Tracking down lipid flippases and their biological functions

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Summary

The various organellar membranes of eukaryotic cells display striking differences in the composition, leaflet distribution and transbilayer movement of their lipids. In membranes such as the endoplasmic reticulum, phospholipids can move readily across the bilayer, aided by membrane proteins that facilitate a passive equilibration of lipids between both membrane halves. In the plasma membrane, and probably also in the late Golgi and endosomal compartments, flip-flop of phospholipids is constrained and subject to a dynamic, ATP-dependent regulation that involves members of distinct protein families. Recent studies in yeast, parasites such as *Leishmania,* and mammalian cells have identified several candidates for lipid flippases, and whereas some of these serve a fundamental role in the release of lipids from cells, others appear to have unexpected and important functions in vesicular traffic: their activities are required to support vesicle formation in the secretory and endocytic pathways.

Key words: Lipid flippase, ABC transporter, P-type ATPase, Vesicle budding, Membrane dynamics

Introduction

Eukaryotic cells are compartmentalized into distinct organelles by lipid bilayers. Each membrane is composed of hundreds of lipids, and there are dramatic differences between organelles – for example, sphingomyelin and sterols are highly enriched at the plasma membrane (Fig. 1). Even within a membrane, lipids are not randomly distributed. Lipids are asymmetrically arranged between the two leaflets of the plasma membrane, and this was first thought to be a static characteristic of membranes (Bretscher, 1972). However, it is now clear that the lipid topology results from a continuous bi-directional movement of lipids between the two leaflets – so-called 'flip-flop' – in which specific membrane proteins have an essential role. Recently, several 'lipid flippases' have been identified. Besides maintaining an asymmetric lipid arrangement, the physiological functions of which are still relatively unclear, these appear to regulate other, more dynamic events, such as the budding of intracellular transport vesicles.

Lipid movement in model membranes

The most prevalent phospholipid in eukaryotic cell membranes is phosphatidylcholine (PC). In water, the cylindrical and amphipathic PC spontaneously forms bilayer membranes: its polar head is exposed to water and the hydrocarbon chains constitute the hydrophobic core of the bilayer. In biomembranes, lipids diffuse laterally over an area of 1 μm^2 within seconds. However, the rate of spontaneous flip-flop between leaflets differs for each lipid. Phospholipids that have polar head groups, such as PC, phosphatidylserine (PS) and phosphatidylethanolamine (PE), and glycolipids that have bulky hydrophilic carbohydrate moieties flip only slowly across a pure lipid bilayer, displaying half-times of hours to days depending on the size and charge of the head group (Kornberg and McConnell, 1971; Buton et al., 2002). By contrast, neutral lipids such as diacylglycerol and charged lipids such as free fatty acids, phosphatidic acid or phosphatidylglycerol in their protonated form can move rapidly from one leaflet to the other in seconds or minutes. Cholesterol is embedded in the membrane; with its polar OH group facing the aqueous phase and the acyl chain pointing towards the bilayer center. Recent experimental evidence supports rapid flip-flop of cholesterol in PC membranes, red cell membranes and, presumably, in most other cellular membranes (reviewed in Hamilton, 2003).

Studies of model membranes have shown that lipid flip-flop is affected by the physical properties of the bilayer. An essential factor is the lipid packing. At temperatures above the solid-liquid phase transition, flip-flop of short-chain phospholipids in human erythrocytes and PC membranes is reduced by cholesterol (Morrot et al., 1989; John et al., 2002). Cholesterol condenses the phospholipid arrangement and increases the thickness of the hydrophobic core. Indeed, plasma membranes are thought to contain less-fluid patches enriched in cholesterol, so-called sphingolipid-cholesterol rafts (Harder and van Meer, 2003). Interestingly, molecular packing defects can enhance the flip-flop rate (de Kruijff and van Zoelen, 1978; John et al., 2002). This may be the case at the border of coexisting liquid-ordered and liquid-disordered phases in the bilayer. Packing defects may also be induced by lipids favoring non-bilayer structures, such as PE, a cone-shaped lipid that has a small head group relative to its large hydrophobic chains. Likewise, phosphopolyisoprenols (dolichols), which function as hydrophobic carriers of glycosyl residues across the ER membrane, destabilize the bilayer and may thus facilitate their flipping (Chojnacki and Dallner,

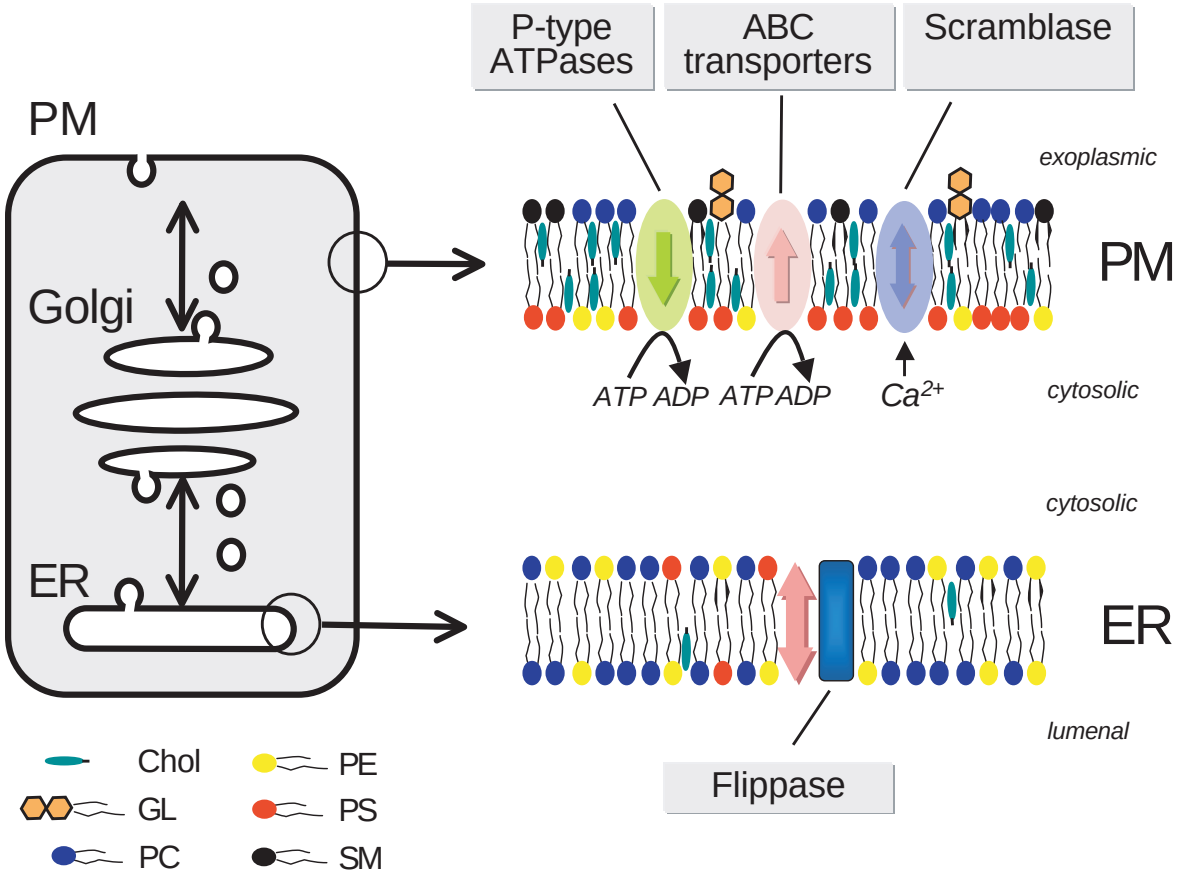

Fig. 1. Regulation of the transbilayer lipid distribution in cellular membranes. In early secretory organelles, such as the endoplasmic reticulum (ER), membrane proteins facilitate rapid flip-flop of lipids and allow them to equilibrate between the two membrane leaflets independently of ATP. This system is unable to accumulate a given lipid in one leaflet, thereby promoting a symmetric lipid distribution across the bilayer. In contrast, flip-flop of phospholipids across the plasma membrane (PM) is constrained owing to high levels of cholesterol and sphingolipids and/or the absence of constitutive bi-directional flippases. Thus, ATP-dependent flippases can maintain an asymmetric lipid distribution by moving specific lipids towards (P-type ATPase family members) or away from the cytosolic leaflet (ABC transporters). Cellular activation triggered by cytosolic calcium can collapse the lipid asymmetry by the transient activity of an ATP-independent scramblase. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; SM, sphingomyelin; GL, glycolipids; Chol, cholesterol.

