Identification of residues defining phospholipid flippase substrate specificity of type IV P-type ATPases

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AUTHOR SUMMARY

The plasma membrane forms a barrier around cells and is comprised of many different types of phospholipid molecules. These phospholipids are organized into a bilayer structure with strikingly different internal (i.e., cytosolic) and external (i.e., extracellular) sides. Proteins called type IV P-type ATPases (P4-ATPases) establish this phospholipid bilayer asymmetry by moving specific phospholipids across the membrane to the cytosolic side (Fig. P1). The underlying mechanism is unclear, however, as most related P-type ATPases transport small ions, known as their “substrate” (1). To determine how P4-ATPases recognize the bulky phospholipid substrate we searched for parts of the protein responsible for substrate preference.

The “phospholipid flipping” property of P4-ATPases, in which a specific phospholipid is unidirectionally translocated across the membrane, is a surprising and controversial activity for a P-type ATPase. Ion transporting P-type ATPases all utilize a very similar substrate binding pocket that transports these ions (2–4), but this region is too small for accommodating phospholipids for transport, a conundrum referred to as the “giant substrate problem” for the P4-ATPases. In addition, P4-ATPases will flip specific types of phospholipids (e.g., phosphatidylserine), to the cytosolic side of the membrane and leave sphingolipids on the external side. While proteins responsible for flippase activities have been identified, very little is known about their mechanisms of substrate selection and transport.

To probe how P4-ATPases recognize and flip phospholipids, we created chimeras (i.e., hybrids) from yeast flippases, one called Dnf1 that preferentially flips phosphatidylcholine and one called Drs2 that preferentially flips phosphatidylserine (1). Dnf1 transmembrane segments were replaced with the analogous segments from Drs2, and the resultant chimeric protein was expressed (its synthesis was induced) in yeast cells lacking their own Dnf1. The chimeras were then tested for the Dnf1 chimera flippase activity at the plasma membrane. We looked for the substituted segments that effectively changed the preferred phospholipid of Dnf1 from its normal phosphatidylcholine to phosphatidylserine, which is the preferred substrate of Drs2. Our rationale was that these segments must be compatible with proper folding of the ATPase structure and would be those that are important for substrate selection.

We assayed a series of chimeras for their ability to flip phosphatidylcholine, phosphatidylserine, or phosphatidylethanolamine, which were fluorescently labeled to allow detection. We found that a Dnf1 chimera containing regions termed transmembrane segments 3 and 4 (TM3-4) from Drs2 preferentially flipped phosphatidylserine. Further analysis of this region indicated that TM3 and residues (or amino acids) in the adjoining loop between TM3 and TM4 were primarily responsible for phosphatidylcholine selection by Dnf1. Amazingly, changing a single residue in TM4, tyrosine 618 to phenylalanine (Dnf1 Y618F), allowed Dnf1 to flip phosphatidylserine without reducing its phosphatidylcholine flippase activity. Based on this observation, we predicted that the reciprocal change in Drs2, phenylalanine 511 to tyrosine (Drs2 F511Y), would specifically perturb the ability of Drs2 to flip phosphatidylserine. In fact, cells expressing Drs2 F511Y as the sole source of Drs2 aberrantly exposed normal phosphatidylserine on the extracellular side of the plasma membrane, but maintained a normal asymmetric distribution of PE. Thus, the presence of a tyrosine or a phenylalanine (amino acid types) in TM4, differing only in the presence (Y) or absence (F) of a hydroxyl group, is a major determinant of phosphatidylserine recognition by these flippases.

What do these observations tell us about the mechanism of phospholipid translocation? Y618 in Dnf1 lies at the “proline + 4” position within a PISLY_Y618 motif that is...
structurally significant. The proline (another amino acid type) in this motif is present in TM4 of all P-type ATPases. Most transmembrane segments are structural features called α-helices and this proline induces a break in the middle of the TM4 α-helix. The analogous proline + 4 residue in the experimentally determined structures of the well characterized ion transporting P-type ATPases is oriented away from the canonical ion binding pocket in the middle of the transmembrane structural domain, and towards a groove at the protein/lipid interface (2–4). As we have also implicated other amino acids lining this groove in phospholipid recognition, we speculate that the groove provides a pathway for the hydrophilic phospholipid headgroup to slide from the extracellular side to the cytosolic side (interior of the cell). Because this pathway is at the interface between the protein and lipid environments, the hydrophobic phospholipid tails could simply reorient within the membrane and would not have to be squeezed into the canonical substrate-binding pocket.

Here, we have mapped key residues and transmembrane segments responsible for selection of phospholipid substrate in P4-ATPases. The residues responsible for substrate selection suggest that P4-ATPases utilize a unique transport pathway for phospholipid flip compared to ion transporting P-type ATPases. This work provides mechanistic insight into how P4-ATPases select their phospholipid substrates representing the first step toward deciphering the giant substrate problem.