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Review

Lipid Traffic: The ABC of Transbilayer Movement

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Membrane lipids do not spontaneously exchange between the two leaflets of lipid bilayers because the polar headgroups cannot cross the hydrophobic membrane interior. Cellular membranes, notably eukaryotic plasma membranes, are equipped with special proteins that actively translocate lipids from one leaflet to the other. In addition, cellular membranes contain proteins that facilitate a passive equilibration of lipids between the two membrane halves. In recent years, a growing number of proteins have been put forward as lipid translocators or facilitators. Unexpectedly, some of these appear to be required for efficient translocation of lipids lacking bulky headgroups, like cholesterol and fatty acids. The candidate lipid translocators identified so far belong to large protein families whose other members include pumps for amphiphilic molecules like bile salts and drugs.

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Lipid Asymmetry and Transbilayer Movement

Lipids are not homogeneously distributed throughout the eukaryotic cell. The membranes of different cellular organelles require distinct lipid (and protein) compositions. Even within a membrane lipids are not equally distributed over both membrane leaflets. The first observations on lipid asymmetry in the early 1970s were made on the erythrocyte membrane (1,2), on blood platelets, plasma membrane-derived membranes, such as viruses and endocytotic organelles (3), and finally on nucleated cells. They have led to the concept of lipid asymmetry as a general property of plasma membranes. In these membranes sphingomyelin (SM), and most of the phosphatidylcholine (PC) is situated in the external half of the bilayer, whereas the aminophospholipids phosphatidylserine (PS), and to a lesser extent phosphatidylethanolamine (PE), are confined to the cytosolic leaflet of the plasma membrane bilayer.

For several years lipid asymmetry was considered a stable property of membranes (3), but quite early on, it became clear that, in order to expand the ER, lipids newly synthesized on the cytosolic surface must move across the ER membrane. The movement of headgroups across the hydrophobic membrane interior is energetically unfavorable (see Spontaneous transbilayer movement below). Thus, in 1973 Bretscher (4) made the insightful proposal for the involvement of a 'flippase', a protein still unidentified today. Only in 1984, an ATP-driven and selective transport of aminophospholipids toward the inner leaflet of the plasma membrane was demonstrated, suggestive of an aminophospholipid translocase (5). It took another 10 years before the identification of the mouse Mdr2 P-glycoprotein (P-gp) as a putative PC-flippase operating in the opposite direction (6), and the cloning of a fatty acid transporter (7) and a putative aminophospholipid translocase (8).

In the years since, a growing number of proteins has been put forward as lipid flippases. Remarkably, these proteins turn out to belong to large protein families, whose other members include transporters of amphiphiles like bile acids and drugs. This implies that flipping of membrane lipids is mechanistically related to drug transport across cellular membranes. However, functional reconstitution of these proteins in model membranes is required to assess whether they are able to move lipids across the membrane by themselves. So far, this has only been achieved in rare cases.

Transbilayer Lipid Movement

Spontaneous transbilayer movement

The most abundant membrane lipid PC is a cylindrical molecule, that consists of two hydrophobic tails and a hydrophilic headgroup (Figure 1). Such amphipathic lipids form a bilayer with their hydrophobic tails towards the hydrophobic interior and their polar headgroup towards the aqueous environment. In pure lipid bilayers, PC moves across the lipid bilayer only slowly ($t_{1/2}$ = days) (9). This can easily be understood from the fact that it is energetically unfavorable to move the hydrophilic headgroup across the lipidic part of the bilayer. Even in the plasma membrane of erythrocytes and in membranes derived from the plasma membrane of nucleated cells, like viruses or phagosomes, transbilayer movement of PC is slow [$t_{1/2}$ = hours to days; see (3,9)]. This shows that membrane proteins, in general, do not induce rapid transbilayer lipid movement.

Some membrane lipids are not cylindrical, but are shaped like cones, e.g. PE, or inverted cones, lysophospholipids (10). Cone-shaped lipids disturb the ordered bilayer and can induce spontaneous transbilayer movement of other lipids. Evidence has been presented for the occurrence of inverted micelles in the ER membrane and in mitochondria, and they

could, in principle, be responsible for the rapid transmembrane lipid movement observed in the ER membrane (10). Inverted cones act as detergents; although, when present in low concentrations, a lipid like lyso-PC (Figure 1) displays slow transbilayer movement (9).