1988). Furthermore, perturbations of the bilayer structure can be triggered by imperfect matching between transmembrane domains of proteins and the boundary lipid phase. This has prompted speculation that the interplay between transmembrane domains and non-bilayer favoring lipids might be sufficient to allow fast flip-flop in the ER (Kol et al., 2001).

Lipid movement in the ER and cis-Golgi: evidence for energy-independent flippases

Lipid movement across cellular membranes is essential for cell growth and survival. Phospholipid biosynthesis in the ER is an asymmetric process that results in the insertion of new lipids into the cytoplasmic leaflet (Bell et al., 1981). To ensure balanced growth, and thus stability, of the ER bilayer, half of the newly synthesized lipids must flip. The same is true for the membranes of peroxisomes and mitochondria, organelles that derive most of their lipids from the ER but not by a vesicular pathway. In regions of close membrane contact, lipids are assumed to be delivered from the outer leaflet of the donor membrane to that of the target membrane (Voelker, 2003). Again, balanced growth of membrane requires lipid redistribution. Indeed, rapid flip-flop has been reported in microsomal membranes at half-times of seconds to minutes (de Kruijff et al., 1979; Bishop and Bell, 1985; Herrmann et al., 1990; Marx et al., 2000; Buton et al., 2002). Because lipid flip-flop is orders of magnitude slower in protein-free lipid bilayers, this has led to the idea that phospholipid flip-flop is protein-mediated and involves one or more phospholipid flippases (Bretscher, 1973). Indeed, phospholipid flip-flop in the ER is sensitive to proteases and protein-modifying reagents (Bishop and Bell, 1985; Buton et al., 1996). The flipping machinery does not require ATP and can translocate most, if not all, phospholipid classes non-vectorially across the bilayer. The bulk of phospholipids in the ER must therefore be distributed symmetrically between the two membrane leaflets.

Besides the glycero-phospholipids, the glyco-phospholipids mannosyl-phosphodolichol, glucosyl-phosphodolichol and an oligosaccharide-diphosphodolichol have to undergo flip-flop in the ER, because the full-length lipid-linked oligosaccharide that is subsequently used in N-linked protein glycosylation is synthesized on the lumenal side. Rush and Waechter have

demonstrated this using water-soluble analogs (Rush and Waechter, 1998). Similarly, synthesis of complex glycosphingolipids in the Golgi lumen requires flip-flop of the precursor glucosylceramide, which is synthesized on the cytoplasmic leaflet of the cis-Golgi. Indeed, monohexosylsphingolipids can flip across the Golgi membrane by an energy-independent, bi-directional mechanism (Burger et al., 1996; Buton et al., 2002). Because the complex glycosphingolipids synthesized at the lumenal leaflet are unable to translocate in Golgi membranes (Lannert et al., 1994; Burger et al., 1996; Buton et al., 2002), a monohexosylsphingolipid flippase might regulate complex glycosphingolipid synthesis.

Although considerable evidence that the bi-directional lipid movement across ER and cis-Golgi membranes is protein-mediated exists, it is not clear whether a dedicated flippase, a family of flippases or the mere presence of membrane proteins facilitates flip-flop. Genetic work in yeast has identified a putative flippase, Rft1p, required for the translocation of lipid-linked oligosaccharides from the cytoplasmic leaflet to the lumenal leaflet of the ER (Helenius et al., 2002). In an attempt to isolate the flippase(s) from microsomal membranes Menon et al. reconstituted fractions of proteins separated on a glycerol gradient or by anion exchange chromatography in proteoliposomes (Menon et al., 2000). This approach yielded protein pools that had increased specific flip-flop activities, suggesting that specific proteins are responsible for the fast lipid movement in the ER. Remarkably, Kol et al. have found that certain α-helical membrane spanning peptides promote transbilayer movement of some classes of phospholipid in synthetic membranes, suggesting that lipid flip-flop proceeds in the absence of a dedicated flippase (Kol et al., 2003). However, this hypothesis can only hold for specific organellar membranes. Flip-flop in the plasma membrane, for example, is a very slow process (Zachowski, 1993). Interestingly, as mentioned above, peptide-induced flip-flop is reduced by cholesterol, an abundant component of the plasma membrane. The gradual accumulation of cholesterol along the organelles of the secretory pathway probably modulates lipid flip-flop rates and may help to explain the transition from constitutive flip-flop in the ER membrane to regulated, energy-driven lipid translocation across the plasma membrane.

Lipid movement across the plasma membrane: a role for ATP-driven flippases

Phospholipid flip-flop in the plasma membranes of eukaryotic cells is constrained, enabling cells to generate an asymmetric distribution of lipids between the inner and outer leaflets. The majority of PC and sphingolipids is in the exoplasmic leaflet, and the aminophospholipids PS and PE are in the cytoplasmic layer (reviewed in Zachowski, 1993). This asymmetry is made possible by a lipid-translocation machinery that hydrolyses ATP to flip aminophospholipids against a concentration gradient: the 'aminophospholipid translocase'. Some cell types display similar transport of PC across their plasma membranes and, therefore, may contain either a PC-specific translocase in addition to the aminophospholipid translocase, or an inward translocase of different specificity that translocates both aminophospholipids and PC (Pomorski et al., 1999; Grant et al., 2001; Araujo-Santos et al., 2003). A less-selective ATP-dependent outward flippase may export phospholipids from the inner to the outer plasma membrane leaflet (Connor et al., 1992). Finally, calcium-dependent activation of an ATP-independent flippase, termed scramblase, rapidly randomizes the phospholipid distribution across the membrane bilayer (Comfurius et al., 1996; Bassé et al., 1996). The various flippase activities are mostly demonstrated by the use of fluorescent and spin-labeled lipids (Fig. 2), and only few studies have attempted to measure the translocation of natural lipids. Nevertheless, there is now convincing evidence to suggest that lipids bearing a reporter moiety are flipped in biological membranes with a selectivity dependent on the head group and backbone and that this reflects a phenomenon that takes place for naturally occurring lipids (Devaux et al., 2002). However, the kinetic constants of flip-flop are affected by the chemical structure of the lipid probe.

