Ion pumps and lipid pumps studied by site-directed mutagenesis: how different are they?

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P-type ATPases constitute a family of membrane pumps believed to be transiently phosphorylated at a conserved aspartate residue during the catalytic cycle. Among the most well-known and best understood P-type ATPases are the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) and the Na^{+},K^{+}-ATPase. To elucidate the transport mechanism of SERCA we have since 1989 characterized the functional consequences of several hundred different SERCA mutations, allowing identification of amino acid residues involved the binding of Ca^{2+}, ATP, and the inhibitors thapsigargin and vanadate, as well as the key conformational changes of the protein. When the first crystal structure of SERCA was published by the Toyoshima group in 2000, it revealed an almost surrealistic agreement between the actual locations of the Ca^{2+} and ATP binding sites and the assignment of the Ca^{2+} and ATP liganding residues made on the basis of mutagenesis work. With the crystal structures, mutagenesis of the Ca^{2+}-ATPase entered a new era in which the major task is to explain the functional relevance of the structural features seen, rather than to predict structure.

For many other P-type ATPases, much less is known regarding the transport mechanism, due to lack of structural and biochemical data. P4-ATPases ("flippases") are a recently discovered subfamily of P-type ATPases with 14 members represented in the human genome, i.e. 40% of the human P-type ATPase genes. They are believed to "flip" phospholipids from the exoplasmic to the cytoplasmic leaflet of biological membranes against a concentration gradient ("lipid pumps"), thus contributing to the creation of the asymmetric lipid distribution enabling important physiological functions including vesicle formation (endocytosis and exocytosis), fertilization, and signalling in connection with initiation of blood coagulation and apoptosis. Mutations in some of the P4-ATPases are linked to severe human disorders. The flipping of a phospholipid molecule, consisting of a polar/charged head group and two hydrocarbon chains ("the tail"), is much more complex than the transport of small ions with a radius of only ~1 Å, because the phospholipid head group has to traverse the hydrophobic core of the membrane together with its large tail (~20 Å long, half of the lipid bilayer thickness) and must reorient during the transport. This mechanistic enigma has been referred to as the "giant substrate problem". We have set out to investigate flippase mechanism by purifying the flippases and using a site-directed mutagenesis strategy based on our previous experience with the ion pumps. Our results have now led to a realistic proposal for a solution to the giant substrate problem, which we are working to validate in continued studies.