In contrast to their comparable transbilayer mobility, lysophospholipids and short-chain lipids, like platelet-activating factor (PAF; 2-C_2-PC), have a higher off-rate from membranes and higher diffusion between membranes through the aqueous phase than the usual membrane lipids with two long chains. Many studies on lipid translocation have exploited this convenient property by using analogs of membrane lipids where one fatty acid has been replaced by a short radiolabeled chain, or a short chain carrying a spin-label or fluorescent moiety. It should be obvious that the validity of these studies for natural lipids must eventually be tested in each case.

When the number of polar or charged moieties in the headgroup is enhanced, as in the higher glycosphingolipids, transbilayer movement is reduced. However, some polar phospholipids, such as phosphatidic acid or phosphatidylglycerol, can move between leaflets with half-times of seconds when the negative charge is neutralized by protonation (11). The same is true for fatty acids, although the $t_{1/2}$s are seconds to minutes (12). Amphiphilic molecules where the polar groups are scattered over the molecule may penetrate through lipid membranes easily or not, depending on their overall hydrophobicity; for example, hydrophobic bile acids ($t_{1/2} < 1$ s) versus taurine-conjugated bile acids ($t_{1/2} > 1$ h) (12).

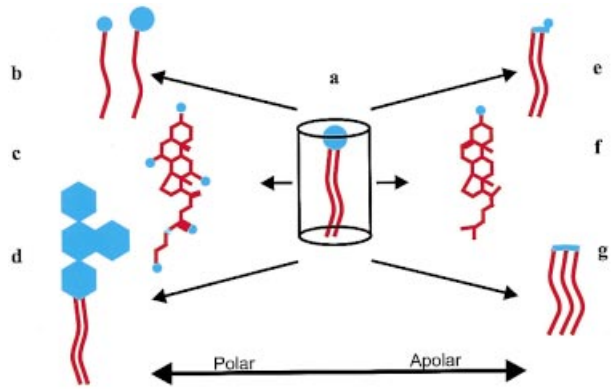

Figure 1: Amphiphilic character of various cellular lipids. Starting from PC (a) as a typical membrane lipid, polarity is increased by a decrease of the apolar tail of the lipid (e.g. fatty acids or lyso-lipids, b), by an increase of the size of the headgroup as for higher glycosphingolipids (e.g. GM_2, d), or by introduction of polar groups, (e.g. taurocholic acid, c). On the contrary, lipids become more apolar by a decrease of the headgroup (e.g. diacylglycerol, e, or cholesterol, f), or by an increase of the apolar tail of the lipid (e.g. triacylglycerol, g). The behavior of the various lipids within a membrane depends on the balance between polar and apolar parts within the lipid. Many drugs are amphiphilic and locate to the left of this spectrum.

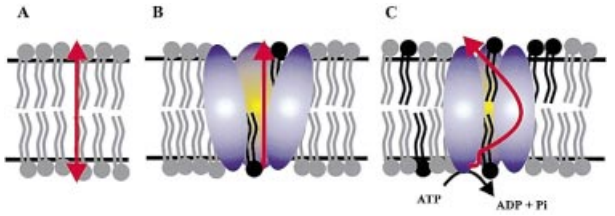

Figure 2: Possible mechanisms of lipid transbilayer movement. Polar headgroups can move across a membrane by various mechanisms. A) Spontaneous transbilayer movement: the rate is determined by the biophysical properties of both the lipid and the membrane. B) ATP-independent protein-mediated transbilayer movement; lipids can move (bi-directionally) along the (polar) surface provided by the protein. The protein could be selective, but cannot move lipids against a gradient. C) ATP-dependent lipid translocation. A specific lipid is translocated by the protein upon hydrolysis of ATP. The use of energy allows the protein to move a lipid against a gradient in the membrane. As discussed in the text, the lipid is collected within one leaflet (or from the aqueous phase) and either directly deposited into the opposite leaflet or extruded, after which it can partition back into the membrane.