Inward lipid transporters

Seigneuret and Devaux first observed an aminophospholipid translocase activity in human erythrocytes, and later it was demonstrated to reside in the plasma membranes of many mammalian cell types (Seigneuret and Devaux, 1984). A second major breakthrough in the field was the cloning of a potential aminophospholipid translocase from bovine chromaffin granules (Tang et al., 1996). This turned out to be the first representative of a novel subfamily of P-type ATPases, and genome sequencing projects have revealed over a dozen family members in eukaryotes. New evidence obtained in yeast supports the notion that these proteins are the lipid translocases responsible for concentrating specific lipids in the cytosolic leaflet of biological membranes (Pomorski et al., 2003). The members of this subfamily have 10 predicted transmembrane domains and differ from the cation-transporting P-type ATPases in that they lack negatively charged residues within transmembrane segments critically involved in ion transport (Catty et al., 1997; Halleck et al., 1998). A few biological disorders have been attributed to mutations in members of this subfamily. Mutations in *FIC1*, for example, cause familial intrahepatic cholestasis, which is a defect in bile secretion (Thompson and Jansen, 2000; Ujhazy et al., 2001). The human *ATP10C* gene has been linked to the neurological disorders Angelman syndrome and autism (Herzing et al., 2001; Meguro et al., 2001). Precisely what cellular process is affected in these diseases is unknown.

Drs2p was the first member of this subfamily studied in yeast. Deletion of the *DRS2* gene was reported to abolish the low-temperature uptake of a fluorescent PS analog at the plasma membrane, which suggests Drs2p has a role in aminophospholipid translocation (Tang et al., 1996; Gomes et al., 2000). However, the uptake defect could not be confirmed in two subsequent studies (Siegmund et al., 1998; Marx et al., 1999). Moreover, Drs2p does not reside in the plasma membrane but in the trans-Golgi (Chen et al., 1999). Genetic screens in yeast by two independent groups identified Ros3p/Lem3p as a protein whose removal markedly reduces uptake of fluorescent analogs of PE and PC across the plasma membrane (Kato et al., 2002; Hanson et al., 2003). However, the protein is unrelated to any known ATPase. Hence, Ros3p is unlikely to function as an independent lipid transporter at the yeast plasma membrane. Instead, it may represent an essential

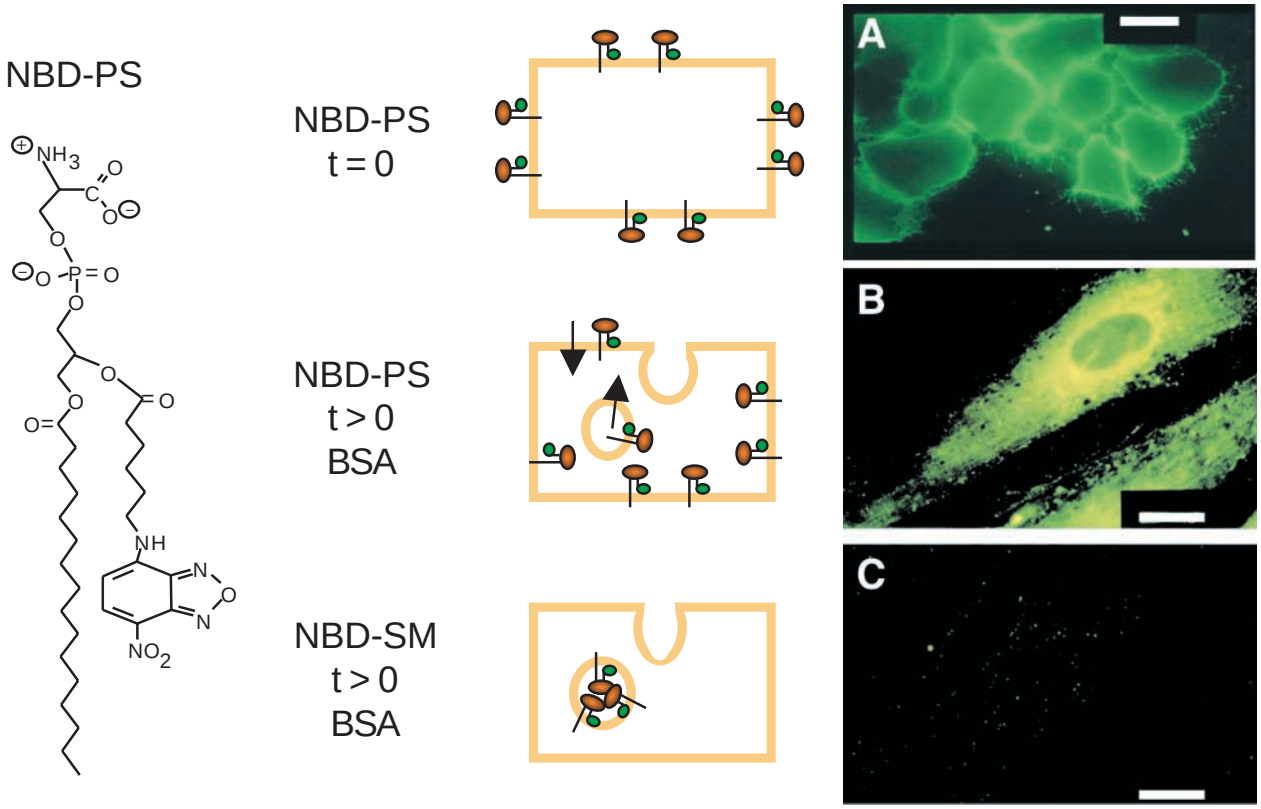

Fig. 2. Characterization of phospholipid movement at a qualitative level in (plasma) membranes is frequently based on phospholipid probes. These analogs have a reporter group attached to a short fatty acid chain and maintain most of the properties of endogenous phospholipids, except that they are more water-soluble, which facilitates incorporation from the medium into the outer monolayer of the membrane (A). Transport of these probes is usually monitored by extracting with bovine serum albumin (BSA) the residual fraction of analogs not transported across the membrane. Depending on the head group and cell type, the lipid analog inserted into the outer plasma membrane leaflet can be internalized by spontaneous flip-flop, by protein-mediated translocation or endocytosis. In human fibroblasts, for example, disappearance of NBD-PS from the cell surface is predominantly due to fast translocation across the plasma membrane (and endosomal membranes), resulting in a labeling of various intracellular membranes (B). In contrast, NBD-SM is internalized via endocytic vesicles resulting in the appearance of intracellular fluorescent spots (C). Bar, 20 μm.

component of the transporter complex or be required for chaperoning components of this complex to the proper membrane.

A new twist to the story came when recent work identified two Drs2p-related ATPases, Dnf1p and Dnf2p, that are essential for the ATP-dependent transport of fluorescent analogs of PS, PE and PC from the outer to the inner plasma membrane leaflet (Pomorski et al., 2003). Although their lipid preference disqualifies these proteins as specific aminophospholipid translocases, fluorescent analogs of sphingolipids, phosphatidic acid and phosphatidylglycerol were not recognized. In a *dnf1 dnf2* deletion mutant, endogenous PE accumulates at the cell surface PE exposure further increases when *drs2* is deleted as well. Another subfamily gene, *LdMT*, was recently identified in the protozoan parasite Leishmania after a genetic screen for parasites resistant to miltefosine, an alkylphosphocholine drug (Pérez-Victoria et al., 2003). The LdMT ATPase is required for the rapid intracellular uptake of not only alkylphosphocholine drugs but fluorescent analogs of PC, PE and PS as well. Transfection experiments clearly demonstrated a direct correlation between protein (over)expression and the level of lipid analog uptake in this parasite. Thus, the members of this subfamily appear to have a general function as lipid translocases at the plasma membrane and, remarkably, also at other cellular locations. This notion is supported by the identification of two other Drs2p-related ATPases, Dnf3p and Neo1p, that are associated with the Golgi complex and perhaps endosomal compartments (Hua et al., 2002; Pomorski et al., 2003; Hua and Graham, 2003). The various mammalian members of the P-type ATPase subfamily may be expressed in different cells types or in different membranes in the same cell, or they may have different biochemical properties (e.g. substrate specificity).

