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Review

P4 ATPases - Lipid flippases and their role in disease

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ABSTRACT

P4 ATPases (type 4 P-type ATPases) are multispan transmembrane proteins that have been implicated in phospholipid translocation from the exoplasmic to the cytoplasmic leaflet of biological membranes. Studies in *Saccharomyces cerevisiae* have indicated that P4 ATPases are important in vesicle biogenesis and are required for vesicular trafficking along several intracellular vesicular transport routes. Although little is known about mammalian P4 ATPases, some members of this subfamily appear to be associated with human disease or mouse pathophysiology. ATP8B1, a phosphatidylserine translocase, is the most extensively studied mammalian P4 ATPase. This protein is important for maintaining the detergent resistant properties of the apical membrane of the hepatocyte. Mutations in ATP8B1 give rise to severe liver disease. Furthermore, a role for Atp8b3 in mouse sperm cell capacitation has been suggested, whereas deficiency of Atp10a and Atp10d leads to insulin resistance and obesity in mice. Here we review the present status on the pathophysiological consequences of P4 ATPase deficiency.

1. Introduction

P-type ATPases form a large family of multispan transmembrane proteins that facilitate the transport of, in most cases, cations at the expense of ATP [6,13,78,93]. Within this family, the subset of type 4 P-type ATPases (P4 ATPases) forms a distinct group based on sequence divergence and their proposed role in transport of phospholipid molecules rather than cations [6,13,78,93]. Indeed, P4 ATPases lack the cation-interacting amino acid residues in transmembrane domains 4 and 6 that are important in cation binding, and that are present in most P-type ATPases. Instead, in P4 ATPases these regions contain hydrophobic residues which may interact with hydrophobic lipid moieties [13,93].

P4 ATPases are putative phospholipid flippases that translocate phospholipids from the exoplasmic to the cytoplasmic leaflet of membrane bilayers. These activities contribute to the maintenance of the asymmetric distribution of phospholipids between the two leaflets of biological membranes. In mammalian plasma membranes, the aminophospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE) are confined to the cytoplasmic leaflet of the membrane bilayer, whereas the choline-containing phospholipids phosphatidylcholine (PC) and (glyco)sphingolipids are concentrated in the exoplasmic leaflet [9]. The non-random distribution of phospholipids is crucial for many physiological processes, including signal transduction, cell morphology, cell movement, activity of membrane proteins, and vesicle biogenesis [7,22,40,97]. On the other hand, phospholipid randomization is essential in initiating phagocytosis, platelet activation, and apoptosis [7,28,99,102].

Much of the present knowledge on the cellular function of P4 ATPases is derived from studies in the yeast *Saccharomyces cerevisiae*. The yeast genome harbors five P4 ATPases. Several groups have demonstrated that these proteins participate in distinct intracellular vesicular trafficking pathways and are important for the biogenesis of exocytic and endocytic transport vesicles [33,54,60,61,81,86]. Importantly, these functions are closely linked to the flipping of phospholipids or phospholipid analogues. Furthermore, it has become clear that P4 ATPases require an interaction with members of the Cdc50p/Lem3p protein family in order to be released from the endoplasmic reticulum and for targeting to the proper membrane domains [71,84]. The role of P4 ATPases in the yeast *S. cerevisiae* will be addressed in more detail elsewhere in this issue.

Mammals express fourteen P4 ATPases, which are summarized in Table 1 (for detailed reviews, see [48,78]). In the past years we have