The polarity of membrane lipids can be reduced by lowering the polarity of the head group and/or by an increase in the lipidic part. Extreme cases are triacylglycerols and cholesterol-esters (Figure 1), which no longer orient at the water-lipid interphase but reside in the hydrophobic membrane interior. Further examples are diacylglycerol and ceramide [$t_{1/2} = 70$ ms and 22 min, respectively, for their analogs containing one fluorescent C_5-fatty acid (13)]. Cholesterol is a special case. In the erythrocyte membrane transmembrane movement was measured with a $t_{1/2}$ of hours (14) whereas others reported 3 s (15). Apparently, the factors that govern the behavior of cholesterol in such assays are not under control. One of these may be its heterogeneous lateral distribution in the plane of the bilayer, whereby a tight lateral interaction with other lipids in liquid-ordered domains can reduce rates of transmembrane movement.

Protein-mediated transbilayer movement

Proteins can stimulate transbilayer movement of lipids in different ways (Figure 2). First of all, a protein can act as a facilitator, e.g. by providing a hydrophilic surface across the bilayer, along which the polar headgroup can slide. A facilitator allows lipids to assume an equilibrium distribution that is determined by the relative affinities for the two leaflets under the prevailing conditions. In principle, such a facilitator could be selective for certain lipids. It cannot move lipids against an energy gradient, e.g. an electro-(chemical) gradient, or lateral pressure.

In contrast, proteins may utilize energy (e.g. ATP) to move a lipid across the bilayer. Such 'translocators' can work against an energy gradient. Different mechanisms of action can be envisaged. (1) The lipid enters the protein channel sideways from one bilayer leaflet. The polar headgroup is then moved across the bilayer under the consumption of ATP, and the

lipid is released in the opposite membrane leaflet [the classical 'flippase' mechanism, which was later also proposed for the translocation of other amphiphiles, like drugs (16)]. (2) The translocator could also deposit the lipid into the medium at the opposite side of the membrane, from where it may partition back into the membrane. (3) Less likely is the possibility that the translocator picks up the lipid from the aqueous phase before moving it across. The latter mechanism is, however, found in many proteins that pump water-soluble substrates, like ions, and might apply to proteins that translocate some of the more water-soluble amphiphiles across membranes. Unfortunately, it is not easy to experimentally address whether a protein that is required for translocation represents a translocator, part of a translocator complex, or simply induces lipid movement indirectly, e.g. by creating an ion gradient.

The removal of a lipid from one bilayer leaflet and the insertion into the opposite leaflet results in an imbalance in lateral pressure. This stress may be relieved by the movement of one other lipid molecule in the opposite direction, or by expansion of one leaflet compared with the other inducing curvature. The latter effect, formulated in the bilayer couple hypothesis (17), does occur in biomembranes. It has been suggested that lipid translocation towards the cytosolic surface might be the driving force in vesicle budding during endocytosis (18,19). Lipid translocation in highly curved (model) membranes may generate pressure to a level where it blocks further action of the translocator. This puts constraints on the use of model membranes to study the activity of (reconstituted) lipid translocators.

Candidate ATP-Dependent Proteins Mediating Transbilayer Lipid Movement

P-type ATPases

P-type ATPases are primarily active transport systems that transport mono- or divalent cations across cellular membranes (20). Members of a novel subfamily of P-type ATPases have been implicated in the transbilayer movement of aminophospholipids (8) and bile acids (21). The proteins belonging to this 'DRS2' subfamily differ from the ion-transporting ATPases in several amino acids within transmembrane segments involved in ion translocation (22).

DRS2 and related proteins: aminophospholipids. The most intensively studied protein-mediated transverse movement of phospholipids is the selective ATP-dependent transport of PS and PE from the exoplasmic to the cytoplasmic leaflet of mammalian plasma membranes. First detected in the human erythrocyte membrane using spin-labeled and fluorescent lipid analogs, and long-chain radioactive phospholipids, the aminophospholipid translocating activity has now been demonstrated in various plasma membranes [for reviews see (3,9,18,23)] as well as in the membranes of secretory vesicles (9). A similar activity was found for both the basolateral and apical membrane of kidney epithelial cells, which unexpectedly also displayed translocation of short-chain (spin-labeled) PC (24).