Outward lipid transporters

ABC transporters form a large, diverse family of ATP-driven pumps. Various family members are attractive candidates for flippases that translocate lipids from the inner to the outer leaflet of the plasma membrane primarily because of genetic evidence. The liver-specific ABC transporter MDR3 (ABCB4) and its mouse homolog Mdr2 specifically transport PC across the canalicular membrane during bile formation. Mice that

have a disrupted *Mdr2* gene show an absence of PC secretion into bile, and cells that overexpress Mdr2 exhibit increased PC translocation (Smit et al., 1993; Ruetz and Gros, 1994; Smith et al., 1994). By contrast, the multi-drug resistance protein MDR1 (ABCB1) expels a variety of short-chain lipids and amphiphilic drugs from the cell (van Helvoort et al., 1996; Raggers et al., 2000) but is unable to restore transport of PC into the bile of *Mdr2*-knockout mice (Smit et al., 1993). Thus, it remains to be established whether natural long-chain PC is an MDR1 substrate. Yet recent studies provided indirect evidence for MDR1-mediated outward transport of natural lipids such as PAF (platelet-activating factor, a short-chain PC), PS, SM and glucosylceramide (Bezombes et al., 1998; Raggers et al., 2001; Pohl et al., 2002), suggesting a physiological role for this protein in the distribution of several endogenous lipids between the leaflets of the plasma membrane. The glutathione-dependent multidrug transporter MRP1 (ABCC1) transports short-chain PC, PS, SM and GlcCer analogs and may help to maintain the outward orientation of natural choline phospholipids in the plasma membrane (Kamp and Haest, 1998; Raggers et al., 1999; Dekkers et al., 2000).

Other ABC transporters have been implicated in the transport of sterols. Mammalian cells synthesize cholesterol de novo, but the majority is made in the liver and delivered to the peripheral cells by lipoproteins. Peripheral cells are unable to degrade cholesterol and, thus, any surplus of cholesterol must be either stored as cholesterol esters in the cytosol or released from the cell. In Tangier disease, the efflux of cholesterol and phospholipids from peripheral cells onto apolipoproteins such as apoA-I is impaired owing to mutations in ABCA1. Initially it was proposed that ABCA1 transports cholesterol across the plasma membrane to the cell surface for efflux. At first glance, this is unexpected, since sterols are believed to flip rapidly across membranes (see above) but rapid movement might be impaired in rigid domains. By contrast, recent studies have suggested that ABCA1 acts primarily as a phospholipid flippases and promotes cholesterol efflux in an indirect fashion by generating a microenvironment facilitating the binding of apoA-1 (Hamon et al., 2000; Chambenoit et al., 2001; Wang et al., 2001). Alternatively, ABCA1 may translocate both lipid classes across the membrane (Wang et al., 2003). Mutations in ABCG5 and ABCG8 are associated with the accumulation of dietary cholesterol in the sterol storage disease sitosterolemia. These two half-transporters presumably act as a heterodimer responsible for the efflux of sterols from epithelial cells into the gut and from the liver to the bile duct (Berge et al., 2000; Graf et al., 2002; Yu et al., 2002). ABCG1, the human homolog of the *Drosophila* White protein, is implicated in the regulation of macrophage cholesterol and phospholipid transport (Schmitz et al., 2001). It remains to be established whether these proteins act as sterol flippases and/or facilitate the exposure of membrane-bound sterol to cognate acceptors (e.g. bile salts) as suggested for ABCG5/ABCG8 (Small, 2003).

Bidirectional translocation: the scramblase

An increase in intracellular calcium due to cell activation, cell injury or apoptosis can induce a progressive loss of plasma membrane phospholipid asymmetry. This has been ascribed to the activation of the lipid scramblase, a putative membrane protein facilitating a rapid bi-directional movement of all major phospolipid classes between the leaflets of the plasma membrane (Smeets et al., 1994; Williamson et al., 1995). However, for PS and PE a vectorial efflux from the inner to the outer leaflet upon activation of a scramblase activity has been reported (Gaffet et al., 1995; Hamon et al., 2000).

A potential phospholipid scramblase (PLSCR1) has been isolated and cloned from human erythrocytes (Sims and Wiedmer, 2001). However, upon reconstitution in liposomes the protein exhibits a very low calcium-activated phospholipid scrambling activity (Bassé et al., 1996). In addition, blood cells from a mouse PLSCR1 knockout do not exhibit defects in lipid scrambling (Zhou et al., 2002), and further clarification of the precise cellular functions of PLSCR1 and other family members is required.

Biological functions of energy-coupled lipid flippases

The energy-independent flippases in organelles of the early secretory pathway are important for the proper assembly of the membrane. They allow lipids to equilibrate rapidly between the two bilayer leaflets. By contrast, ATP-driven flippases are responsible for a net transfer of specific lipids to one side of a membrane and thereby regulate the transbilayer lipid arrangement in the plasma membrane, and probably the late Golgi and endosomal compartments. The most obvious function of those flippases could be the generation and maintenance of an asymmetric transbilayer lipid distribution that provides the two membrane leaflets of organelles with different characteristics necessary for their respective physiological functions. In the case of the exoplasmic leaflet of the plasma membranes, the high level of PC and cholesterol provides a rather inert surface essential for the stability and barrier function. The enrichment of glycosphingolipids on the cell surface not only plays an important structural role protecting cells but is also essential for intercellular recognition and signal transduction (reviewed by Hakomori, 2003). By contrast, the preferential orientation of aminophospholipids in the cytoplasmic leaflet forms an interface promoting membrane-membrane interactions and fusion.

More important than concentrating aminophospholipids in the cytosolic leaflet may be to deplete them from the outer leaflet of plasma membranes. The exposure of PS on the cell surface signifies apoptosis and results in engulfment of the cells by macrophages bearing PS receptors (Fadok et al., 2000). In addition, an aminophospholipid translocase activity in the canalicular membrane of hepatocytes is essential for preserving the specific phospholipid composition of bile (Tannert et al., 2003): although aminophospholipids and PC each represent some 35% of the canalicular membrane lipids, aminophospholipids are virtually absent from bile, whereas PC accounts for 95% of bile phospholipids. In blood coagulation, rapid cell surface exposure of PS is an essential determinant in the assembly of coagulation factors on the activated platelet membrane (Lentz, 2003). Sperm cells might also, when stimulated, change their transbilayer lipid arrangement to favor fusion (Flesch et al., 2001; Gadella and Harrison, 2002). Thus, bilayer asymmetry and its rapid change, e.g. by activation of the scramblase provide a system to modulate the

