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Isoform specificity of Na^+,K^+-ATPase regulation by protein kinase C

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P-Type ATPases are membrane-bound pumps of ions, lipids or other small present in all living organisms. They serve numerous crucial physiological roles such as maintaining cell volume, providing the energy to drive the absorption of nutrients and maintaining the lipid asymmetry of cellular membranes. 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. Mutations in animal pumps are also known to be the cause of hereditary neurological diseases, such a rapid onset dystonia Parkinsonism and acute hemiplegic migraine.

The Na^+,K^+-ATPase is an archetypal P-type ATPase and the first of the family to be identified. For its discovery Jens Christian Skou from the University of Aarhus, Denmark, was awarded the 1997 Nobel Prize in Chemistry. In animal cells, the Na^+,K^+-ATPase maintains osmotic homeostasis, preventing cell swelling or shrinkage, it regenerates the Na^+ electrochemical potential gradient across nerve membranes after each action potential and it provides the energy to drive every animal secondary transporter. An interesting structural feature of the protein is a long lysine-rich cytoplasmic N-terminal tail, which is unresolved in all published structures (either via X-ray crystallography or cryo-electron microscopy) because it is an intrinsically disordered region of the protein. Earlier investigations (Jiang et al, 2017) have provided evidence that the positively-charged lysines of the N-terminus interact electrostatically with negatively-charged phosphatidylserine headgroups of the surrounding lipid membrane, thus stabilising a particular conformation of the rest of the protein. Another clear feature of the N-terminus are two residues that are conserved across all vertebrate species, a tyrosine and a serine residue (Diaz and Clarke, 2018), which are potential targets for regulatory phosphorylation by some form of Src kinase and protein kinase C (PKC), respectively. Phosphorylation of one or both of these residues would introduce negative charge onto the N-terminus, thus weakening its interaction with the membrane and allowing a conformational change of the Na^+,K^+-ATPase via an electrostatic switch mechanism (Clarke et al, 2020).

The purpose of this study is to provide clues as to which of the ten different isoforms of PKC (α, β1, β2, γ, δ, ε, η, θ, ζ, or λ) is the most likely regulatory kinase that interacts with the Na^+,K^+-ATPase. The logic on which our study is based is that two proteins that interact functionally are mostly to co-evolve, so that mutations in one protein are compensated for by mutations in its partner protein in order to maintain the functional interaction. To test for co-evolution, we have applied two different bioinformatic methods: 1) mirror tree analysis, whereby the phylogenetic trees of the two proteins are compared for their similarity, and 2) phylogenetic distribution analysis, where the distributions of the two proteins across different animal classes are compared.

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, and studies such as this will help to identify the most promising targets.

Clarke RJ, Hossain KR and Cao K (2020) Biochim Biophys Acta – Biomembr **1862**: 183382.
Diaz D and Clarke RJ (2018) *J Membr Biol* **251**: 653-666.
Jiang Q, Garcia A, Han M, Cornelius F, Apell H-J, Khandelia H and Clarke RJ (2017) *Biophys J* **112**: 288-299.
Morth, JP, Pedersen BP, Buch-Pedersen MJ, Andersen JP, Vilsen B, Palmgren MG and Nissen P (2011) *Nat Rev Mol Cell Biol* **12**: 60-70.