Lipid Asymmetry of the Eukaryotic Plasma Membrane: Functions and Related Enzymes
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Received December 12, 2005

1. INTRODUCTION

Lipids of the plasma membrane are distributed asymmetrically between two leaflets, and the presence of glycerophospholipids and sphingolipids contribute to this asymmetry (Fig. 1). Of the glycerophospholipids, phosphatidylcholine (PC) is located mainly in the outer (extracytosolic) leaflet, and the aminophospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE), as well as minor lipids like phosphatidylglycerol (PG) and phosphatidic acid (PA), are abundant in the inner (cytosolic) leaflet. The sphingolipids, sphingomyelin (SM) and glucosylsphingolipids, are confined to the outer leaflet. Translocation of lipids from one leaflet to the other is called 'flip-flop', with 'flip' being the movement from the extracytosolic leaflet to cytosolic leaflet, and 'flop' being the reverse. Since the polar head groups of glycerophospholipids and sphingolipids make a large contribution to this asymmetry, flipping of phosphatidylserine (PS) and phosphatidylethanolamine (PE) is mediated by P-type ATPases/aminophospholipid translocases. Flopping of glycerophospholipids is catalyzed by ABC transporters. Maintenance of proper lipid asymmetry is required for the mechanical stability of the membrane and for vesicular transport. On the other hand, local or global changes in lipid asymmetry are important for cell cycle progression, apoptosis, and platelet coagulation. Three classes of lipid translocases, P-type ATPases, ABC transporters, and scramblases, are known to be involved in the regulation of lipid asymmetry. In this review, we describe the physiological and pathological functions of lipid asymmetry and the current knowledge of lipid translocases.

Key words lipid translocase; plasma membrane; glycerophospholipid; sphingolipid; flip-flop

Biological membranes are composed of lipid bilayers. Major lipid components of the eukaryotic plasma membrane include glycerophospholipids, sphingolipids, and cholesterol. Lipids are irregularly distributed between the two leaflets, thus causing lipid asymmetry, or within the same leaflet, forming a lipid microdomain. Glycerophospholipids and sphingolipids both contribute to the lipid asymmetry, whereas cholesterol and sphingolipids form lipid microdomains. Maintenance of proper lipid asymmetry is required for the mechanical stability of the membrane and for vesicular transport. On the other hand, local or global changes in lipid asymmetry are important for cell cycle progression, apoptosis, and platelet coagulation. Three classes of lipid translocases, P-type ATPases, ABC transporters, and scramblases, are known to be involved in the regulation of lipid asymmetry. In this review, we describe the physiological and pathological functions of lipid asymmetry and the current knowledge of lipid translocases.

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![Fig. 1. Lipid Asymmetry of the Plasma Membrane and Translocases of Glycerophospholipids](image)

Lipids of the plasma membrane (PM) are distributed asymmetrically between the two leaflets, and the presence of glycerophospholipids and sphingolipids contributes to this asymmetry. Flipping of phosphatidylserine (PS) and phosphatidylethanolamine (PE) is mediated by P-type ATPases/aminophospholipid translocases. Flopping of glycerophospholipids is catalyzed by ABC transporters. Scramblases randomize and collapse the asymmetry of the lipids. In yeast, Cdc50 family members are known to be regulatory components of P-type ATPases. GSL, glycosphingolipid; PC, phosphatidylcholine; Chol, cholesterol; SM, sphingomyelin.

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transmembrane helix, whereas within the cholesterol-high plasma membrane is mediated by specific translocases.\textsuperscript{9}

2. \textbf{INWARD TRANSLOCATION OF GLYCEROPHOSPHOLIPIDS BY P-TYPE ATPASES/AMINOPHOSPHOLIPID TRANSLOCASES AND CDC50 FAMILY MEMBERS}

Seigneuret and Devaux found that in human erythrocytes spin-labeled analogs of aminophospholipids (PS and PE) rapidly translocated to the cytosolic leaflet, while a PC analog remained in the outer leaflet of the plasma membrane.\textsuperscript{10} This translocation correlated strongly with properties of a 115 kDa Mg\textsuperscript{2+} -ATPase purified from bovine chromaffin granules\textsuperscript{11} or human erythrocytes,\textsuperscript{12} properties such as sensitivity to inhibitors. In 1996, Tang \textit{et al.} cloned the gene responsible for this activity from bovine chromaffin granules, and found that it encodes a member of a subfamily of the P-type ATPases, ATP8A\textsubscript{1}/ATPase 11.\textsuperscript{13} In addition, they also reported that yeast mutant lacking the ATP8A\textsubscript{1} homolog, Drs2, exhibited a loss in the uptake of NBD-PS.\textsuperscript{13} The P-type ATPase superfamily, which is widely conserved from prokaryotes to eukaryotes, is genealogically categorized into 5 subclasses.\textsuperscript{14} ATP8A\textsubscript{1} and Drs2 belong to the type 4/aminophospholipid translocase subfamily. At present, 14 human proteins and 5 yeast proteins have been identified as members of this subfamily.\textsuperscript{14} Overproduction of another member, APT8B1, which was identified as a gene mutated in patients with forms of cholestasis,\textsuperscript{15} also resulted in elevated NBD-PS incorporation.\textsuperscript{16}