Whereas several independent lines of evidence have indicated that maintenance of phospholipid asymmetry is dependent on the presence of an 'aminophospholipid translocase' (9,18,23), the molecular identity of this activity has not been

Table 1: Lipid synthesis, sidedness and transbilayer movement

Lipid	Site of synthesis[1]	Sidedness	Flip[2]
Glycerophospholipids			
Phosphatidylcholine	ER	Cytosolic	ER,PM
Phosphatidylethanolamine	ER	Cytosolic	ER,PM
	IMM	Intermembrane Space	M[2]
Phosphatidylserine	ER	Cytosolic	ER,PM,M[2]
Phosphatidylinositol	ER	Cytosolic	?
Sphingolipids			
Ceramide	ER	Cytosolic	ER,G
Sphingomyelin	Golgi	Lumenal	?
Glycosphingolipids			
Galactosylceramide	ER	Lumenal	ER
Glucosylceramide	Golgi	Cytosolic	G,PM
Higher glycolipids	Golgi	Lumenal	?
Cholesterol	ER/peroxisome		PM,G
Dolichol-P-X	ER	Cytosolic	ER
Phosphatidylinositol-glucosamine-Y	ER	Cytosolic	ER
Glucuronides	ER	Lumenal	ER,PM

[1] ER, endoplasmic reticulum; G, Golgi apparatus; IMM, inner mitochondrial membrane; M, mitochondria; PM, plasma membrane; X, glucose, mannose, or P-$(GlcNAc)_2Man_5$; Y, oligosaccharide or oligosaccharide-P-ethanolamine.
[2] PS decarboxylation occurs on the cytosolic surface of the IMM, thus ER-derived PS must flip across the outer membrane. The new PE must flip back to reach the ER.

unequivocally established yet. One candidate proposed is a 30–32 kDa integral membrane protein, which was identified via photoaffinity labeling with photoreactive PS, and found to bear the Rh antigens (25). As this protein lacks an ATP-binding site (26), it can only represent a subunit of the transporter. Another candidate, a 115–120 kDa Mg^{2+}-dependent-ATPase, has been purified from erythrocytes (27), synaptic vesicles and chromaffin granules (8). Cloning of the gene encoding this ATPase from bovine chromaffin granules revealed it to be a member of an ancient and previously unrecognized subfamily of P-type ATPases (8,22). Disruption of a homologous gene in yeast, the *DRS2* gene, abolished the internalization of a fluorescent PS analog (C_6-NBD-PS) at low temperature (8). This finding was interpreted as evidence for the biological function of this subfamily of ATPases as aminophospholipid translocases. However, in two subsequent studies, deletion of *DRS2* in yeast had no (specific) effect on the uptake or distribution of fluorescent PS or PE analogs (28,29), nor on the amount of endogenous aminophospholipids exposed on the exoplasmic leaflet of the plasma membrane (29). These observations argue against the idea that Drs2p is the exclusive or major aminophospholipid translocase in the plasma membrane of yeast. Interestingly, *DRS2* and four yeast homologs of unknown function form a distinct subgroup, cluster II, within the P-type ATPase family (22). Whether the homologs encode additional aminophospholipid translocases that can compensate for a loss of Drs2p remains to be established.

FIC1: bile acids. Recently, the first human member of the DRS2 subfamily of P-type ATPases was identified by mapping the gene mutated in two forms of familial intrahepatic cholestasis (21). The *FIC1*-encoded ATPase is expressed in several epithelial tissues, more strongly in small intestine than in liver (21). It is not the human counterpart of the bovine aminophospholipid translocator, and supposedly plays a role in the enterohepatic circulation of bile acids, possibly as a translocator of bile acids.

