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Review

Structure and mechanism of ATP-dependent phospholipid transporters ☆

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ABSTRACT

Background: ATP-binding cassette (ABC) transporters and P4-ATPases are two large and seemingly unrelated families of primary active pumps involved in moving phospholipids from one leaflet of a biological membrane to the other.
Scope of review: This review aims to identify common mechanistic features in the way phospholipid flipping is carried out by two evolutionarily unrelated families of transporters.
Major conclusions: Both protein families hydrolyze ATP, although they employ different mechanisms to use it, and have a comparable size with twelve transmembrane segments in the functional unit. Further, despite differences in overall architecture, both appear to operate by an alternating access mechanism and during transport they might allow access of phospholipids to the internal part of the transmembrane domain. The latter feature is obvious for ABC transporters, but phospholipids and other hydrophobic molecules have also been found embedded in P-type ATPase crystal structures. Taken together, in two diverse groups of pumps, nature appears to have evolved quite similar ways of flipping phospholipids.
General significance: Our understanding of the structural basis for phospholipid flipping is still limited but it seems plausible that a general mechanism for phospholipid flipping exists in nature. This article is part of a Special Issue entitled Structural biochemistry and biophysics of membrane proteins.

1. Background

Biological membranes are the basis for highly defined and separated functional units. The cell membrane, or plasma membrane, defines the external boundary of every cell separating the cytoplasm from the surrounding environment. Eukaryotic cells contain in addition numerous subcellular membranes that divide the cytoplasm into multiple organelles, thereby allowing different functions to occur efficiently and simultaneously in different parts of the cell. Almost all biological membranes are organized as bilayers consisting of two leaflets structurally formed by phospholipids. Depending on their (sub)cellular location, they might also contain other types of phospholipids, including glycolipids and sterols. A remarkable feature of many biological membranes is that their phospholipids are asymmetrically distributed across the lipid bilayer, a phenomenon known as transbilayer lipid asymmetry. A prominent example is the plasma membrane of animal cells where the phospholipids phosphatidylcholine (PC) and sphingomyelin (SM) are concentrated in the exoplasmic leaflet while phosphatidylserine (PS) and phosphatidylethanolamine (PE) are restricted to the cytosolic leaflet [1]. Transbilayer lipid asymmetry is essential for several vital cellular functions, including regulation of membrane protein activity, signaling, and vesicle formation in the secretory and endocytic pathways [2–5]. In animals, loss of transbilayer lipid asymmetry has been related to processes like blood coagulation [6], macrophage recognition [7] and apoptosis [8]. Establishing and maintaining the asymmetry is thus crucial for the cells, and a number of proteins have evolved to fulfill a role as cross-bilayer phospholipid transporters.

Transbilayer lipid asymmetry is largely controlled by a diverse group of membrane proteins that catalyze the movement of phospholipids across membranes. Lipid translocators can be classified into two categories: (i) energy-independent transporters such as scramblases that randomize the distribution of lipids across the bilayer and (ii) ATP-driven, vectorial transporters that actively translocate specific lipids from one leaflet to the other. The latter class of transporters includes ATP-dependent flippases and floppases, which catalyze inward phospholipid movement to and outward phospholipid movement from the cytoplasmic leaflet of cellular membranes, respectively. Current genetic and biochemical evidence indicates that these proteins are primarily members of the ATP-binding cassette (ABC) and P-type family of transporters

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(Tables 1 and 2). However, measuring phospholipid translocation is not a trivial task, as the transporters are trapped in an environment (cellular membranes) formed by their own substrate (lipids). Therefore, in intact cells or organisms, assignment of phospholipid translocating activity is in most cases based on the use of fluorescent phospholipid analogs; only few studies have attempted to measure transport of natural phospholipids (Tables 1 and 2). Phospholipid transport observed in vivo need not be directly linked to the activity of ABC transporters or P4-ATPases, and might represent indirect effects. Only recently, advances in purification and reconstitution techniques have allowed demonstrating the capacity of some ABC transporters and P-type ATPases to directly translocate fluorescent phospholipid analogs, providing the best evidence so far that these transporters indeed have phospholipids as a substrate.

2. Scope of review

Several excellent reviews have surveyed the physiological relevance of phospholipid transporters recently [3,5,9]. Here, we will focus on recent advancements in the determination of the structure and mechanism of putative ATP-dependent phospholipid transporters.

