Quick guide

The elusive flippases

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What do they do? In water, phospholipids like phosphatidylcholine spontaneously form closed bilayers with the polar head group exposed to water and the hydrocarbon chains forming the hydrophobic membrane interior. Although phospholipids display a fast lateral diffusion, their 'flip-flop' movement between the two membrane halves is very slow, with half times up to several days. Yet phospholipids in cells are mainly produced in the cytoplasmic leaflet of the endoplasmic reticulum (ER). To ensure bilayer stability, half of the phospholipids must flip to the other side at a rate close to that of their production. Thus, in 1973, Marc Bretscher proposed the involvement of a 'flippase', a protein that facilitates the energetically unfavourable movement of a phospholipid's polar head group through the hydrophobic membrane interior.

Is the reaction energy-driven? Yes and no. ER flippases do not require metabolic energy and mediate a rapid transverse movement of most phospholipid classes in either direction, promoting a symmetrical transbilayer lipid distribution. This is contrary to the situation at the plasma membrane, where the aminophospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE) are concentrated in the cytoplasmic leaflet. This lipid asymmetry is generated by an energy-dependent flippase, the aminophospholipid translocase (APLT), which uses ATP hydrolysis to catalyse a fast, inward translocation of PS and PE. First described in human erythrocytes, APLTs have now been detected in the plasma membranes of many nucleated cells as well as in secretory vesicles and the trans-Golgi compartment. Loss of lipid asymmetry is a hallmark of cells undergoing apoptosis and involves a Ca^{2+}-activated 'scramblase'. PS externalized by this activity is thought to promote the engulfment of apoptotic cells by macrophages.

Why are they so elusive? Although flippases have been studied for more than two decades, their identity is still uncertain. First steps in the purification of an ER flippase have been reported. But the mere presence of transmembrane proteins may be sufficient to catalyze rapid flip-flop in the ER. A putative scramblase, PLSCR1, has been identified that catalyses Ca^{2+}-activated lipid scrambling *in vitro*; but this protein is not required for PS exposure during apoptosis. As PLSCR1 belongs to a protein family, the absence of a scrambling defect *in vivo* might be due to redundancy.

Prime candidate APLTs are members of a subfamily of P-type ATPases, the P4 ATPases (Figure 1). Indeed, studies in yeast revealed that P4 ATPases are essential for inward aminophospholipid transport. But P4 ATPases are closely related to Ca^{2+}-pumping P-type ATPases, and it is somewhat difficult to imagine how these pumps could transport both cations and phospholipids. Members of the unrelated CDC50 family are also required for aminophospholipid transport and may be components of a P4 ATPase-dependent translocation machinery, analogous to the β-subunit of X^+, K^+-ATPases. Functional reconstitution of purified candidate flippases in model membranes is a necessary step to prove their ability to move lipids across the bilayer. So far, this has only been achieved in rare cases.

Can we live without them? Probably not. Flippases play a key role in membrane stability as well as in the mechanism by which cells avoid being killed by macrophages. By expanding one membrane leaflet at the expense of the other, unidirectional flippases may participate in the formation of transport vesicles and cell surface protrusions. Moreover, candidate