ABC transporters

ATP-binding cassette (ABC) transporters (16) or traffic ATPases (30) form a superfamily of proteins that translocate a wide range of substrates across a variety of cellular membranes. Substrates include sugars, amino acids, peptides, proteins, metals, (in)organic ions, toxins, and antibiotics. ABC transporters have been associated with different clinical manifestations in man, such as cystic fibrosis (*CFTR*), multidrug resistance (*MDR1*, *MRP1*), intrahepatic cholestasis (*MDR3*), adrenoleukodystrophy (*ALD*) and hyperinsulinism (*SUR1*). Recently, various ABC transporters have been implicated in the transport of amphiphilic substances, including an increasing number of membrane lipids, across membranes. The ABC transporter family is found in organisms throughout evolution. The functional transport unit comprising two ATP-binding domains typical of ABC-transporters (31) and two hydrophobic domains, each containing five to eight membrane-spanning regions, can occur as one complete transporter (MDR1 P-gp), two half-transporters (ALDP), or four polypeptides (some bacterial transporters).

MDR3 P-glycoprotein: PC. Mice homozygous for a disruption of the *mdr2* gene, an ABC transporter of the multidrug transporter P-glycoprotein (P-gp) subfamily of unknown function, were found unable to transport PC into bile (32). A subtype of progressive familial intrahepatic cholestasis with a similar lack of phospholipids in bile has recently been found to be caused by mutations in the *MDR3* gene, the human homolog of the mouse *mdr2* (33). MDR3 P-gp with a MW of 140 kDa (32) is highly expressed in the bile canalicular membrane of hepatocytes, and to some extent in the adrenal, heart, striated muscle, spleen, and tonsil (33). Evidence that mdr2/MDR3 P-gp is a PC translocator was obtained when it was found that secretory vesicles from yeast transfected with *mdr2* can accumulate the short-chain PC analog C_6-NBD-PC (34) [however, cf. (35)]. Similarly, epithelial cells transfected with human *MDR3* translocated intracellularly synthesized C_6-NBD-PC towards the outer leaflet of the plasma membrane, as measured in the absence of vesicular transport (36). While MDR3 P-gp-mediated translocation was specific for C_6-NBD-PC versus the NBD-analogs of PE, SM and glucosylceramide (GlcCer), C_8C_8-PC was not translocated, suggesting a specificity for distinct PC species. Translocation of endogenous PC was addressed in MDR3 P-gp transfected fibroblasts from *mdr2* knock out mice (6). Accessibility of newly synthesized PC to an exogenous PC transfer protein was enhanced after transfection (but this approach cannot exclude differences in vesicular PC transport). In addition to MDR3 P-gp, evidence has been presented for an energy-independent PC flippase in the bile canalicular membrane (37).

MDR3 P-gp displays 80% homology with MDR1 P-gp, a multidrug transporter, and it has been suggested that MDR3 P-gp also pumps certain drugs (38). Actually, translocation of PC by MDR3 P-gp is inhibited by verapamil (34,36), a modulator and substrate of MDR1 P-gp (39) demonstrating that MDR3 P-gp has, at least, a binding site for drugs.

MDR1 P-glycoprotein: broad specificity. The human ABC-transporter MDR1 P-gp has been identified as one major protein causing the multidrug resistance phenotype, the resistance of cancer cells against chemotherapy. MDR1 P-gp is ubiquitously expressed at low levels. It is concentrated in the apical membrane of many epithelial and endothelial cells (and at the cell surface of many tumor cells). It extrudes a wide variety of chemically diverse drugs from the cell that share a few basic features (39).

Quite unexpectedly, human MDR1 P-gp and mouse mdr1a (= mouse mdr3) P-gp, when overexpressed, translocated NBD-PC, very similar to MDR3 P-gp (36,40). This was different to earlier findings (34). C_{12}-NBD-PS was not an MDR1-substrate (although the assay could not exclude that the PS was not available to MDR1 P-gp for different reasons), whereas MDR1 P-gp, but not MDR3 P-gp, also translocated C_6-NBD- and C_6-analogs of the sphingolipids GlcCer and SM. Translocation depended on ATP and was inhibited by verapamil and PSC 833. GlcCer is an interesting candidate for