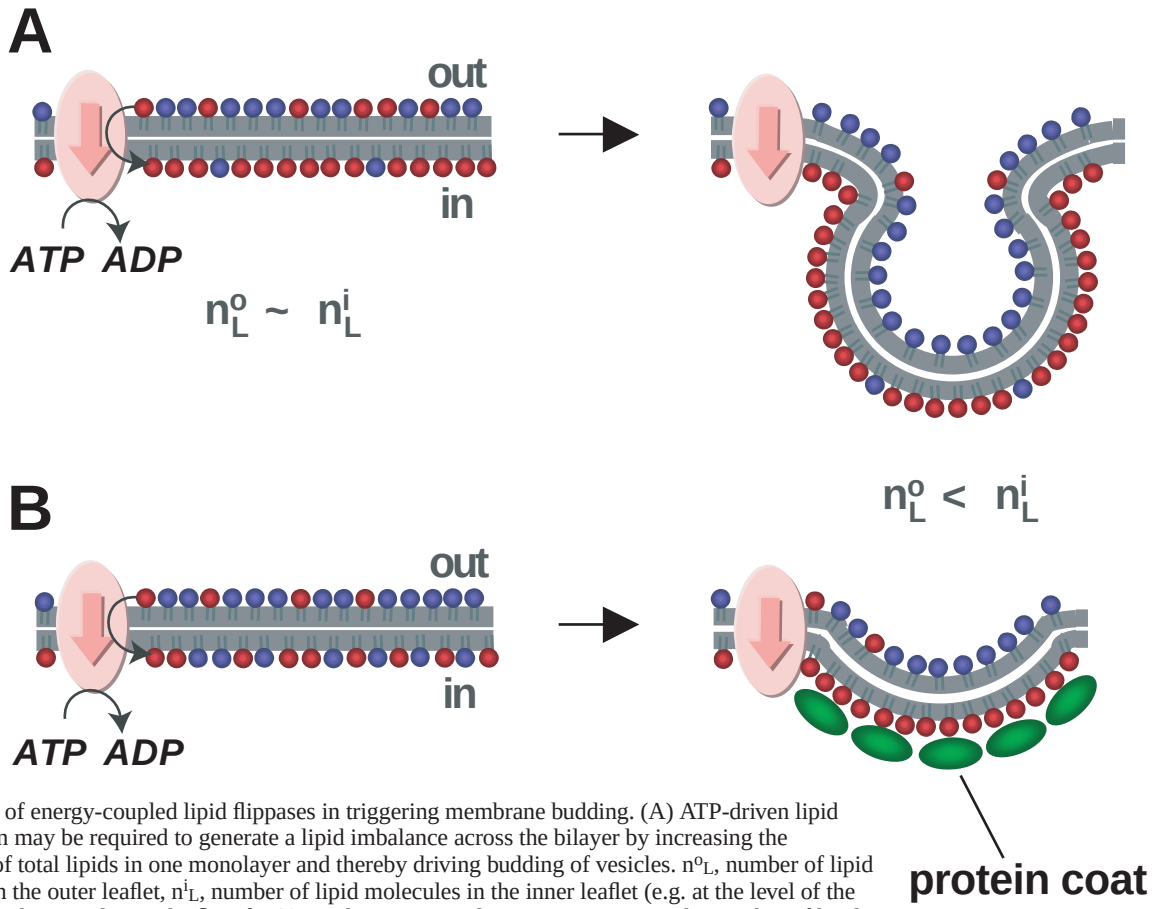

Fig. 3. Role of energy-coupled lipid flippases in triggering membrane budding. (A) ATP-driven lipid translocation may be required to generate a lipid imbalance across the bilayer by increasing the proportion of total lipids in one monolayer and thereby driving budding of vesicles. n^o_L, number of lipid molecules in the outer leaflet, n^i_L, number of lipid molecules in the inner leaflet (e.g. at the level of the head groups, the cytoplasmic leaflet of a 60 nm diameter vesicle contains 1.5 times the number of lipid molecules of the lumenal leaflet). (B) ATP-driven lipid translocation may help to create a high local concentration of aminophospholipids in the cytosolic leaflet favorable for recruitment of peripheral proteins, such as ARF, clathrin, amphiphysin and endophilins.

biological activity of the exoplasmic leaflet. The system shares many common features with the regulation of ion movements across membranes by ion pumps and ion channels.

In the absence of a compensatory flux, unidirectional transport of lipids by energy-coupled flippases creates a mass imbalance between the two membrane leaflets. Remarkably, this appears to have a critical role in membrane budding and endocytosis. In fact, membrane bending and the generation of a lipid imbalance across the bilayer is a prerequisite for vesicle budding. Experiments with synthetic membrane vesicles and erythrocytes have shown that insertion of small amounts of additional lipids (less than 1%) in one of the membrane leaflets is sufficient to induce dramatic shape changes (Sheetz and Singer, 1974; Farge and Devaux, 1992; Mui et al., 1995; Mathivet et al., 1996). Adopting a transbilayer lipid arrangement permissive for vesicle formation might not pose a problem to ER and cis-Golgi membranes, where phospholipids can rapidly cross the bilayer in both directions owing to the presence of energy-independent, bi-directional flippases. Here, assembly of a protein coat may exert a force sufficient to deform the bilayer into a bud. In the plasma membrane, the late Golgi and endosomes, however, the free ‘flip-flop’ of phospholipids across the bilayer is constrained. In these organelles, it would be hard to accomplish the transbilayer lipid imbalance required for vesicle budding without assistance of ATP-driven lipid flippases (Fig. 3). In this case, coat assembly would determine the site on the membrane where budding occurs. Direct participation of ATP-driven lipid flippases in vesicle budding is supported by the observation that inward translocation of short-chain PS and PE by the aminophospholipid translocase in the plasma membrane of human erythroleukemia cells stimulates endocytosis (Farge et al., 1999; Rauch and Farge, 2000). Intriguingly, yeast lacking the Dnf1p, Dnf2p and Drs2p display a general defect in endocytosis (Pomorski et al., 2003). Moreover, loss of Drs2p results in a decrease in clathrin-coated vesicle budding from the trans-Golgi (Chen et al., 1999; Gall et al., 2002). Conversely, overexpression of ABC transporters with outward-directed lipid translocase activity causes a defect in endocytosis (Kean et al., 1997; Decottignies et al., 1998), and loss of ABCA1 function in Tangier fibroblasts is associated with enhanced endocytosis (Zha et al., 2001). Collectively, these data suggest a functional link between lipid transport and vesicle biogenesis. Notably, in this process the type of lipid used as transport substrate would not be important.

These functions of energy-coupled lipid flippases are not mutually exclusive. The enrichment of aminophospholipid head groups in the cytoplasmic leaflet of mammalian plasma membranes may support recruitment or activity of peripheral membrane proteins that have a critical function in vesicle

budding as well as the docking and subsequent fusion of intracellular vesicles with the plasma membrane in exocytosis. At the same time, the transport of lipids to the cytoplasmic leaflet may trigger bending of the plasma membrane, which might resemble an early step of endocytosis (as discussed above). In the former case, the properties of the head groups create a supportive interface for membrane-membrane interaction, while in the latter case the headgroup may be irrelevant. The existence of 12 or more potential aminophospholipid translocases in man (Halleck et al., 1999) and the fact that each of the five family members in yeast is selectively located in one organelle of the late secretory and endocytotic recycling pathways raises the possibility that these proteins independently regulate the rate of vesicle budding from each organelle.

Perspectives

Now that several potential lipid flippases have been identified, functional reconstitution into proteoliposomes with natural, unlabeled lipids is an obligatory step for proving that these proteins are directly responsible for lipid transport. The next important challenge will be to unravel the precise nature of lipid transport mediated by the various flippases. Depending on the type of transporter, different mechanisms can be envisaged. The flippase could bind the lipid substrate in the cytoplasmic leaflet and flip it across the membrane to deliver the molecule to the exoplasmic leaflet. Instead of flipping it between the leaflets, the transporter could move the molecule onto a membrane-bound acceptor, as suggested for many ABC transporters. In the latter case, the transporter would be involved in lipid efflux rather than in the maintenance of membrane lipid asymmetry. Furthermore, the presence of ATP-dependent lipid transporters is not sufficient to ensure transbilayer asymmetry of lipids. Lipid asymmetry can persist only in the absence of non-specific rapid flip-flop, and this also depends on the membrane lipid composition. Thus, insight into how cells regulate the lipid composition of each membrane will be essential for a complete understanding of the lipid arrangements in the membranes of all organelles. Additional complexity comes from the fact that the lipid arrangement in cellular membranes clearly relies on the interplay between selective and non-selective flippases and each membrane may contain more than one flippase. A challenge for the future is to map the subcellular locations of these flippases, to evaluate their transport efficiency and to establish whether and how their activities are regulated in the living cell.

