Potassium channel gating

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K^+ channels are essential for life and exist in countless forms in nature. Major families appear throughout the evolutionary tree, and their diversity reflects both a multitude of cellular functions and tissue/developmental specificity. K^+ channels are distinguished chiefly by the regulatory messages they respond to, and pore gating is in many cases mediated by domains or subunits surrounding the pore in a functional assembly. Although a small number of K^+ channel structures have now been reported, there is still no rational basis on which the molecular conformation observed in a crystal can be classified "closed" or "open" - other than by eyeballing the width of the pore aperture at the intracellular face. Judgements applied on this basis are ill advised, except in truly self-evident cases (*e.g.*, the MthK Ca^{2+}-gated channel). Removal of integral membrane proteins from the lipid bilayer can, and commonly does, lead to structural artefact that confuses the picture.

The research outlined here is aimed at correlating K^+ channel physiology with the structural rearrangement(s) accompanying opening. It will serve as a platform for interpreting the various means by which auxiliary domains of K^+ channels influence action. Our (continuing) experimental goal has been to obtain crystal structures of a K^+ channel of one type in conducting and non-conducting configurations, and, if possible, in intermediate gating states.

A successful collaboration with Dr D.A. Doyle (Oxford, U.K.) in 2003 allowed us to determine and publish the three-dimensional structure of a complete prokaryotic potassium channel assembly of the inward rectifier family (Kuo *et al*., 2003). KirBac1.1 was the first X-ray structure of an integral membrane protein to have been determined in Australia. The pore of KirBac1.1 was unequivocally closed to ion conduction, and the structure established that intracellular regions are coupled to the activation gate of the channel. In collaboration with Doyle we have since determined structures of a close homologue, KirBac3.1, in different conformations (PDB codes for two sets of deposited coordinates: 1XL4 and 1XL6). These provide the first structural data on the binding sites for divalent ions and polycations during current rectification. There are subtle but significant differences in the conformers, which we suggest represent a progression of changes during the closed to open transition. These global rearrangements, encompass systematic changes in the ion selectivity filter right through to the distal cytoplasmic domains. The study reveals that gating of KirBac channels proceeds *via* an asymmetric intermediate. Our results indicate one structure technically represents an open pore (conducting) state, despite a relatively narrow aperture at the cytoplasmic face. Further widening of the aperture is prohibited due to constraints imposed by the lattice packing. To confirm the parameters of a conducting channel, a wide-open conformer must also be determined and conform to the same pattern of molecular changes. Only in this way can we establish firm criteria that define the gating states. Our group is now working at crystallising an unambiguously open channel, and a closed channel of KirBac3.1. We are producing inward rectifier K^+ channels as recombinant proteins in *E. coli*, and extracting them from the membrane fraction. Whereas the KirBac3.1 structures were obtained from soaking additives into crystals of one particular form, the underlying crystal lattice is, as mentioned, unfortunately incompatible with obtaining a conformer with a wider pore aperture. We have, however, recently obtained crystals under completely different precipitant conditions. Our next step is to test these for diffraction and put them through a series of iterative improvement steps.

This study will provide a starting point for deciphering whether a common mechanism for gating the pore is maintained in all K^+ channels, evidenced by the nature of conformational changes in the pore and adjoining regions, or if the family genres markedly differ.

Kuo A, Gulbis JM & Doyle DA.(2003) *Science*, **300:** 1922-6.