Table 2: Proteins involved in transbilayer lipid movement

Protein	Lipid	Movement	Disease
ATP-dependent			
DRS2	PS, PE	In, PM	
FIC1	Bile acids	?, PM	Familial intrahepatic cholestasis
MDR3 P-gp *(ABCB4)*	PC	Out, canalicular membrane	Familial intrahepatic cholestasis
MDR1 P-gp *(ABCB1)*	Drugs, (glyco)lipids	Out, PM (apical)	Multidrug resistance
SPGP *(ABCB11)*	Bile salts	Out, canalicular membrane	
MRP1 *(ABCC1)*	Drugs, anions	Out, PM (basolateral)	Multidrug resistance
MRP2 *(ABCC2)*	Organic anions	Out, canalicular	
ABC1 *(ABCA1)*	Cholesterol	Out, PM	Tangier disease, FHA
ALDP *(ABCD1)*	Fatty acids	Import in peroxisomes	X-linked adreno-leukodystrophy
ATP-independent			
Scramblase	Lipids	In/out	Scott syndrome (23)
FATP	Fatty acids	In, PM	

being an MDR1 P-gp substrate. It is synthesized from ceramide on the cytosolic surface of the Golgi and ends up in the outer leaflet of the plasma membrane. GlcCer levels were only half in cells lacking multidrug transporters, and MDR1 P-gp appears to rescue GlcCer from hydrolysis by a cytosolic hydrolase (R.J. Raggers et al., unpublished). In earlier studies, multidrug resistance has been linked to GlcCer accumulation, and in one case this has been explained via the removal of ceramide (41).

MDR1 P-gp has also been proposed to be involved in the intracellular trafficking of cholesterol, but it is unclear whether the effect is related to MDR1 P-gp's drug transport activity (42,43). Alternatively, it might be linked to translocation of GlcCer or another sphingolipid as cholesterol preferentially interacts with sphingolipids. A third member of the P-gp subfamily, the sister of P-gp (SPGP) is now known to be the pump of the canalicular membrane responsible for exporting amphiphilic bile salts (44).

MRP1-MRPx: organic anions, glycosphingolipids and glycerophospholipids. The multidrug resistance-related protein MRP1 was shown to be a drug-efflux pump in multidrug resistant cells, not overexpressing MDR1 P-gp (45). MRP1, like MDR1 P-gp, translocated C_6-NBD-GlcCer and C_6-NBD-SM across the plasma membrane in transfected epithelial cells. However, translocation by MRP1 occurred at the basolateral, not the apical, plasma membrane, it depended on glutathione and was sensitive to MRP inhibitors like sulfinpyrazone and indomethacin, but not to the MDR1 inhibitor PSC 833 (46). In contrast to MDR1 P-gp (see above), MRP1 translocated C_6-NBD-PS towards the outer leaflet in erythrocytes, but did not affect endogenous PS distribution (23). MRP1 did not translocate radiolabeled C_6-sphingolipids (46). Thus, normal long-chain lipids are most likely not translocated by MRP1. Fluorescent lipids may be recognized as xenobiotics, and results on these analogs should not be extrapolated to natural lipids.

MRP2 (cMOAT) is highly similar to MRP1 in substrate specificity, but is highly expressed in the canalicular, not the sinusoidal basolateral, membrane of hepatocytes (47). Like MRP1 and MRP2, MRP3, mainly present in liver, is an organic anion and multidrug transporter, while MRP5 is thought to be an anion transporter that is ubiquitously expressed (48). So far, we have not observed translocation of C_6-NBD-lipids by any MRP besides MRP1 (R.J. Raggers et al., unpublished), but this does not exclude the possibility of specific natural lipids being substrates of a particular MRP.

ABC1: cholesterol. ABC1, quite unexpectedly, was found to be the protein that is mutated in Tangier disease, an autosomal recessive disorder of lipid metabolism (49–51). Tangier patients have very low plasma HDL levels and accumulate massive amounts of cholesterol-ester within the macrophages of various tissues. Their fibroblasts display a defect in HDL- or apolipoprotein A1-induced efflux of cholesterol (and of PC and SM) (49–51). Familial HDL deficiency (FHA) is also associated with mutations in the ABC1 gene (50). The possibility exists that the rate of spontaneous transmembrane movement of cholesterol (see Spontaneous transbilayer movement) is insufficient for HDL loading, and that ABC1 is a cholesterol translocator. However, an indirect role of ABC1, at present, cannot be excluded. ABC1 has been proposed to be involved in the engulfment and clearance of dead cells (52), in the secretion of leaderless proteins like IL-1β (53), and in the generation of a regulated anion flux (54).

