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Review

# P4 ATPases – The physiological relevance of lipid flipping transporters

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ABSTRACT

**P4 ATPases are integral transmembrane proteins implicated in phospholipid translocation from the exoplasmic to the cytosolic leaflet of biological membranes. Our present knowledge on the cellular physiology of P4 ATPases is mostly derived from studies in the yeast *Saccharomyces cerevisiae*, where P4 ATPases play a pivotal role in the biogenesis of intracellular transport vesicles, polarized protein transport and protein maturation. In contrast, the physiological and cellular functions of mammalian P4 ATPases are largely unexplored. P4 ATPases act in concert with members of the CDC50 protein family, which are putative β-subunits for P4 ATPases. This review highlights the current status of a slowly emerging research field and emphasizes the contribution of P4 ATPases to the vesicle-generating machinery.**


## 1. Introduction

Lipid flippases are proteins that catalyze the transport of lipid molecules from the exoplasmic to the cytosolic leaflet of membrane bilayers. Lipid flippases are crucial for maintaining a non-randomized distribution of phospholipids over the two hemi-leaflets in many biological membranes. Already since the early 1970-ties it is known that phospholipids are non-randomly distributed in biological bilayers. In most eukaryotic cells, phosphatidylcholine (PC) and (glycero)sphingolipids are enriched in the exoplasmic leaflet, whereas the aminophospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE) are largely confined to the cytosolic leaflet of the endomembrane system [1–3]. Furthermore, the less abundant phospholipids, phosphatidylinositol (and derivatives) and phosphatidic acid, are also concentrated in the cytosolic leaflet [4–6]. An exception are the membranes of the endoplasmic reticulum in which the phospholipids are distributed randomly over the two leaflets. Phospholipids tend to equilibrate between the two leaflets of a bilayer (a process termed 'scrambling') at very slow rates (reviewed in [7]). However, due to extensive membrane fusion and budding events in the intracellular trafficking pathways, phospholipid scrambling is accelerated. Scrambling is of physiological importance for e.g. the activation of the blood coagulation, sperm capacitation, and the engulfment of apoptotic cells and can be accelerated by $Ca^{2+}$-regulated scramblases [8,9]; However, phospholipid randomization also interferes with membrane dynamics and may impair membrane-associated protein structure and function [10]. Thus, maintaining and dissipating the non-random distribution of phospholipids is crucial for normal regulated cell function, and requires the activity of proteins that are able to catalyze the intramembranous transport phospholipids. Lipid flippases and lipid floppases are ATP-dependent proteins that are implicated in the generation and preservation of the non-random distribution of phospholipids (reviewed in [11,12]). Lipid floppases transport lipids from the cytosolic to the exoplasmic leaflet of bilayers, and several members of the ATP-binding cassette (ABC) transporter protein family display such an activity [13]. The role of ABC transporters in lipid flopping will be discussed elsewhere in this issue. In 1984, the first ATP-dependent lipid flippase activity was identified in erythrocyte membranes [14]. In 1989, a second flippase activity was identified in Golgi-derived chromaffin granules from bovine adrenal glands, which would be required for the generation of fusion-competent membrane vesicles [15]. However, the gene(s) encoding these activities, and the nature of the protein(s) remained elusive. In 1996, the cDNA encoding the bovine chromaffin granule flippase, termed ATPaseII and presently known as ATP8A1, was isolated [16].

## 2. The P4 ATPase subfamily

ATP8A1 and its *Saccharomyces cerevisiae* ortholog Drs2p were identified as the first members of the type 4 subfamily of the P-type ATPase superfamily (abbreviated to P4 ATPase) (Fig. 1). Both proteins were identified as aminophospholipid flippases for their ability to translocate fluorescently-labeled PS (NBD-PS) [16]. P4