Yeast have 5 members (Dnfl, Dnf2, Dnf3, Drs2, and Neo1) of the type 4 subfamily of P-type ATPases, which differ in their intracellular localization. Dnfl and Dnf2 are found in the plasma membrane; Dnf3 in the late Golgi; Drs2 in the late Golgi, late endosome, and plasma membrane;\textsuperscript{17-19} and Neo1 in the endosome, ER, and Golgi.\textsuperscript{19-21} Of these proteins, only Neo1 is essential for growth. Deleting \textit{DNF1}, \textit{DNF2}, \textit{DNF3}, or \textit{DRS2} individually does not affect growth, but the quadruple deletion is lethal,\textsuperscript{18} suggesting that the proteins they encode are functionally redundant.

Yeast also carry three members of the highly conserved (yeast to mammals) Cdc50 family, Lem3, Dnfl, and Neo1, which are known to form complexes.
with Dnf1 and Drs2, respectively. Without Lem3 or Cdc50, the respective P-type ATPase cannot exit the ER, so these proteins are essential for the function of those enzymes. A deletion mutant of Lem3 exhibited a significant reduction of flip activity for NBD-PE and NBD-PC at the plasma membrane, while the translocation of NBD-PS was unchanged.

3. OUTWARD TRANSLOCATION OF GLYCEROPHOSPHOLIPIDS BY ABC TRANSPORTERS

In eukaryotic cells ABC transporters function as ATP-dependent efflux pumps. To date, 49 human and 30 yeast ABC transporters have been identified. Overexpression of certain ABC transporters confers multidrug resistance to cells. Additionally, some ABC transporters function as lipid transporters/translocases.

The ABC transporter ABCB4 (mouse mdr2, human MDR3) is highly expressed in hepatic bile canalicular membranes and, through studies of knockout mice, has been shown to be involved in PC release from the liver into bile. A subsequent study revealed that ABCB4 is in fact a PC-specific transporter/translocase that cannot externalize PE. In contrast, ABCB1 exhibits rather broad substrate specificity, and in ABCB1-overproducing cells, enhanced distribution of both metabolically labeled PC and PE was observed in the outer leaflet of the plasma membrane. A third transporter, ABCA1, mediates the secretion of cholesterol from cells into high-density lipoproteins (HDLs). A mutation in ABCA1 causes Tangier disease, a severe HDL deficiency syndrome. Recent studies demonstrated that overexpression of ABCA1 also enhanced the translocation of PS and PE but not PC. A photoreceptor-specific ABC transporter, ABCA4 (ABCR), is thought to be involved in the translocation of N-retinylidene-PE, a natural PE derivative, from the inner to the cytosolic side of the rod outer segment disc membrane in the mammalian eye. Mutations in the ABCA4 gene are responsible for Stargardt's disease, the most common form of juvenile macular degeneration. Thus, several ABC transporter members are recognized as glycerophospholipid translocases/transporters and have been linked to diseases. Genetic studies in yeast have also suggested that some yeast ABC transporters, such as Pdr5, Yor1, and Ste6, function as lipid translocases.

4. BIDIRECTIONAL TRANSLOCATION OF GLYCEROPHOSPHOLIPIDS BY SCRAMBLASES

In 1996, a 37 kDa, Ca²⁺-dependent, integral membrane protein was isolated from human erythrocytes and found to non-selectively enhance bidirectional translocation of NBGC glycerophospholipids. The PLSCR1 gene was cloned from its peptide sequence in 1997, and its scramblase activity was confirmed using proteoliposomes reconstituted with the purified recombinant PLSCR1 protein. However, in erythrocyte membranes prepared from PLSCR1 knockout mice, the Ca²⁺-dependent externalization of PS was normal. Thus, it is unclear whether PLSCR1 does indeed function as a scramblase in vivo. It is possible, however, that other members of the PLSCR family (PLSCR2 to PLSCR4) have overlapping functions.

5. SPHINGOLIPID ASYMMETRY IN MEMBRANES

Sphingolipids also contribute to plasma membrane asymmetry, as complex sphingolipids such as SM and glycosphinogolipids localize exclusively in the outer leaflet. SM is synthesized from ceramide in the lumen of the Golgi, Glucosyleramide, the simplest glycosphingolipid, is synthesized in the cytosolic side of the Golgi, yet further modification with galactose and other sugars also occurs in the luminal side of the Golgi. Thus, glucosyleramide must traverse the Golgi membrane to be a substrate for the production of complex sphingolipids. ABCB1 is thought to be the translocase in this reaction. Additionally, SM and glycosphingolipids synthesized in the lumen of the Golgi are thought to be delivered to the extracytosolic leaflet of the plasma membrane by vesicular transport while keeping their membrane topology.

