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Review

The yeast plasma membrane P_4-ATPases are major transporters for lysophospholipids☆

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ABSTRACT

The transbilayer movement of phospholipids plays an essential role in establishing and maintaining the asymmetric distribution of lipids in biological membranes. The P4-ATPase family has been implicated as the major transporters of the aminoglycerophospholipids in both surface and endomembrane systems. Historically, fluorescent lipid analogs have been used to monitor the lipid transport activity of the P4-ATPases. Recent evidence now demonstrates that lyso-phosphatidylethanolamine (lyso-PtdEtn) and lyso-phosphatidylcholine (lyso-PtdCho) are *bona fide* biological substrates transported by the yeast plasma membrane ATPases, Dnf1p and Dnf2p, in consort with a second protein Lem3p. Subsequent to transport, the lysophospholipids are acylated by the enzyme Ale1p to produce PtdEtn and PtdCho. The transport of the lysophospholipids occurs at rates sufficient to support all the PtdEtn and PtdCho synthesis required for rapid cell growth. The lysophospholipid transporters also utilize the anti-neoplastic and anti-parasitic ether lipid substrates related to edelfosine. The identification of biological substrates for the plasma membrane ATPases coupled with the power of yeast genetics now provides new tools to dissect the structure and function of the aminoglycerophospholipid transporters.

1. Introduction

Membrane phospholipids are asymmetrically distributed across the bilayers of multiple cellular membranes, and this physical arrangement has important implications for membrane function. The asymmetric distribution of lipid species is largely the result of ATP-driven, intramembrane lipid translocation, catalyzed by ABC transporters and P-type ATPases in specific membrane compartments [1]. The first putative lipid-translocase to be identified at the primary sequence level, and the founding member of the P_4-ATPase class, was the bovine adrenal gland chromaffin granule protein ATPase II, now named ATP8A1. ATP8A1 was shown to be a phosphatidylserine (PtdSer) stimulated ATPase, providing the first clue that this protein might act as a lipid translocase responsible for establishing or maintaining phospholipid asymmetry. Cloning of the yeast gene encoding the ATPase II homolog, Drs2p, and subsequent functional analysis of this and other family members, revealed these translocases are conserved across all eukaryotic phyla and probably act to directly produce membrane phospholipid asymmetry. Additional analyses revealed that the Drs2p was principally localized in the Golgi apparatus, and played an important role in regulating constitutive protein secretion through the organelle [2].

Since the initial discoveries uncovering the Golgi-localized P_4-ATPases and their roles in protein secretion, the genes and cDNAs encoding multiple other members of this family have been cloned and their actions characterized. Members of this transporter family exhibit diverse functions and when mutated, disrupt multiple cellular and tissue-specific functions, including bile excretion [3], lysophospholipid uptake [4,5], endocytosis [6], drug metabolism [7–10], and cold sensitivity [11]. This review will focus on the discovery, biochemical properties, and cellular functions of the P_4-family of P-type ATPases, and their requisite β-subunits of the Cdc50p family, with a particular emphasis on their role as lysophospholipid translocases and their functions as transporters of lysophospholipid-like drugs, the structures of which are given in Fig 1.

2. The Dnf1/2p-Lem3p complexes as plasma membrane phospholipid translocases

After the identification and initial characterization of the bovine ATPase II (ATP8A1) gene [12], it was recognized that the yeast genome contains five closely related homologs, with the Drs2p product showing the closest sequence identity to the ATP8A1 transporter