ALDP and related proteins: fatty acids. X-linked adrenoleukodystrophy (X-ALD), a lethal neurodegenerative disorder, is characterized by accumulation of very-long-chain fatty acids (VLCFA; $>$C22:0) in serum, due to decreased β-oxidative degradation of VLCFAs in the peroxisomes (55). The defective gene, *ALD*, encodes the peroxisomal membrane half ABC transporter ALDP (56). Genetic and biochemical studies in *Saccharomyces cerevisiae* revealed that two peroxisomal homologs of ALDP, Pat1p (Pxa2p) and Pat2p (Pxa1p) play a critical role in the β-oxidation of long-chain fatty acids (LCFAs) like oleate, C18:1, and palmitate, C16:0, but not medium-chain fatty acids like myristate, C14:0. Because β-oxidation activities in detergent lysates from mutants with disrupted *PAT1* or *PAT2* genes were normal, Pat1p and Pat2p

may import LCFAs into the yeast peroxisome (57). In yeast, LCFAs are activated in the cytosol. Long-chain acyl-CoAs are therefore the candidate substrate for translocation (58). The structural similarity between Pat1p, Pat2p, and human ALDP combined with the striking resemblance in the defects observed in pat mutants and X-ALD patients suggests that ALDP is involved in the uptake of VLCFAs into human peroxisomes. If, in contrast to yeast, activation of VLCFAs by acyl-CoA synthetase in man would occur inside the peroxisome (59), ALDP would transport free VLCFAs, instead of very long-chain acyl-CoAs, across the peroxisomal membrane.

Three structurally related half ABC transporters have been identified in the human peroxisomal membrane: the ALDP-related protein (ALDRP); the 70 kDa peroxisomal membrane protein (PMP70); and the PMP70-related protein (P70R). Overexpression of PMP70 or ALDRP restored the β-oxidative capacity of peroxisomes in X-ALD fibroblasts and in ALDP-deficient mice, reflecting a significant degree of functional similarity between these proteins (60,61). The half transporters presumably function as homo- or heterodimers with potentially distinct substrate specificities.

Candidate ATP-Independent Proteins Mediating Transbilayer Lipid Movement

The scramblase: a plasma membrane blender

A rise in intracellular [Ca^{2+}] causes a loss of phospholipid asymmetry in the plasma membrane of blood cells, or lipid scrambling (23). Lipid analogs of the major glycerophospholipids PS, PE and PC [but also platelet activating factor (62) and palmitoylcarnitine (63)] rapidly equilibrated between the two plasma membrane leaflets, with slower mobility for SM. The resulting exposure of PS on the cell surface plays a critical role in blood coagulation and in the clearance of aged blood cells by macrophages, and has been recognized as a hallmark of apoptosis in many different cell types, including yeast (64). Phospholipid scrambling is ATP-independent, bidirectional and not solely due to the inhibition of the aminophospholipid translocase since erythrocytes depleted of ATP or treated with inhibitors of the aminophospholipid translocase retain an asymmetric lipid distribution for many hours (23). A membrane protein fraction from platelets with scramblase activity was reconstituted into proteoliposomes (65). A type II membrane protein of 37 kDa from erythrocytes was identified by molecular cloning, and found capable of mediating calcium-dependent transbilayer movement of phospholipids in reconstituted liposomes, although the apparent rate of phospholipid scrambling was rather low ($t_{1/2}$ of 2 h) (66,67). The mRNA of the putative scramblase was detected in a variety of cells (67), expression levels of the protein in various cells corresponding with scrambling activity (68). Whether all membrane lipids are scramblase substrates remains to be established.