3. ATP-dependent phospholipid transporters

Cellular, biochemical and recent reconstitution studies demonstrate that various ABC transporters and P-type ATPases couple ATP hydrolysis to translocation of specific phospholipids from one leaflet to the other and thereby help generate membrane lipid asymmetry (Tables 1 and 2). An important question arises when comparing the two classes of phospholipid flipping pumps: are their transport mechanisms the same or has nature evolved different ways of translocating phospholipids across the bilayer? In the next sections, we will review the main features of ABC transporters and P-type ATPases implicated in phospholipid transport and compare their putative transport mechanism(s).

3.1. ABC transporter family

The ABC transporter family is found in organisms throughout evolution and constitutes one of the largest superfamilies of integral membrane transporters. The family includes a wide range of proteins that share common structural features and transport a variety of organic and inorganic substrates, including phospholipids (Table 1). Several of these transporters are mutated in human disorders related to phospholipid transport and metabolism, including Tangier disease [10], Stargardt disease [11] and progressive familial intrahepatic cholestasis [12]. The functional transport unit comprises two nucleotide-binding domains (NBD), typical of ABC transporters, and two transmembrane domains (TMDs), each containing five to ten membrane-spanning regions (Fig. 1A). They can occur as one complete transporter, two half-transporters, or four polypeptides.

3.1.1. General structural features of ABC transporters

The NBDs of all ABC transporters contain highly conserved amino acid motifs. Each NBD contains two ATP-binding motifs: the Walker A motif (GXXGXGK(S/T)) and the Walker B motif (hhhhDE, where h is a

Table 1
Evidence for ABC transporter-catalyzed phosholipid transport.

Transporter	Lipid[1]	Movement, membrane[2]	Approach	Reference
Bacteria				
MsbA	**Lipid A**, PLs?	Out, PM	Intact cells; ATPase stimulation; Reconstitution	[72,137–139]
PglK	**Lipid-linked oligosaccharides**	Out, PM	Intact cells	[140]
LmrA	PE, **Lipid A**	Out, PM	Reconstitution; *E. coli* complementation; ATPase stimulation	[141,142]
Fungi				
Pdr5p, Yor1p	**PE,**	Out, PM	Intact cells	[143,144]
Ybt1p	PC	Out, vacuolar	Intact cells; Isolated vacuoles	[145]
Cdr1p	PE,PC,PS	Out, PM	Intact cells; Reconstitution	[63,146,147]
Cdr2p	PE,PC,PS	Out, PM	Intact cells	[63]
Cdr3p	PE,PC,PS	In, PM	Intact cells	[63]
Leishmania donovani				
LABCA1	PLs	Out, PM	Intact cells	[148]
LABCA2	PLs	Out, PM	Intact cells	[149]
LABCB4	PC	Out, PM	Intact cells	[150]
LABCG2	PS	Out, PM	Intact cells	[151]
LABCG4	PC	Out, PM	Intact cells; yeast vesicle assay	[152]
LABCG6	PLs	Out, PM	Intact cells	[153]
Mammals				
ABCA1	PC, PS, SM	Out, PM	Intact cells; reconstitution	[65,91]
ABCA3	PC, SM	Out, lysosomal vesicles	Expression in HEK-293 and A549	[154–156]
ABCA4	PE, N-retinylidene-PE	In, Disk	Reconstitution	[64,65,157]
ABCA7	PS, (PC, SM)	Out, PM	Intact cells; reconstitution	[65,158–160]
ABCB1 (MDR1)	PLs, SLs, **PAF**	Out, PM (apical)	Intact cells; yeast vesicle assay; reconstitution	[70,71,89,90,161–164]
ABCB4 (MDR3,Mdr2)	**PC**	Out, canalicular	Intact cells; yeast vesicle assay	[89,165–167]
ABCC1 (MRP1)	PLs	Out, PM (basolateral)	Intact cells; reconstitution	[168–171]

[1] PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine. PLs, phospholipids; SLs, sphingolipids; PAF, platelet-activating factor (1-Hexadecyl-2-acetyl-sn-glycero-3-phosphocholine); SM, sphingomyelin. Substrate specificities are mostly demonstrated by the use of fluorescent lipid probes. Evidences for translocation of natural lipids are indicated in bold.
[2] PM, plasma membrane; Disk, photoreceptor disk membranes.

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