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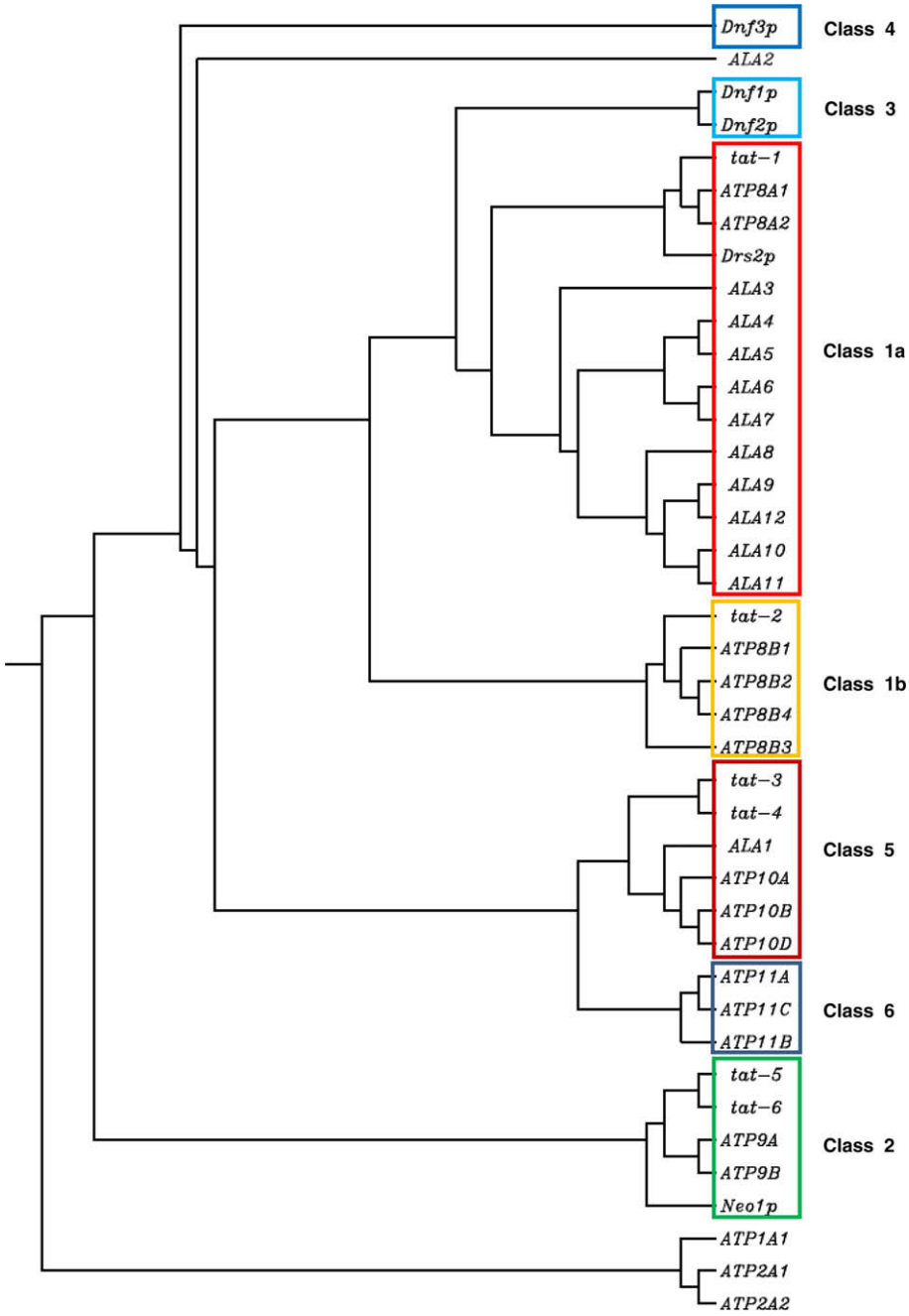


**Fig. 1.** Phylogenetic analyses of the P4 ATPase protein family of mammalian, *Arabidopsis thaliana*, *S. cerevisiae*, and *C. elegans* using ClustalW sequence alignment software (http://align.genome.jp/clustalw/). The P4 ATPases are subdivided into classes, based on amino acid consensus sequences [17,110]. ALA2 is closest related to Dnf3p (class 4) but does not contain any of the class-specific consensus sequences. Database accession numbers: *C. elegans*: tat-1 (NP_001022894), tat-2 (NP_001023252), tat-3 (NP_499363), tat-4 (NP_495244), tat-5 (NP_001021457), tat-6 (NP_503858); *Arabidopsis thaliana*: ALA1 (P98204), ALA2 (P98205), ALA3 (Q9XIE6), ALA4 (Q9LNQ4), ALA5 (Q9SGG3), ALA6 (Q9SLK6), ALA7 (Q9LVK9), ALA8 (Q9LK90), ALA9 (Q9SX33), ALA10 (Q9LI83), ALA11 (Q9SAF5), ALA12 (P57792); *S. cerevisiae*: Drs2p (P39524), Dnf1p (P32660), Dnf2p (Q12675), Dnf3p (Q12674), Neo1p (P40527). Human: ATP8A1 (P70704), ATP8A2 (P98200), ATP8B1 (O43520), ATP8B2 (P98198), ATP8B3 (O60423), ATP8B4 (Q8TF62), ATP9A (O75110), ATP9B (O43861), ATP10A (O60312), ATP10B (O94823), ATP10D (Q9P241), ATP11A (P98196), ATP11B (Q9Y2G3), ATP11C (Q8NB49). Type 2 (non-P4) ATPase accession numbers: ATP1A1 (P05023), ATP2A1 (O14983), ATP2A2 (P16615).

ATPases are exclusively expressed in eukaryotic cells and are deviant from the other P-type ATPase subfamilies in that they are implicated in the transport of phospholipids rather than in the transport of cations [12,17,18]. Studies in *S. cerevisiae*, *Arabidopsis thaliana*, and *Caenorhabditis elegans* suggest an important role for P4 ATPases in the biogenesis of transport vesicles in the biosynthetic and endocytic pathways. In particular, the mammalian P4 ATPase subfamily is poorly studied. However, inactivation of one member causes severe human disease, while inactivation of some murine P4 ATPases suggests roles in fertility-related disorders, insulin resistance and obesity (reviewed in [19]). Recently, several groups have demonstrated an important chaperone function for members of the evolutionary conserved Cdc50 protein family. It is suggested that CDC50 proteins are β-subunits for P4 ATPases, analogous to the β-subunits for other P-type ATPases such as the Na,K-ATPase, and are important for regulation of trafficking and activity of the P4 ATPase. In this review, we will highlight the present knowledge of this slowly emerging and very exciting family of proteins.