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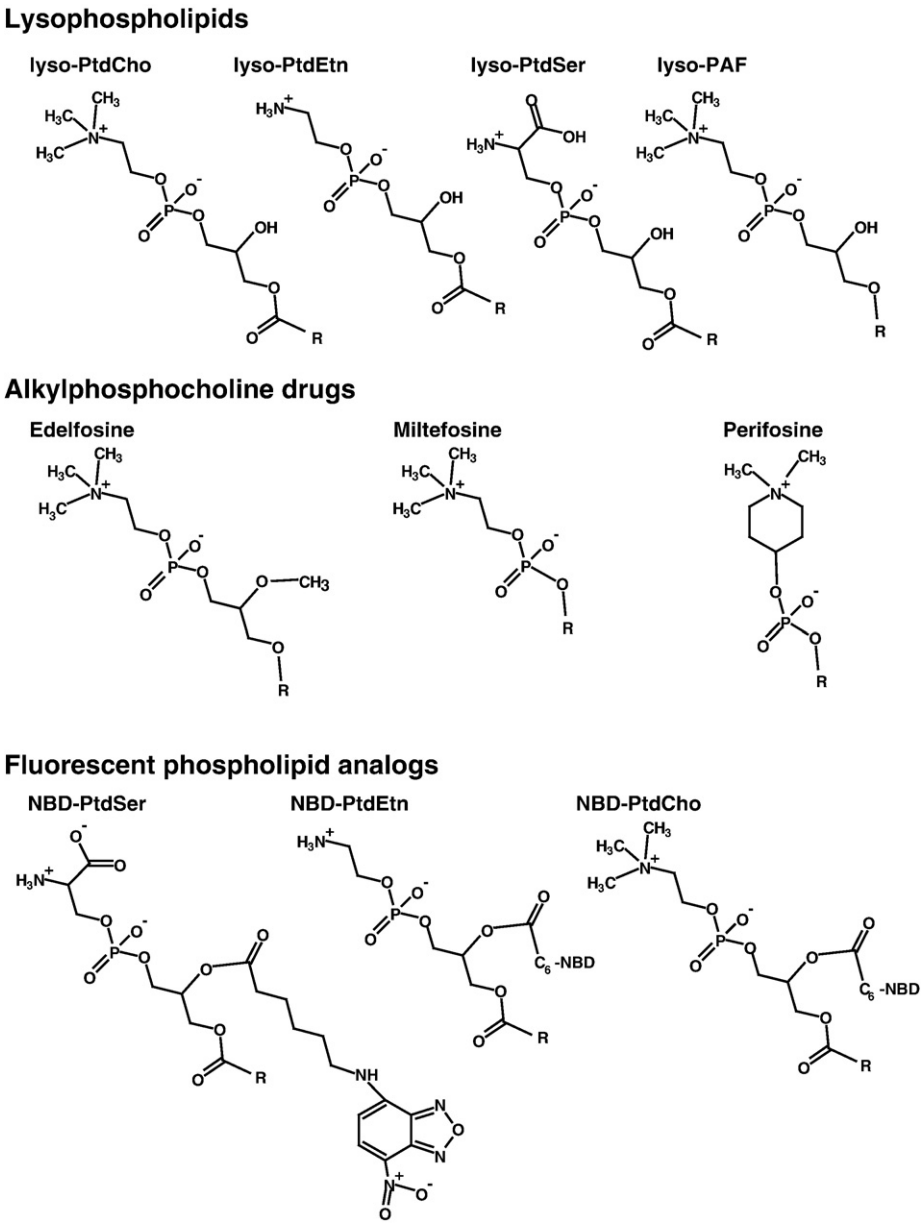

Fig. 1. Structures of substrates for P_4-family ATPases. The structures of the P_4-ATPase substrates discussed in the text are given, and abbreviations are: NBD-, 7-nitrobenz-2-oxa-1,3-diazol-4-yl; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdSer, phosphatidylserine; R, saturated hydrocarbon chain of 15–17 carbons. The structure of the C_6-NBD moiety is given in the structure for NBD-PtdSer, and abbreviated in the other lipids.

(Fig. 2). Drs2p was shown to exert its function in the Golgi and to be essential for proper vesicular trafficking [13]. Drs2p was also shown to directly interact with, and require for its proper function, the accessory protein Cdc50p [14]. Members of the Cdc50p family in yeast include the homologs Lem3p and Crf1p. These proteins are ~450 amino acids, with two transmembrane helices at the N- and C-termini, a large, glycosylated, exoplasmic loop between the transmembrane domains, and short, cytoplasmic N- and C-terminal domains at the ends [2]. The functional Drs2p–Cdc50p transporter complex has been characterized as preferring PtdSer as a substrate, but this specific PtdSer transport function is not essential in vivo [15]. The body of work describing the initial characterization of Drs2p, Cdc50p, and the other yeast P_4-ATPases and their β-subunits or accessory factors has been reviewed recently by several authors [1–3]. It is important to note at the outset that we refer to the P_4-ATPases as lipid translocases throughout this review, but this activity has not been formally demonstrated in an unequivocal way. For example, unlike some of the mineral ion transporters of the P-type superfamily, the P_4-lipid translocases have not been purified and reconstituted in liposomes for activity assays measuring native substrates. Thus, it remains a formal possibility that the lipid translocase activity associated with the presence or absence of a particular P_4-ATPase is due to a secondary effect resulting from loss of the transporter. We believe that biological parsimony coupled with the preponderance of evidence argues for the direct action of these ATPases as lipid translocases.

In addition to Drs2, the proteins Neo1p and the *D*rs2–*N*eo1 *f*amily 1, 2, and 3 proteins (Dnf1–3p) are also P_4-ATPases in yeast, with specific functions described below. The internalization of the fluorescent phospholipid analogs NBD-PtdCho and NBD-PtdEtn by yeast was initially described by the Nichols laboratory [16,17]. Elucidation of the activities and subcellular location of Drs2p and Dnf3p and their accessory proteins [13,18,19] clearly demonstrated these complexes do not function as the principal PtdCho and PtdEtn translocases of the plasma membrane, as originally postulated in yeast [20]. Two other members of the yeast P_4-ATPase family encoded by the *DNF1* and *DNF2* genes have now been implicated in plasma