Membrane ion channels: Visualizing the invisible

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The pioneering work of Hodgkin and Huxley in the 1950s suggested that the permeability of ions through the membrane is controlled by changes in membrane potential such as that occurs when the membrane of a nerve or a neuron fires an action potential. As early as the 1950s (refs. 1, 2) with much ingenuity and foresight, they recognized that the permeability had to be preceded by movement of charged 'gating particles' in the membrane. They could not, however, provide clear electrophysiological evidence for actual movement of charged gating particles, although from their observations they could suggest the involvement of charges in the gating process.

It was Armstrong and Bezanilla[3] who first clearly demonstrated a current component hidden in the capacitive transient (owing to charging of the membrane capacity), preceding the current originating from ionic flux. This was demonstrated by careful leak-subtraction, and they named this current component as the 'gating current'. The observation was much debated and initially considered with much scepticism. The existence of the gating current component preceding the ionic current is now firmly established for voltage-gated ionic channels.

With the introduction of recombinant DNA technology in the field of voltage-gated ionic channels in the 1980s, the primary structure of a variety of voltage-gated ionic channels has now become available and the importance of the membrane spanning segments in channel function is being worked out. It is now fairly accepted that the S4 membrane spanning segment of the voltage-gated ionic channels is the 'voltage sensor'. The voltage sensing arises from the positively charged arginine (R) or lysine (K) residues at every third position, with non-polar or hydrophobic residues in between, and this basic pattern is conserved among the voltage-gated Na, K and Ca channels, which are important in patterning action potential waveforms in excitable cells.

There have been interesting models to suggest the conformational rearrangement of the S4 on changing the electri-

cal field across the membrane. An important one is the 'sliding-helix' model[4,5], which suggests that the S4 segment exists as an α-helix in the membrane and is pushed out as a twisting rod inside a cylinder of corresponding negatively charged sites of the neighbouring helices (see Figure 1). Opening of the channel requires an outward movement of the S4 helix, and

thereby movement of charged residue through the electrical field across the membrane. The 'propagating heli model[6] on the other hand, suggests the S4 segment undergoes an α-helic to β-sheet transition, that moves ou ward carrying the charged residue across the membrane.

The physical movement of S4 and i residues, however, has so far bee
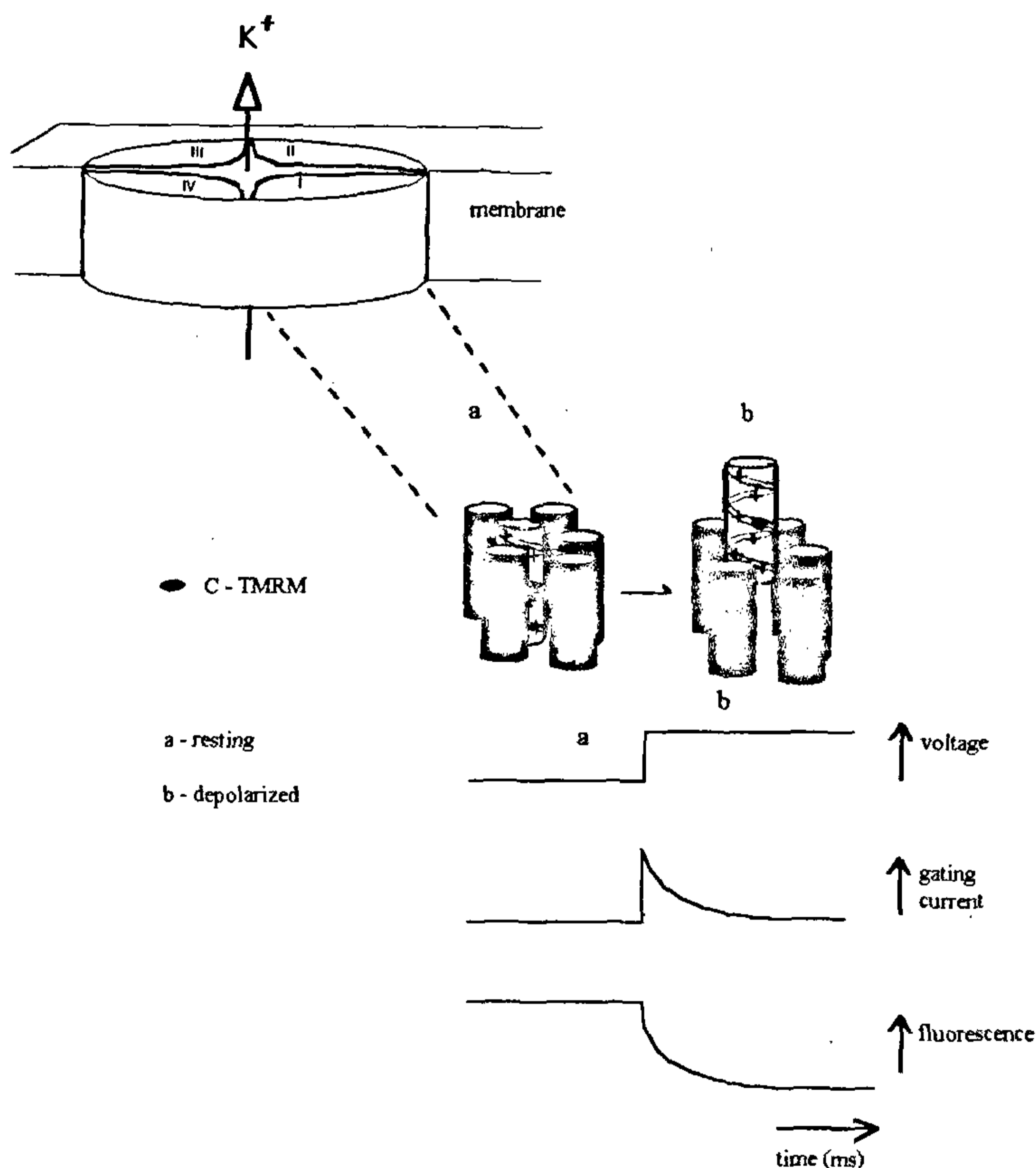