ER flippase: mixing lipids

The cytosolic surface of the ER is the site of synthesis for numerous membrane lipids (see Tables 1 and 2). For expansion of the ER membrane to occur, the newly synthesized lipids should redistribute across the ER membrane, possibly aided by a protein. Although, originally, the transbilayer movement was reported only for PC (4,69), later work found no evidence for the suggested specificity (70,71). The bilayer movement appears to equilibrate the lipids across both leaflets (70). Measured $t_{1/2}$s of movement range from less than 25 s (71), to 2–3 min (70,72) and 45 min (73). Recently, by a new assay, the redistribution of spin-labeled and fluorescent phospholipid analogs appeared to be even more rapid than previously assumed with $t_{1/2}$ of 16 s and 2 min, respectively (U. Marx, G. Laßmann, H.-G. Holzhuetter, D. Wuestner, P. Mueller, A. Hoehlig and A. Herrmann, personal communication). Interestingly, rapid movement of phospholipid analogs and endogenous PE (74,75) has also been reported for bacterial membranes, which are similar to the ER in that new lipids are synthesized at their cytoplasmic leaflet.

The successful reconstitution of transport-active proteoliposomes from detergent-solubilized ER vesicles under conditions where protein-free liposomes were inactive [(4) and A. Menon, personal communication], supports the notion of an ER-flippase. However, the involvement of non-bilayer arrangements of lipids in the ER may facilitate lipid translocation (10). Apart from the phospholipids, highly complex lipids flip across the ER membrane in the biological processes of the synthesis of glycosylphosphatidylinositol (GPI)-anchored proteins and the N-glycosylation of proteins on the lumenal surface of the ER, while the glycosylated phosphatidylinositol and phosphodolichol precursors are synthesized on the cytosolic surface. Interestingly, after glucuronation of bilirubin by lumenal glucuronyltransferases, for example, the resulting bilirubin glucuronide must be transported towards the cytosol to become available for MRP2-mediated pumping across the plasma membrane. In addition, C_6-NBD-galactosylceramide, synthesized in the lumen of the ER, also became rapidly available at the cytosolic leaflet (76).

FATP: fatty acid uptake

A long search for the protein(s) responsible for the saturable uptake of LCFAs by adipocytes resulted in the identification of a protein, by expression cloning, that is used by adipocytes for the efficient import of LCFAs (7). The FATP, with an apparent MW of 63 kDa, was found to be highly expressed in skeletal muscle, heart and fat, moderately expressed in brain, kidney, lung and liver, and not in spleen or intestine. It does not contain an ATP-binding motif.

A Golgi glucosylceramide translocator?

An as yet unidentified protein is involved in the transbilayer movement of GlcCer in the Golgi membrane. GlcCer is synthesized at the cytosolic surface of the Golgi complex. Since the galactosyltransferase that converts GlcCer to LacCer and the enzymes acting in later steps of glycosphingolipid biosynthesis have their active centers in the Golgi lumen, GlcCer must flip from the cytosolic leaflet of the Golgi membrane to the lumenal leaflet. Based on the use of short-chain lipid analogs on enriched Golgi membranes, it was suggested that

this translocation is ATP-independent (76,77). The translocator is not MDR1 P-gp (R.J. Raggers et al., unpublished). The exact localization of GlcCer translocation is unknown, but has been suggested to be in the same compartment in which GlcCer is converted into LacCer, the *trans* Golgi (77). Alternatively, the ER-flippase may be involved in this process in the *cis* Golgi. Possibly, the translocase forms a complex with the GlcCer synthase (78).

From ABC to . . . . XYZ

Various proteins have now been identified that are involved in translocating lipids across cellular membranes. In the short-term, two major challenges must be met in order to establish that a candidate lipid translocator actually translocates natural lipids: (i) the development of assays to measure transbilayer movement of these lipids; and (ii) measurements in reconstituted systems with defined lipid and protein compositions. The latter should reveal whether a translocator consists of a single or of multiple polypeptides acting in a complex. Translocators appear to be members of large families. Presumably, the identification of other members will follow, maybe even of new families of translocators and facilitators. For example, proteins involved in phospholipid translocation have so far not been characterized in mitochondria and peroxisomes, despite the fact that these membranes must grow by the import of lipids via their cytosolic surface and subsequent redistribution over the bilayer(s). Each cellular membrane may well contain more than one type of translocator or facilitator. The next challenge will therefore be to understand how their actions are regulated and coordinated under different physiological conditions. Although the translocation of a lipid seems a small step on the map of intracellular trafficking, it turns out that we have underestimated its significance for the functioning of cells.

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