The sphingolipid precursors, sphingoid base (long-chain base) and ceramide, are synthesized in the ER. Although, the precursors' transbilayer distribution in the ER is not known, ceramide seems to be localized in both the luminal and cytosolic leaflets of the ER membrane. Ceramide can be converted to galactosyleramide in the luminal side or be transported from the cytosolic leaflet of the ER to the Golgi by CERT, a ceramide-transfer protein. Although how ceramide traverses the ER membrane is unclear, its spontaneous flip-flop is much faster than that of SM in model membranes. In giant unilamellar vesicles ceramide flip-flop occurs spontaneously, once every 30 s at 37°C.

Recently, Rsbl was isolated in yeast as an ATP-dependent transporter/translocase of sphingoid base, one of two hydrophobic chains constituting ceramide. This protein is localized in the plasma membrane and the ER, suggesting that Rsbl translocates sphingoid base from the cytosolic to extracytosolic leaflets of these membranes. Interestingly, although Rsbl expression is low in normal conditions, it is significantly increased by a change in glycerophospholipid asymmetry, such as that caused by mutations in genes involved in either the flip or the flop of these lipids. In addition, Rsbl overproduction promotes the flip and represses the flop of NBD-PE and NBD-PC. These results suggest some crosstalk between sphingolipids and glycerophospholipids in the maintenance of the functional lipid asymmetry of the plasma membrane.

6. PHYSIOLOGICAL ROLES OF LIPID ASYMMETRY

Maintenance of lipid asymmetry is important for certain cellular processes. For example, interactions between PS located in the inner leaflet and skeletal proteins like spectrin improve the mechanical stability of the membranes of red blood cells. In yeast, mutational analyses demonstrated that members of the aminocephospholipid translocate family function in intracellular trafficking, maintenance of organelle structure, and cell polarity. Local or global changes in lipid asymmetry also cause a variety of cellular responses. For instance, transient PE exposure and a complete loss of cell surface SM have been observed at the cleavage furrow during cytokinesis in cultured cells. Moreover, when cell surface PE was trapped by ei-
ther a PE-binding peptide conjugated to streptavidin, or a mutation in PE biosynthesis, cell division stopped at the late stage of cytokinesis due to inhibited disassembly of the contractile ring. These results suggest that locally and temporally regulated exposure of PE is essential for cell cycle progression. Likewise, in budding yeast, PE is predominantly located on the extracytosolic side of the plasma membrane at the bud neck. When cell surface PE was trapped, actin filaments accumulated at the bud neck and small bud, suggesting that redistribution of PE at specific regions is involved in cell polarity.

Global changes in lipid asymmetry have been observed in apoptotic cells as well. Elevated Ca²⁺-dependent scramblase activity or a reduction in aminophospholipid translocase activity is thought to cause the collapse of lipid asymmetry. PS exposure on the apoptotic cells as a result of the collapse is used as a recognition signal by phagocytes. Several receptors for this ligand have been reported, including the PS receptor (PSR), lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1), scavenger receptor type I (SR-BI), milk fat globule-EGF-factor 8 (MFG-E8), and developmental endothelial locus-1 (Del-1). Although the physiological relevance of these receptors is not clear in most cases, PSR knockout mice do exhibit developmental abnormalities associated with accumulations of apoptotic cells in the lung and brain. Furthermore, MFG-E8-deficient macrophages cannot efficiently engulf apoptotic lymphocytes.

PS exposure on phagocytic cells is also important. The ABC transporter ABCA1 promotes phagocytosis by redistributing aminophospholipids on the macrophage membrane surface. Likewise, CED-7, an ortholog of ABCA1 in Caenorhabditis elegans, is required for optimal engulfment during apoptosis.

PS is also exposed on the cell surface in the course of blood coagulation, and this externalization is essential for the formation of the procoagulant surface. The clinical condition associated with a defect in procoagulant activity is best understood from the studies of Scott syndrome, a rare inherited disease linked to a lack of PS exposure in activated platelets and other blood cells. Recently, a missense substitution in the ABCA1 gene and a significant reduction of ABCA1 mRNA were found in Scott syndrome patients.

PS translocation to the external leaflet is also considered to be important for myotubule formation, sperm capacitation, and signal transduction. van den Eijnde et al. observed that PS is exposed on the outer leaflet during myoblast differentiation. When PS was trapped in the outer leaflet of the plasma membrane by annexin V, a PS-specific binding protein, myotubule formation was inhibited. PS is also exposed on the cell surface of activated boar sperm cells during sperm capacitation. Elliott et al. found that in T lymphocytes transient PS externalization is involved in signal transduction that leads to activation of the P2X₇ receptor, an ATP-gated cation channel.

7. CONCLUSION AND PERSPECTIVE

Lipid asymmetry must be maintained to preserve certain cellular functions, yet its alternation is also required to induce several biological processes as described in this review. Pathologically, the genes involved in lipid asymmetry have been directly or indirectly linked to a variety of diseases. ATP8B1 has been associated with benign recurrent intrhepatic cholestasis and progressive familial intrahepatic cholestasis type 1; ABCA1 with tangier disease and Scott syndrome; ABCB4 with progressive familial intrahepatic cholestasis type 3; and ABCA4 with Stargardt's disease. However, in most cases it is not clear how lipid asymmetry regulates cellular functions or causes the diseases. Further elucidation of regulatory mechanisms of lipid translocases will be needed.

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August 2006


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