilepsy (70), and behavioral disorders, such as autism (71), have been linked to ATP10C. A mouse homolog of ATP10C (pfatp) has been associated with obesity and may be a new model for Type 2 diabetes (72). How the putative role of the P4-ATPases in membrane structure and function influences these diseases remains to be determined.

Conclusions

The generation and maintenance of the transfat bilayer distribution of lipids require the action of a number of specific and nonspecific transbilayer lipid transporters. Although collectively these transporters are referred to as "flippases," they comprise distinct sets of transporter families. In addition to serving a maintenance function, phospholipid flippases may also drive intracellular vesicle trafficking either by inducing membrane vesiculation or by creating an environment favorable for the binding of vesicle coat proteins. The selective retention or transport of each of these diverse families of lipid transporters along the membrane trafficking pathmay be responsible for the generation of lipid asymmetry as the membranes traffic from the ER to the plasma membrane. Although a substantial amount of indirect evidence has implicated a number of proteins as "flippases," positive identification awaits their purification, reconstitution, and demonstration of transport activity.

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