References

Araujo-Santos, J. M., Gamarro, F., Castanys, S., Herrmann, A. and Pomorski, T. (2003). Rapid transport of phospholipids across the plasma membrane of Leishmania infantum. *Biochem. Biophys. Res. Commun.* **306**, 250-255.

Bassé, F., Stout, J. G., Sims, P. J. and Wiedmer, T. (1996). Isolation of an erythrocyte membrane protein that mediates Ca^{2+}-dependent transbilayer movement of phospholipid. *J. Biol. Chem.* **271**, 17205-17210.

Bell, R. M., Ballas, L. M. and Coleman, R. A. (1981). Lipid topogenesis. *J. Lipid Res.* **22**, 391-403.

Berge, K. E., Tian, H., Graf, G. A., Yu, L., Grishin, N. V., Schultz, J., Kwiterovich, P., Shan, B., Barnes, R. and Hobbs, H. H. (2000). Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science* **290**, 1771-1775.

Bezombes, C., Maestre, N., Laurent, G., Levade, T., Bettaieb, A. and Jaffrezou, J. P. (1998). Restoration of TNF-alpha-induced ceramide generation and apoptosis in resistant human leukemia KG1a cells by the P-glycoprotein blocker PSC833. *FASEB J.* **12**, 101-109.

Bishop, W. R. and Bell, R. M. (1985). Assembly of the endoplasmic reticulum phospholipid bilayer: the phosphatidylcholine transporter. *Cell* **42**, 51-60.

Bretscher, M. S. (1972). Asymmetrical lipid bilayer structure for biological membranes. *Nat. New Biol.* **236**, 11-12.

Bretscher, M. S. (1973). Membrane structure: some general principles. *Science* **181**, 622-629.

Burger, K. N., van der Bijl, P. and van Meer, G. (1996). Topology of sphingolipid galactosyltransferases in ER and Golgi: transbilayer movement of monohexosyl sphingolipids is required for higher glycosphingolipid biosynthesis. *J. Cell Biol.* **133**, 15-28.

Buton, X., Morrot, G., Fellmann, P. and Seigneuret, M. (1996). Ultrafast glycerophospholipid-selective transbilayer motion mediated by a protein in the endoplasmic reticulum membrane. *J. Biol. Chem.* **271**, 6651-6657.

Buton, X., Hervé, P., Kubelt, J., Tannert, A., Burger, K. N., Fellmann, P., Muller, P., Herrmann, A., Seigneuret, M. and Devaux, P. F. (2002). Transbilayer movement of monohexosyl sphingolipids in endoplasmic reticulum and Golgi membranes. *Biochemistry* **41**, 13106-13115.

Catty, P., de Kerchove d'Exaerde, A. and Goffeau, A. (1997). The complete inventory of the yeast Saccharomyces cerevisiae P-type transport ATPases. *FEBS Lett.* **409**, 325-332.

Chambenoit, Y., Hamon, D., Marguet, D., Rigneault, H., Rosseneu, M. and Chimini, G. (2001). Specific docking of apolipoprotein A-I at the cell surface requires a functional ABCA1 transporter. *J. Biol. Chem.* **276**, 9955-9960.

Chen, C. Y., Ingram, M. F., Rosal, P. H. and Graham, T. R. (1999). Role of Drs2p, a P-type ATPase and potential aminophospholipid translocase, in yeast late Golgi function. *J. Cell Biol.* **147**, 1223-1236.

Chojnacki, T. and Dallner, G. (1988). The biological role of dolichol. *Biochem. J.* **251**, 1-9.

Comfurius, P., Williamson, P., Smeets, E. F., Schlegel, R. A., Bevers, E. M. and Zwaal, R. F. (1996). Reconstitution of phospholipid scramblase activity from human blood platelets. *Biochemistry* **35**, 7631-7634.

Connor, J., Pak, C. H., Zwaal, R. F. and Schroit, A. J. (1992). Bidirectional transbilayer movement of phospholipid analogs in human red blood cells. Evidence for an ATP-dependent and protein-mediated process. *J. Biol. Chem.* **267**, 19412-19417.

Decottignies, A., Grant, A. M., Nichols, J. W., de Wet, H., McIntosh, D. B. and Goffeau, A. (1998). ATPase and multidrug transport activities of the overexpressed yeast ABC protein Yor1p. *J. Biol. Chem.* **273**, 12612-12622.

Dekkers, D. W., Comfurius, P., van Gool, R. G., Bevers, E. M. and Zwaal, R. F. (2000). Multidrug resistance protein 1 regulates lipid asymmetry in erythrocyte membranes. *Biochem. J.* **350**, 531-535.

De Kruijff, B. and van Zoelen, E. J. (1978). Effect of the phase transition on the transbilayer movement of dimyristoyl phosphatidylcholine in unilamellar vesicles. *Biochim. Biophys. Acta* **511**, 105-115.

Devaux, P. F., Fellmann, P. and Herve, P. (2002). Investigation on lipid asymmetry using lipid probes: Comparison between spin-labeled lipids and fluorescent lipids. *Chem. Phys. Lipids* **116**, 115-134.

Fadok, V. A., Bratton, D. L., Rose, D. M., Pearson, A., Ezekewitz, R. A., Henson, P. M. (2000). A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* **405**, 85-90.

Farge, E. and Devaux, P. F. (1992). Shape changes of giant liposomes induced by an asymmetric transmembrane distribution of phospholipids. *Biophys. J.* **61**, 347-357.

Farge, E., Ojcius, D. M., Subtil, A. and Dautry-Varsat, A. (1999). Enhancement of endocytosis due to aminophospholipid transport across the plasma membrane of living cells. *Am. J. Physiol.* **276**, C725-C733.

Flesch, F. M., Brouwers, J. F., Nievelstein, P. F., Verkleij, A. J., van Golde, L. M., Colenbrander, B. and Gadella, B. M. (2001). Bicarbonate stimulated phospholipid scrambling induces cholesterol redistribution and enables cholesterol depletion in the sperm plasma membrane. *J. Cell Sci.* **114**, 3543-3555.

Gadella, B. M. and Harrison, R. A. (2002). Capacitation induces cyclic adenosine 3′,5′-monophosphate-dependent, but apoptosis-unrelated, exposure of aminophospholipids at the apical head plasma membrane of boar sperm cells. *Biol Reprod.* **67**, 340-350.

Gaffet, P., Bettache, N. and Bienvenue, A. (1995). Transverse redistribution of phospholipids during human platelet activation: evidence for a vectorial outflux specific to aminophospholipids. *Biochemistry* **34**, 6762-6769.

Gall, W. E., Geething, N. C., Hua, Z., Ingram, M. F., Liu, K., Chen, S. I.

and Graham, T. R. (2002). Drs2p-dependent formation of exocytic clathrin-coated vesicles in vivo. *Curr. Biol.* **12**, 1623-1627.

Gomès, E., Jakobsen, M. K., Axelsen, K. B., Geisler, M. and Palmgren, M. G. (2000). Chilling tolerance in Arabidopsis involves ALA1, a member of a new family of putative aminophospholipid translocases. *Plant Cell* **12**, 2441-2454.

Graf, G. A., Li, W. P., Gerard, R. D., Gelissen, I., White, A., Cohen, J. C. and Hobbs, H. H. (2002). Coexpression of ATP-binding cassette proteins ABCG5 and ABCG8 permits their transport to the apical surface. *J. Clin. Invest.* **110**, 659-669.

