Premonitions of ion channel gating

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Understanding the gating mechanism by which voltage-sensitive ion channels open and close has been a central goal of ion channel research. Channel biophysicists have been making detailed structural models of the gating mechanism for years — in the absence of any real structural data. These elaborate fantasies, which continue to be borne out by new structural discoveries, were made possible by the abundance of conceptual landmarks in ion channels (see Fig. 1). The central landmark is the *pore*, which traverses the membrane and permits extremely high rates of ion transport. The pore has a narrow *selectivity filter*, which shows a high degree of selectivity for the favored permeant ion. This selectivity filter is flanked at each end by a non-selective *vestibule*, into which relatively large organic cations can enter and inhibit permeation by obstructing the pore.

These organic pore blockers proved to be extremely useful probes of channel gating. Pioneering experiments by Armstrong[1] took advantage of the ability to measure K^+ channel currents on a millisecond time scale while applying blockers by intracellular perfusion of squid axons. He found that intracellularly applied quaternary ammonium (QA) blockers interacted with the voltage-sensitive activation gating mechanism of K^+ channels. Although the blockers were applied long in advance of the measurement, the blockade did not begin until channels were opened by a voltage stimulus, as though the blockers could not reach their binding site when the channel gates were closed. Once the blockers had bound, they impaired the ability of the channel to close, as though the gate could not close while the block site was occupied (the 'foot-in-the-door' effect). These experiments showed that activation gating involved a change in access to the intracellular mouth of the pore.

A secondary effect seen by Armstrong, which can sometimes be the dominant interaction between blockers and the activation gate[2], is blocker trapping. In this case, the channel gates are able to close when the blocker is bound, and in doing so they trap the blocking molecule in a cavity located between the intracellular gate and the selectivity filter located closer to the extracellular end of the channel. Only re-opening of the gate allows the trapped blocker to exit.

With the cloning of potassium channels and a flurry of site-directed mutagenesis, the cartoons of channel gating began to take on more molecular substance. The binding sites for various pore blockers[3–5], as well as the selectivity filter[6], were located in the primary sequence. More recently, the use of cysteine substitution mutagenesis together with chemical modification has permitted the identification of positions that line the intracellular mouth of the pore to which access is regulated by activation gating[7].

Very recently the first crystal structure of a K^+ channel was reported[8], and although the gating mechanism of this bacterial K^+ channel is obviously different (it is activated by extracellular protons[9] rather than by changes in the transmembrane voltage) it provides a beautiful physical embodiment for the gating cartoon. On the intracellular side of the selectivity filter is a water-filled cavity with a hydrophobic lining, and further toward the intracellular side is a criss-cross bundle of the pore-lining inner helices. Based on homology with functional measurements in voltage-gated K channels[7], the bundle crossing is the most likely point of gating (at least in the voltage-gated channels). The EPR measurements on the bacterial channel by Perozo and colleagues[10] in this issue of *Nature Structural Biology* now provide the first direct physical evidence for motion of the protein in this region, results that continue the steps toward converting the static cartoon into a full moving picture of the gating mechanism.

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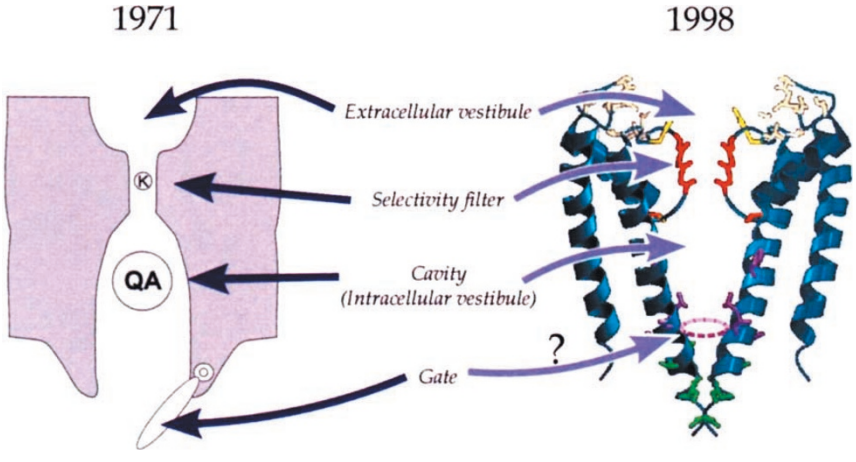

Fig. 1. Evolution of K^+ channel gating concepts. ***a***, The gating cartoon by Armstrong circa 1971 (adapted from ref. 11), showing a cross-section through the pore with the selectivity filter, extracellular and intracellular vestibules, and an intracellular activation gate. Top is extracellular, bottom is intracellular, and the membrane extends to either side of the protein. ***b***, Structure of a bacterial proton-gated K^+ channel (adapted from ref. 8; copyright *Science* magazine), with the same features noted. Only two of the four subunits are shown (those in front and back have been removed); the closest approach of the four inner helices is marked approximately by the dashed ring. Voltage-gated K^+ channels have an additional four transmembrane helices per subunit, and these include the voltage sensor that is energetically coupled to activation gating.