

Voltage-Gated Ion Channels and Electrical Excitability

Review

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Our ability to do gymnastics, to perceive a colorful world, and to process language relies on rapid communication among neurons. Such signaling, the fastest in our bodies, involves electrical messages produced as ion channels in cell membranes open and close. Various ion channels mediate sensory transduction, electrical “computations,” propagation over long distances, and synaptic transmission. Here, we focus on voltage-gated ion channels, the family of channels that includes the familiar Na^+ , K^+ , and Ca^{2+} channels of nerve and muscle excitability. In the computer metaphor of the brain, the voltage-gated ion channels are like the transistors of logical circuits, detecting, amplifying, and reshaping electrical messages. Our basic understanding of these proteins maintains the framework and rigor established 50 years ago by Hodgkin and Huxley (1952), enriched by much new molecular information and by insights gained from patch-clamp methods.

Although we have had full amino acid sequences of voltage-gated channels for over a decade, we still lack even modest resolution three-dimensional information. All three-dimensional diagrams in the literature derive from functional studies without the benefit of crystallography or NMR. Figure 1A represents widely accepted functional information, much of it deriving from early biophysical and pharmacological work that will be described in this article. An overriding conclusion is that ion channels are aqueous pores. Proceeding through the pore from the outside, an ion would find a wide outer vestibule, a narrow selectivity filter, a voluminous inner vestibule, and finally, at the cytoplasmic end, the gating machinery that closes the pore. Highly charged voltage sensors control activation of the channel but are less important for inactivation. All of the voltage-gated channels have a 4-fold symmetry, with the pore formed at the central line of contact of four channel-forming domains. Unlike ligand-gated channels of fast chemical synapses, much more of the mass of the voltage-gated channels lies on the intracellular side of the membrane than on the extracellular side.

In this review, we first consider how this field was initiated six decades ago and then see how functions of voltage-gated ion channels have been uncovered and mapped onto the linear amino acid sequence of the protein. The rigor of the original analysis set the tone for a new discipline that now produces more than 5000 papers a year.

Early Biophysics and Voltage Clamp Revealed Voltage-Gated Membrane Permeabilities

The period from 1939 to 1952 was a heroic time in the study of membrane biophysics. During this period, Hodgkin and Huxley explained the propagated action potential. Their definitive description of ionic permeability changes in the axon membrane in 1952 was closely preceded by five important discoveries. Four of them occurred shortly before the Second World War. Hodgkin showed that local circuit currents from an excited region of nerve are needed to bring the next region into activity. This meant that depolarization is the natural stimulus for action potential propagation. J. Z. Young (1936) rediscovered the giant axon of the squid. It provided the first convenient way to place electrodes and even electrode arrays inside an excitable cell. Cole and Curtis (1939) showed that the membrane of the squid giant axon increases its conductance 40-fold during an action potential, in apparent agreement with Bernstein's earlier theory of membrane breakdown. Hodgkin and Huxley (1939) discovered with intracellular electrodes that the peak of the action potential overshoots 0 mV by a significant margin. The overshoot was a serious problem for the Bernstein theory, and Hodgkin and Katz (1949) eventually provided the crucial resolution: the overshoot is determined by the Na^+ equilibrium potential and must be due to Na^+ entry during the action potential.

With this background, Hodgkin and Huxley sought to understand how excitation regulates the entry of Na^+ ions. They developed the voltage clamp to measure ion movements as electric currents. The clamp records revealed an inward current followed by an outward current during step depolarizations. In a major conceptual leap, Hodgkin and Huxley (1952) deduced that these membrane currents could be assigned to Na^+ and K^+ permeability mechanisms whose conductances are functions of time and membrane potential. The assumption of separable permeability components and the realization that membrane potential is the controlling variable were the paradigm shifts that opened a new field of inquiry. Their five seminal papers systematically extracted kinetic constants for an empirical description of the conductance changes, showed that this detailed kinetic description—the Hodgkin-Huxley model—suffices to explain all of the classical properties of action potential excitation and propagation, and even offered a plausible physical basis for the control by membrane potential. The stage was brilliantly set.

Consider a few of the findings of these papers. Today, we like to emphasize that ion channels have two major properties: permeation and gating. This separation was clear in the original papers. With respect to permeation, the principal emphasis was that each component of current obeyed Ohm's law with a reversal potential at the Nernst potential for Na^+ and K^+ ions. When extracellular Na^+ concentration was changed, they argued that the flux in either direction across the membrane was proportional to the concentrations on either side. The clear implication was that ion movement was strictly diffusion down an electrochemical gradient without additional forces.