Grant, A. M., Hanson, P. K., Malone, L. and Nichols, J. W. (2001). NBD-labeled phosphatidylcholine and phosphatidylethanolamine are internalized by transbilayer transport across the yeast plasma membrane. *Traffic* **2**, 37-50.

Hakomori, S. (2003). Structure, organization, and function of glycosphingolipids in membrane. *Curr. Opin. Hematol.* **10**, 16-24.

Halleck, M. S., Pradhan, D., Blackman, C., Berkes, C., Williamson, P. and Schlegel, R. A. (1998). Multiple members of a third subfamily of P-type ATPases identified by genomic sequences and ESTs. *Genome Res.* **8**, 354-361.

Halleck, M. S., Lawler, J. F. Jr, Blackshaw, S., Gao, L., Nagarajan, P., Hacker, C., Pyle, S., Newman, J. T., Nakanishi, Y., Ando, H., Weinstock, D., Williamson, P. and Schlegel, R. A. (1999). Differential expression of putative transbilayer amphipath transporters. *Physiol. Genomics* **1**, 139-150.

Hamilton, J. A. (2003). Fast flip-flop of cholesterol and fatty acids in membranes: implications for membrane transport proteins. *Curr. Opin. Lipidol.* **14**, 263-271.

Hamon, Y., Broccardo, C., Chambenoit, O., Luciani, M. F., Toti, F., Chaslin, S., Freyssinet, J. M., Devaux, P. F., McNeish, J., Marguet, D. and Chimini, G. (2000). ABC1 promotes engulfment of apoptotic cells and transbilayer redistribution of phosphatidylserine. *Nat. Cell Biol.* **2**, 399-406.

Hanson, P. K., Malone, L., Birchmore, J. L. and Nichols, J. W. (2003). Lem3p is essential for the uptake and potency of alkylphosphocholine drugs, edelfosine and miltefosine. *J. Biol. Chem.* **278**, 36041-36050.

Harder, T. and van Meer, G. (2003). Lipid-based membrane domains: Physics meets immunology. *Traffic* **4**, 812-820.

Helenius, J., Ng, D. T., Marolda, C. L., Walter, P., Valvano, M. A. and Aebi, M. (2002). Translocation of lipid-linked oligosaccharides across the ER membrane requires Rft1 protein. *Nature* **415**, 447-450.

Herrmann, A., Zachowski, A. and Devaux, P. F. (1990). Protein-mediated phospholipid translocation in the endoplasmic reticulum with low lipid specificity. *Biochemistry* **29**, 2023-2027.

Herzing, L. B., Kim, S. J., Cook, E. H. Jr and Ledbetter, D. H. (2001). The human aminophospholipid-transporting ATPase gene ATP10C maps adjacent to UBE3A and exhibits similar imprinted expression. *Am. J. Hum. Genet.* **68**, 1501-1505.

Hua, Z., Fatheddin, P. and Graham, T. R. (2002). An Essential Subfamily of Drs2p-related P-Type ATPases Is Required for Protein Trafficking between Golgi Complex and Endosomal/Vacuolar System. *Mol. Biol. Cell* **13**, 3162-3177.

Hua, Z. and Graham, T. R. (2003). Requirement for Neo1p in Retrograde Transport from the Golgi Complex to the Endoplasmic Reticulum. *Mol. Biol. Cell* **12**, 4971-4983.

John, K., Schreiber, S., Kubelt, J., Herrmann, A. and Muller, P. (2002). Transbilayer movement of phospholipids at the main phase transition of lipid membranes: implications for rapid flip-flop in biological membranes. *Biophys J.* **83**, 3315-3323.

Kamp, D. and Haest, C. W. (1998). Evidence for a role of the multidrug resistance protein (MRP) in the outward translocation of NBD-phospholipids in the erythrocyte membrane. *Biochim. Biophys. Acta.* **1372**, 91-101.

Kato, U., Emoto, K., Fredriksson, C., Nakamura, H., Ohta, A., Kobayashi, T., Murakami-Murofushi, K., Kobayashi, T. and Umeda, M. (2002). A novel membrane protein, Ros3p, is required for phospholipid translocation across the plasma membrane in Saccharomyces cerevisiae. *J. Biol. Chem.* **277**, 37855-37862.

Kean, L. S., Grant, A. M., Angeletti, C., Mahe, Y., Kuchler, K., Fuller, R. S. and Nichols, J. W. (1997). Plasma membrane translocation of fluorescent-labeled phosphatidylethanolamine is controlled by transcription regulators, PDR1 and PDR3. *J. Cell Biol.* **138**, 255-270.

Kol, M. A., de Kroon, A. I., Rijkers, D. T., Killian, J. A. and de Kruijff, B. (2001). Membrane-spanning peptides induce phospholipid flop: a model for phospholipid translocation across the inner membrane of E. coli. *Biochemistry* **40**, 10500-10506.

Kol, M. A., van Laak, A. N., Rijkers, D. T., Killian, J. A., de Kroon, A. I. and de Kruijff, B. (2003). Phospholipid flop induced by transmembrane peptides in model membranes is modulated by lipid composition. *Biochemistry* **42**, 231-237.

Kornberg, R. D. and McConnell, H. M. (1971). Inside-outside transitions of phospholipids in vesicle membranes. *Biochemistry* **10**, 1111-1120.

Lannert, H., Bünning, C., Jeckel, D. and Wieland, F. T. (1994). Lactosylceramide is synthesized in the lumen of the Golgi apparatus. *FEBS Lett.* **342**, 91-96.

Lentz, B. R. (2003). Exposure of platelet membrane phosphatidylserine regulates blood coagulation. *Prog. Lipid Res.* **5**, 423-438.

Marx, U., Polakowski, T., Pomorski, T., Lang, C., Nelson, H., Nelson, N. and Herrmann, A. (1999). Rapid transbilayer movement of fluorescent phospholipid analogues in the plama membrane of endocytosis-deficient yeast cells does not require the Drs2 protein. *Europ. J. Biochem.* **263**, 254-263.

Marx, U., Lassmann, G., Holzhutter, H. G., Wustner, D., Muller, P., Hohlig, A., Kubelt, J. and Herrmann, A. (2000). Rapid flip-flop of phospholipids in endoplasmic reticulum membranes studied by a stopped-flow approach. *Biophys J.* **78**, 2628-2640.

Mathivet, L., Cribier, S. and Devaux, P. F. (1996). Shape change and physical properties of giant phospholipid vesicles prepared in the presence of an AC electric field. *Biophys J.* **70**, 1112-1121.

Meguro, M., Kashiwagi, A., Mitsuya, K., Nakao, M., Kondo, I., Saitoh, S. and Oshimura, M. (2001). A novel maternally expressed gene, ATP10C, encodes a putative aminophospholipid translocase associated with Angelman syndrome. *Nat. Genet.* **28**, 19-20.

Menon, A. K., Watkins, W. E., III and Hrafnsdóttir, S. (2000). Specific proteins are required to translocate phosphatidylcholine bidirectionally across the endoplasmic reticulum. *Curr. Biol.* **10**, 241-252.

Morrot, G., Herve, P., Zachowski, A., Fellmann, P. and Devaux, P. F. (1989). Aminophospholipid translocase of human erythrocytes: phospholipid substrate specificity and effect of cholesterol. *Biochemistry* **28**, 3456-3462.

Mui, B. L., Dobereiner, H. G., Madden, T. D. and Cullis, P. R. (1995). Influence of transbilayer area asymmetry on the morphology of large unilamellar vesicles. *Biophys J.* **69**, 930-941.

Pérez-Victoria, F. J., Gamarro, F., Ouellette, M. and Castanys, S. (2003). Functional cloning of the miltefosine transporter: a novel P-type phospholipid translocase from Leishmania involved in drug resistance. *J. Biol. Chem.* **278**, 49965-49971.

