Are chloride intracellular ion channel proteins (CLICs) really channels? Exploring their membrane structure

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Most proteins adopt a well-defined three-dimensional structure, however, it is increasingly recognized that some proteins can exist with at least two or more stable conformations. Recently, a class of Chloride Intracellular ion Channel proteins (CLICs) has been shown to exist in both soluble and integral membrane forms. Members of this class of ion channels have a CLIC domain of approximately 240 amino acids in length and vary widely in their cellular and sub-cellular distribution. They are associated with a variety of intracellular membranes and are involved in numerous diverse physiological processes including cell cycle regulation, bone re-absorption, tubular formation and apoptosis. However, the function of CLICs as ion channels is still controversial because of their unusual dual-environmental nature and because none of the family members show a clear identifiable transmembrane domain.

Our group has now determined the structure of the soluble conformation/s for several members of this family. Despite this knowledge and because of their auto-inserting nature, the membrane channel structure is still proving difficult to determine using traditional atomic resolution structural techniques. It is therefore necessary to establish how these ubiquitous, soluble proteins can unfold, insert into membrane bilayers and refold to form ion channels. Furthermore, the processes that control this mechanism in the cell also require clarification but may include regulation by oxidation and disulphide bond formation, as in the case of CLIC1. The CLIC family members may also be modulated by pH, alteration in lipid composition, divalent cations, phosphorylation and interaction with other proteins.

A combination of structural studies including Electron Paramagnetic Resonance (EPR) and fluorescence spectroscopy, with functional studies performed in parallel, was used to investigate the insertion of CLIC1 into the membrane bilayer. The results demonstrate a role for the conserved cysteine residue at position 24 for insertion of CLIC1 into the membrane and subsequent chloride channel activity. The transmembrane region has been confirmed as comprising residues 24-46 in the N-domain of CLIC1. EPR experiments also show that insertion is likely to involve a large conformational re-arrangement of the C-terminal domain of CLIC to allow the N-domain to span the membrane bilayer.