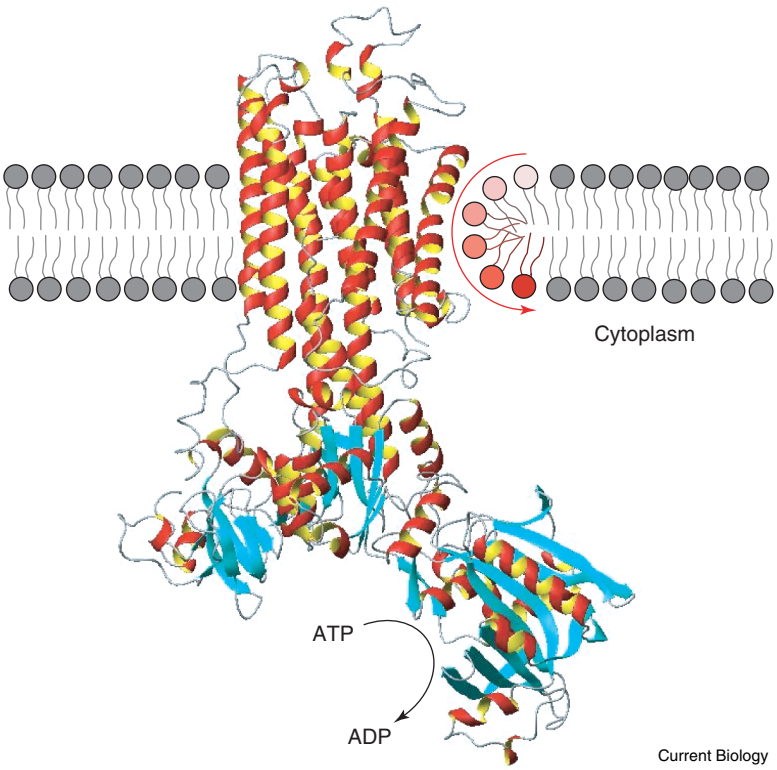

Figure 1. Snapshot of a P4 ATPase at work.

flippases have been implicated in human diseases that include intrahepatic cholestasis, Angelman syndrome, autism, Tangier disease, macular dystrophy and adrenoleukodystrophy.

What is the outlook for researchers working on flippases? Not too bad. Cells contain a whole battery of flippases and these activities can be increasingly attributed to specific proteins. Establishing the primary function of candidate flippases and how they contribute to cell function and human disease is becoming a central issue in biology.

Where can I find out more?

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Human cilia proteome contains homolog of zebrafish polycystic kidney disease gene qilin

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Cilia are found on most cells of the body and are thought to play important roles in physiology and development. Not surprisingly, it is now understood that a very wide range of human diseases and developmental defects results from defects in ciliary assembly or function [1]. The steadily increasing number of disease genes related to cilia suggests that identification of ciliary proteins is a productive way to identify new candidate disease genes. Recent bioinformatics studies have followed this strategy by using comparative genomics to identify likely ciliary genes found only in organisms that have cilia and flagella. This ingenious approach has turned out to be highly productive, most notably by revealing the identity of the Bardet-Biedl Syndrome BBS5 gene [2]. In a recent *Current Biology* Dispatch [3], Greg Pazour presented a carefully thought-out analysis of these approaches, and after discussing several potential limitations, concluded that there is an urgent need for proteomic analysis to determine directly which proteins are contained in cilia and flagella.

In fact, a proteomic analysis of human cilia was completed and published two years ago [4], but curiously this prior work is not referred to, either in the Dispatch on the subject or in the recent comparative genomics studies of flagella. One likely reason for this is that the list of candidate proteins consisted mostly of hypothetical proteins or proteins with no obvious connection to ciliary structure or function, raising the specter, present in all proteomic analyses, of cross-contamination with undesired proteins. However, the human cilia proteome was annotated strictly by searching databases of human sequences, whereas the vast majority of the work on cilia and flagella composition has been done in other model organisms. Inspired by Pazour's article, we re-assessed the annotations for the proteins in the human cilia proteome, by comparing each published entry to the *Chlamydomonas* genome sequence. We chose *Chlamydomonas* as the reference organism because by far the most is known about flagellar components in this particular system.

By searching the *Chlamydomonas* genome using the sequences from the human cilia proteome, we found that 31 of the previously uncharacterized proteins can now be recognized as homologs of either *bona fide* ciliary proteins or proteins identified in comparative genomics analysis of flagellar genomes (Table 1). These include intraflagellar transport proteins, axonemal structural proteins, and proteins required for motility. The presence of a significant number of proteins known to be important for ciliary assembly and function increases our confidence in the validity of these published proteome data, and raises the possibility that some of the other uncharacterized genes could be candidate genes for cilia-related diseases.

A recent forward genetic screen in zebrafish has revealed three novel genes, mutants of which cause polycystic kidney disease (PKD) [5]. Although the PKD phenotype suggested a potential involvement of cilia, only one gene found in the screen showed an obvious ciliary defect. The question was thus posed whether the remaining two genes, *seahorse* and *qilin*, were somehow involved with cilial function. Importantly, upon detailed re-examination, we found that the human cilia proteome contains a homolog of