A possible Achilles heel of ion pumps

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P-Type ATPases are ion pumps present in all living organisms. They serve crucial roles of maintaining cell volume and providing the energy to drive the absorption of nutrients. Their activity is, therefore, vital to an organism's survival. The blocking of pump activity of pathogenic bacterial, protozoan or fungal organisms, therefore, represents a potentially powerful approach to the treatment of infectious diseases which up to now has not been widely investigated or exploited.

Using the Na^+,K^+-ATPase as an archetype of a P-type ATPase, we have found from eosin fluorescence measurements and stopped-flow kinetic studies that the enzyme's E2 conformation is stabilized by electrostatic interactions, most likely between the cytoplasmic N-terminus of the protein's catalytic α-subunit and the adjacent membrane. This conclusion is supported by UV/visible absorbance measurements on the membrane-bound voltage-sensitive probe RH421. It was found that a very similar response of the probe to phosphorylation of the protein by ATP and its conversion from the E1 to the E2P conformation could be achieved by the addition of poly-L-lysine to Na^+,K^+-ATPase-containing membrane fragments. Poly-L-lysine can be considered as a model of the protein's lysine-rich N-terminus. That the membrane-protein interaction is mediated by lysine residues of the N-terminus is also consistent with amino acid sequence analysis, showing the presence of conserved lysine residues within the N-terminus of the Na^+,K^+-ATPase from all vertebrate species.

The electrostatic interactions which stabilize the E2 conformation can be screened by increasing ionic strength, as described quantitatively by the Gouy-Chapman theory. This represents an ideal situation for effective regulation of the Na^+,K^+-ATPase's enzymatic activity, since protein modifications, such as regulatory phosphorylation of a conserved serine residue within the N-terminus, which could perturb the E1-E2 equilibrium in either direction can then easily lead to activation or inhibition. Weakening of the electrostatic interactions and a shift towards E1 causes a significant increase in the rate of ATP phosphorylation. Electrostatic stabilisation of the Na^+,K^+-ATPase's E2 conformation, thus, could play an important role in regulating the enzyme's physiological catalytic turnover.

Other ion pumps also contain extramembranous sequences in either their N- or C-terminus, which could similarly play important roles in pump activity and regulation (Morth *et al.*, 2011). This is most notably the case for the H^+,K^+-ATPase of the stomach mucosa, whose lysine-rich N-terminus shows great similarity to that of the Na^+,K^+-ATPase. The search for drugs which interfere with the interaction of such sequences with the membrane or with their regulatory modification could, thus, be a fruitful direction of future research.

Morth JP, Pedersen BP, Buch-Pedersen MJ, Andersen JP, Vilsen B, Palmgren MG & Nissen P. (2011). *Nat Rev Mol Cell Biol* **12,** 60-70.