Abbreviations: ArfGEF, Arf guanine nucleotide exchange factor; ASBT, apical sodium-dependent bile salt transporter; ATP, adenosine triphosphate; BRIC, benign recurrent intrahepatic cholestasis; *C. elegans*, *Cenorrhabditis elegans*; CA, cholic acid; cAMP, cyclic adenosine monophosphate; cDNA, complementary DNA; CHO, Chinese hamster ovary; eGFP, enhanced green fluorescent protein; FXR, Farnesoid X Receptor; GAP, GTPase activating protein; GPI, glycosylphosphatidylinositol; GTP, guanosine triphosphate; ICP, intrahepatic cholestasis of pregnancy; i.e., id est; NBD, 7-nitro-2,1,3-benzoxadiazol-4-yl; P4 ATPase, type 4 P-type ATPase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PFIC, progressive familial intrahepatic cholestasis; PKCζ, protein kinase C zeta; PS, phosphatidylserine; SM, sphingomyelin; SNP, single nucleotide polymorphism

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Table 1
Overview of the mammalian P4 ATPase subfamily, and the tissue distribution and (potential) involvement of P4 ATPases in pathophysiologic processes.

Class	P4 ATPase	Pathophysiology	Tissue distribution	Ref
1-Σ	ATP8A1		Ubiquitous; high in skeletal muscle and thyroid	[63]
	ATP8A2	Tumorigenicity?	High in testis; low in heart and brain; not in liver, lung, kidney	[43,91]
1-Φ	ATP8B1	Intrahepatic cholestasis	Ubiquitous; high in small intestine and pancreas; low in brain	[11,55]
	ATP8B2		Ubiquitous; high in brain, bladder and uterus; not in kidney and skeletal muscle	[46]
	ATP8B3	Sperm capacitation anomalies	Testis	[46,98]
	ATP8B4	Alzheimer disease?	Ubiquitous at moderate levels, including brain, liver, kidney, testis	[58,68]
2	ATP9A		Ubiquitous at moderate levels; not in spleen	[51]
	ATP9B		Ubiquitous; high in testis; not in spleen en muscle	[43,44]
5	ATP10A	Insulin resistance, obesity	Ubiquitous; high in brain, kidney, lung pancreas, not in small intestine	[23–25,62]
	ATP10B		Low in brain and testis	[66]
	ATP10D	Obesity	Ubiquitous; moderate in liver, kidney, spleen and ovary; low in brain	[30,67,92]
6	ATP11A		Ubiquitous; moderate in liver, heart, kidney and muscle; low in brain and spleen	[53]
	ATP11B		Ubiquitous (low); moderate in kidney	[65]
	ATP11C		Ubiquitous; high in liver, pancreas, kidney; low in brain, skeletal muscle	[3]

See also [78].

extensively studied the molecular and physiological functions of one of these P4 ATPases, ATP8B1 (see below). Mutations in the *ATP8B1* gene cause severe liver disease. We have recently demonstrated that ATP8B1 mediates the translocation of PS from the exoplasmic leaflet to the cytoplasmic leaflet of the plasma membrane. In addition, we have shown that ATP8B1 also requires a subunit or chaperone for endoplasmic reticulum exit and plasma membrane localization. However, the cellular function and biochemical activity of most mammalian P4 ATPases still need to be elucidated. This review highlights the present knowledge on the physiological role of mammalian P4 ATPases and their (potential) roles in human disease.

2. The identification of ATP8A1 — a historical overview

In 1984 Seigneuret and Devaux were the first to describe an inward aminophospholipid translocase activity in erythrocyte membranes [87]. Spin-labeled PE and PS were internalized via an ATP-dependent mechanism, whereas hardly any PC translocation was observed. Interestingly, the inward flipping of PE and PS was accompanied by a change in the shape of the erythrocyte membrane. After incorporation of the spin-labeled phospholipids, the normal, discoid-shaped erythrocytes adopted a crenate-shaped morphology. When the steady-state was achieved after the transfer of PE and PS to the inner membrane leaflet, the cells rapidly converted into stomatocytes [19,87,88]. The crenated shape is explained by the higher ratio of phospholipid molecules in the outer leaflet compared to the inner leaflet, and indicates that the relative distribution of phospholipids between the leaflets of the membrane bilayer may determine membrane curvature. In analogy, inward phospholipid translocation by P4 ATPases may facilitate endosome biogenesis by increased inward membrane bending due to increased amounts of phospholipid molecules in the inner leaflet [29,33,54,60,61,81,86].