Figure 1. The schematic shows a K⁺ channel consisting of 4 homo monomers (tetramer) embedded in the membrane. One of the monomers (I) is enlarged to show each monomer consisting of 6 membrane spanning segments which are represented as cylinders. The fourth segment (S4) is shown in lighter shade, and the spiral represents the a.as arranged to form an α-helix. The dark dot within the S4 represents the fluorescent label TMRM, tagged to a cysteine residue (C-TMRM) of the mutated K⁺ channel. Changing the membrane potential from resting (hyperpolarized), *a* to a depolarized *b* potential, causes an outward movement of the S4, resulting in the movement of charged a.a. residues (+) and the TMRM-tagged cysteine.

The outward movement of S4 is monitored electrophysiologically as a gating current, and by fluorescence quantitation as a decrease in fluorescence intensity owing to the movement of the TMRM labelled cysteine from the non-polar environment of the membrane to the polar environment of the extracellular recording medium.

'invisible', lending much doubt to the acceptance of either the 'sliding helix' or the 'propagating helix' models.

In a recent issue of *Science*, Mannuzzu, Moronne and Isacoff[7] report the development of an important fluorescent technique along with electrophysiological measurements of gating currents, whereby they have ascertained the physical outward movement of the S4.

For fluorescence, they used the membrane impermeant dye, tetramethyl rhodamine maleimide (TMRM) which is sensitive to solvent polarity, viz., changing from a non-polar environment to a more polar one, causes a substantial decrease in the fluorescence. They reasoned that if the dye could be conjugated to specific 'reporter sites' within the S4, then outward movement of the S4 pulling the reporter site along with the tagged dye, would be signaled as a decrease in fluorescence. In order to conjugate the dye to specific sites within the S4, they mutated specific amino acid (a.a.) residues in S4 while retaining the charged residues. A preliminary confirmation of the feasibility of such an approach was done by confocal imaging, where the depolarization was induced by exchanging a hyperpolarizing extracellular solution (containing *N*-methyl D-glucamine) with a depolarizing one (containing high KCl).

The relation between changes in the environment of S4 and displacement of gating charge was done by measuring in parallel, the changes in fluorescence (using a photomultiplier tube connected to a microscope with epi-fluorescence attachment), and the gating currents using two-electrode voltage-clamp. Experiments were done on frog oocytes, transiently expressing a mutated non-conducting, non-inactivating shaker ShH4 K^{+} channel containing cysteine residues in the S4 segment. Selection of a non-conducting K^{+} channel type was indeed clever, since the additional contribution of current through the ionic channel would not affect the recording of fast-gating currents which is indeed a problem with these electrophysiological measurements, and for conducting channels these measurements have to be done by using specific channel blockers. To make the binding of the fluorescent dye more specific to the ionic channels themselves, they incubated the xenopus oocytes pre-injected with the mRNA coding for the mutant K^{+} channel mentioned above, with tetraglycine maleimide for 1 h which blocks sulfhydryl groups of native membrane proteins. Following this, they allowed a waiting period of 12–14 h to permit newly-synthesized channels to get inserted into the plasma membrane. They added the fluorescent label TMRM to the extracellular medium, and monitored the fluorescent intensity changes upon electrical activation of the membrane with rectangular voltage pulses. The observations are schematically represented in Figure 1.

Briefly, the depolarizing voltage steps moved the gating charges (+vely charged R & K), measured as a gating current, and parallely evoked a graded and saturated decrease in the fluorescence. For the mutants with the largest fluorescence change (M356C, A359C and R365C), the fluorescence–voltage relation (F–V) closely correlated with the gating charge (integral of gating current)–voltage (Q–V) relation (not shown in the figure).

They conclude that a stretch of at least 10 a.a. residues (356 to 365) experiences a change of environment from the hydrophobic membrane to the hydrophilic exterior. The large scale S4 movement agrees with the models which propose movement of the S4 and is consistent with the large charge movements that occur during gating.

While Yang and Horn[8] attempted to demonstrate the gating movement of S4, their measurements which utilized extracellular treatment with MTS-ethyl trimethyl ammonium (MTSET), which adds positively charged group to cysteine residues by disulfide linkage, were indirect and limited by the method reporting the end points of the gating transition. The combination of quantitative fluorescence and gating current measurements reported by Mannuzzu, Moronne and Isacoff[7] has, however, provided a completely newer information about the transition through gating intermediates between the end points, as the gating actually proceeds. This is indeed a very significant contribution, and would help us get a better understanding of the conformational rearrangements in the different domains of the channel protein upon changing the electrical field across the membrane.

Application of fluorescence technique to quantitate ionic concentrations such as Ca inside a single cell, combined with electrophysiological measurements of ionic transport at the membrane level, has now been around for sometime giving us interesting insights about the intracellular signalling mechanisms in single living cells. Application of a similar approach to understand the change in conformational rearrangements within channel proteins as they open in response to a change in electrical field, and its successful demonstration has been long awaited, but not entirely unthought of. The trio's work is indeed very significant and opens up many more interesting experimental possibilities.

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