Pohl, A., Lage, H., Muller, P., Pomorski, T. and Herrmann, A. (2002). Transport of phosphatidylserine via MDR1 (multidrug resistance 1) P-glycoprotein in a human gastric carcinoma cell line. *Biochem. J.* **365**, 259-268.

Pomorski, T., Herrmann, A., Muller, P., van Meer, G. and Burger, K. (1999) Protein-mediated inward translocation of phospholipids occurs in both the apical and basolateral plasma membrane domains of epithelial cells. *Biochemistry* **38**, 142-150.

Pomorski, T., Lombardi, R., Riezman, H., Devaux, P. F., van Meer, G. and Holthuis, J. C. M. (2003). Drs2p-related P-type ATPases Dnf1p and Dnf2p are required for phospholipid translocation across the yeast plasma membrane and serve a role in endocytosis. *Mol. Biol. Cell* **14**, 1240-1254.

Raggers, R. J., van Helvoort, A., Evers, R. and van Meer, G. (1999). The human multidrug resistance protein MRP1 translocates sphingolipid analogs across the plasma membrane. *J. Cell Sci.* **112**, 415-422.

Raggers, R. J., Pomorski, T., Holthuis, J. C. M., Kälin, N. and van Meer, G. (2000). Lipid Traffic: The ABC of transbilayer movement. *Traffic* **1**, 226-234.

Raggers, R. J., Vogels, I. and van Meer, G. (2001). Multidrug-resistance P-glycoprotein (MDR1) secretes platelet-activating factor. *Biochem. J.* **357**, 859-865.

Rauch, C. and Farge, E. (2000). Endocytosis switch controlled by transmembrane osmotic pressure and phospholipid number asymmetry. *Biophys J.* **78**, 3036-3047.

Ruetz, S. and Gros, P. (1994). Phosphatidylcholine translocase: a physiological role for the mdr2 gene. *Cell* **77**, 1071-1081.

Rush, J. S. and Waechter, C. J. (1998). Topological studies on the enzymes catalyzing the biosynthesis of Glc-P-dolichol and the triglucosyl cap of Glc3Man9GlcNAc2-P-P-dolichol in microsomal vesicles from pig brain: use of the processing glucosidases I/II as latency markers. *Glycobiology* **8**, 1207-1213.

Schmitz, G., Langmann, T. and Heimerl, S. (2001). Role of ABCG1 and other ABCG family members in lipid metabolism. *J. Lipid Res.* **42**, 1513-1520.

Seigneuret, M. and Devaux, P. F. (1984). ATP-dependent asymmetric distribution of spin-labeled phospholipids in the erythrocyte membrane: Relation to shape changes. *Proc. Natl. Acad. Sci. USA* **81**, 3751-3755.

Sheetz, M. P. and Singer, S. J. (1974). Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions. *Proc. Natl. Acad. Sci. USA* **71**, 4457-4461.

Siegmund, A., Grant, A., Angeletti, C., Malone, L., Nichols, J. W. and Rudolph, H. K. (1998). Loss of Drs2p does not abolish NBD-labeled phospholipid transfer across the plasma membrane of Saccharomyces cerevisiae. *J. Biol. Chem.* **273**, 34399-34405.

Sims, P. J. and Wiedmer, T. (2001). Unraveling the mysteries of phospholipid scrambling. *Thromb. Haemost.* **86**, 266-275.

Small, D. M. (2003). Role of ABC transporters in secretion of cholesterol from liver into bile. *Proc. Natl. Acad. Sci. USA* **100**, 4-6.

Smeets, E. F., Comfurius, P., Bevers, E. M. and Zwaal, R. F. (1994). Calcium-induced transbilayer scrambling of fluorescent phospholipid analogs in platelets and erythrocytes. *Biochim. Biophys. Acta* **1195**, 281-286.

Smit, J. J., Schinkel, A. H., Oude Elferink, R. P., Groen, A. K., Wagenaar, E., van Deemter, L., Mol, C. A., Ottenhoff, R., van der Lugt, N. M., van Roon, M. A. et al. (1993). Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipids from bile and to liver disease. *Cell* **75**, 451-462.

Smith, A. J., Timmermans-Hereijgers, J. L., Roelofsen, B., Wirtz, K. W., van Blitterswijk, W. J., Smit, J. J., Schinkel, A. H. and Borst, P. (1994). The human MDR3 P-glycoprotein promotes translocation of phosphatidylcholine through the plasma membrane of fibroblasts from transgenic mice. *FEBS Lett.* **354**, 263-266.

Tang, X., Halleck, M. S., Schlegel, R. A. and Williamson, P. (1996). A subfamily of P-type ATPases with aminophospholipid transporting activity. *Science* **272**, 1495-1497.

Tannert, A., Wustner, D., Bechstein, J., Muller, P., Devaux, P. F. and Herrmann, A. (2003). Aminophospholipids have no access to the lumenal side of the biliary canaliculus – Implications for the specific lipid composition of the bile fluid. *J. Biol. Chem.* **278**, 40631-40639.

Thompson, R. and Jansen, P. L. (2000). Genetic defects in hepatocanalicular transport. *Semin. Liver Dis.* **20**, 365-372.

Ujhazy, P., Ortiz, D., Misra, S., Li, S., Moseley, J., Jones, H. and Arias, I. M. (2001). Familial intrahepatic cholestasis 1: studies of localization and function. *Hepatology* **34**, 768-775.

van Helvoort, A., Smith, A. J., Sprong, H., Fritzsche, I., Schinkel, A. H., Borst, P. and van Meer, G. (1996). MDR1 P-glycoprotein is a lipid translocase of broad specifity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. *Cell* **87**, 507-517.

Voelker, D. R. (2003). New perspectives on the regulation of intermembrane glycerophospholipid traffic. *J. Lipid Res.* **44**, 441-449.

Wang, N., Silver, D. L., Thiele, C. and Tall, A. R. (2001). ATP-binding cassette transporter A1 (ABCA1) functions as a cholesterol efflux regulatory protein. *J. Biol. Chem.* **276**, 23742-23747.

Wang, N., Lan, D., Gerbod-Giannone, M., Linsel-Nitschke, P., Jehle, A. W., Chen, W., Martinez, L. O. and Tall, A. R. (2003). ATP-binding cassette transporter A7 (ABCA7) binds apolipoprotein A-I and mediates cellular phospholipid but not cholesterol efflux. *J. Biol. Chem.* **278**, 42906-42912.

Williamson, P., Bevers, E. M., Smeets, E. F., Comfurius, P., Schlegel, R. A. and Zwaal, R. F. (1995). Continuous analysis of the mechanism of activated transbilayer lipid movement in platelets. *Biochemistry* **34**, 10448-10455.

Yu, L., Hammer, R. E., Li-Hawkins, J., von Bergmann, K., Lutjohann, D., Cohen, J. C. and Hobbs, H. H. (2002). Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc. Natl. Acad. Sci. USA* **99**, 16237-16242.

Zachowski, A. (1993). Phospholipids in animal eukaryotic membranes: transverse asymmetry and movement. *Biochem. J.* **294**, 1-14.

Zha, X., Genest, J., Jr and McPherson, R. (2001). Endocytosis is enhanced in Tangier fibroblasts: possible role of ATP-binding cassette protein A1 in endosomal vesicular transport. *J. Biol. Chem.* **276**, 39476-39483.

Zhou, Q., Zhao, J., Wiedmer, T. and Sims. P. J. (2002). Normal hemostasis but defective hematopoietic response to growth factors in mice deficient in phospholipid scramblase 1. *Blood* **99**, 4030-4038.