In 1989 Zachowski et al. demonstrated ATP-dependent translocation of a PS analogue in bovine adrenal gland granules [101]. They observed similar translocation kinetics in these chromaffin granules as in inside-out erythrocyte membrane vesicles, and suggested that the so called Mg-ATPase II was involved in this translocation. They hypothesized this activity to be important in making the granules fusion-competent for exocytosis. Five years later, the same group purified ATPase II (presently assumed to be the gene product of *ATP8A1*) from erythrocyte membranes and reconstituted the protein in artificial lipid membrane vesicles [5]. They measured a rapid ATPase II-dependent redistribution of PS and PE, whereas no translocation of PC was observed.

In 1996, the Mg-ATPase II was cloned from the bovine chromaffin granules [93]. Three years later the human homologue was cloned from a human skeletal muscle library [63], and in 2006 two mouse isoforms of the protein, called ATP8A1 by then, were identified in erythroblasts [89]. *Atp8a1* is ubiquitously expressed (see Table 1). In analogy with the preferential translocation of PS observed for the purified Mg-ATPase II, reconstitution of bovine and murine Atp8a1 isoforms showed the highest activation of ATPase activity in PS containing micelles [26,75]. However, PS translocation mediated by this gene product has not unambiguously been demonstrated yet.

3. P4 ATPases in human disease

Thus far, only a few diseases have been described which are associated with loci harboring P4 ATPase genes. Mutations in the *ATP8B1* gene cause severe human liver disease, as will be discussed below. Atp8b3 has been implicated in sperm capacitation in mice, and may underlie fertility-related disorders in humans (see below). A recent report described a significant association of the locus containing the *ATP8B4* gene with Alzheimer disease, since one of the SNPs described localized very close to the *ATP8B4* gene on chromosome 15 [58]. Thus, mutations in *ATP8B4* might play a role in Alzheimer disease. *ATP8A2* has previously been implicated in tumorigenesis [91]. Non-tumorigenic cells isolated from human tumors can be converted to tumorigenic cells in nude mice after treatment with *ATP8A2* antisense cDNA. However, thus far no follow-up studies on these observations have been published. Finally, both *Atp10a* and *Atp10d* have been implicated in obesity, type 2 diabetes, and fatty liver disease in the mouse (see below).

4. Mutations in ATP8B1 cause cholestatic disease

One of the crucial functions of the liver is bile formation. The major components of bile are bile salts, phospholipids, cholesterol, bilirubin, electrolytes and water. The apical (canalicular) membranes of adjacent hepatocytes form a canalicular lumen which is the site of primary bile formation. The main driving force of bile flow is the excretion of bile salts from the hepatocytes into the bile canaliculus by the bile salt export pump ABCB11 [38,90]. This, together with the excretion of electrolytes, creates an osmotic gradient over the canalicular membrane of the hepatocyte, and causes water flow into the bile canaliculus [4]. However, because of the strong detergent properties of bile salts and their potential to dissolve membranes, bile is an extremely toxic body fluid [18]. The canalicular membrane of hepatocytes is protected from being dissolved by two mechanisms which both require phospholipid translocase activity. The first mechanism aims to neutralize the detergent action of the bile salts in the canalicular lumen. Biliary bile salt secretion is coupled to PC and cholesterol excretion, processes mediated by ABCB4 and the heterodimer ABCG5/ABCG8, respectively. Consequently, bile salts form, together with PC and cholesterol, mixed micelles, which have strongly reduced detergent properties [73].

A second mechanism of protection appears to involve the function of ATP8B1. Mutations in the *ATP8B1* gene give rise to Progressive Familial Intrahepatic Cholestasis type 1 (PFIC1) and Benign Recurrent Intrahepatic Cholestasis type 1 (BRIC1) [11,55]. These disorders are