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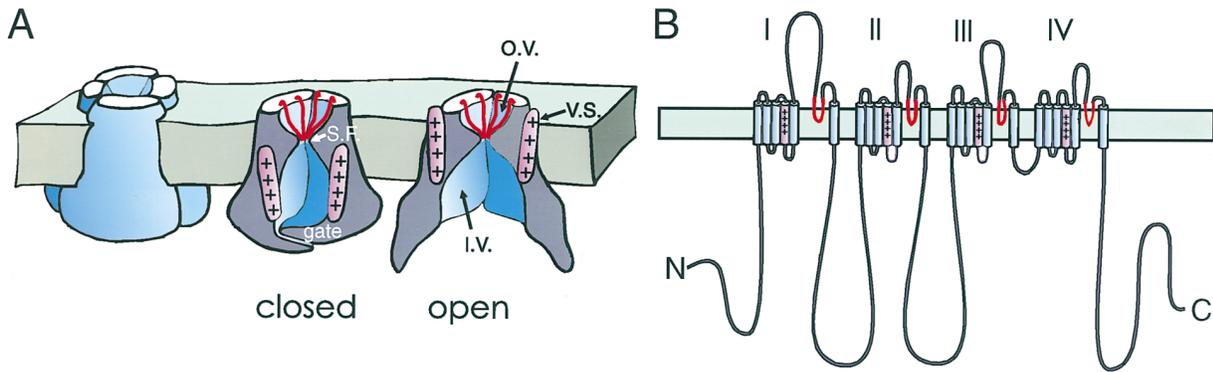


Figure 1. Structure of Voltage-Gated Ion Channels

Functional components (A) and peptide folding (B) are shown diagrammatically with P regions in red and the S4 segment in pink. Abbreviations: S. F., selectivity filter; V. S., voltage sensor; O. V., outer vestibule; and I. V., inner vestibule. The folding diagram is for an Na⁺ channel with four similar domains (I-IV). A K⁺ channel is a homo-tetramer of subunits similar to a single Na⁺ channel domain.

With respect to gating, much more was discovered. Notably, the permeabilities to Na⁺ and to K⁺ had quite different time courses. The Na⁺ conductance rose and inactivated quickly during a depolarization, and in the axon the K⁺ conductance rose more slowly without inactivation. Changing ion concentrations changed the direction of current flow but not the time course: "The changes in membrane permeability appear to depend on membrane potential, and not on membrane current." In addition, Hodgkin and Huxley succeeded in describing the permeability changes as a set of chemical reactions whose rate constants are a function of voltage. The rate of change of each permeability depended quite steeply on the membrane potential, and a 10-fold increase in opening occurred with membrane potential depolarizations as small as 7–12 mV.

What did Hodgkin and Huxley say about mechanism? About permeation, they said very little except that flux was downhill. They did not mention the concept of an aqueous pore, nor did they use the word channel. About gating, a word they also did not use, they argued, "Details of the mechanism will probably not be settled for some time, but it seems difficult to escape the conclusion that the changes in ionic permeability depend on the movement of some component of the membrane which behaves as though it had a large charge or dipole moment." These charged controlling "particles" were envisioned to move under the influence of the electric field in the membrane and to "allow ions to pass when they occupy particular sites in the membrane." The distribution of the particles was compared to a Boltzmann distribution, exactly as is done today, and the steepness of the voltage dependence required that as many as six electronic charges move fully across the membrane during activation. To account for the sigmoid time course of activation of the conductances, the model supposed the movement of three gating particles for Na⁺ and four for K⁺. These hypothetical particles combined the functions that we now separately assign to the voltage sensors, the gates, and the conducting pore. Their multiplicity in the model was a harbinger of the modern finding of four structurally separate voltage sensors in each channel. The novelty, depth, and durability of these insights are a monument to the powerful physical intuition of these two great scientists.

Channels Become Molecules

Cloning of the pore-forming α subunits of voltage-gated Na⁺, K⁺, and Ca²⁺ channels was reported in 1984–1988. It showed that, fundamentally, these channels are all members of the same gene superfamily with the same overall structure. This good news confirmed that there should be much mechanistic similarity among the channels and that we were free to generalize—as we had already been doing in the biophysical work of the previous decades.

The Na⁺ and Ca²⁺ channel clones, first reported by the Numa laboratory, predict large peptides (>2000 amino acids) containing four homologous repeats (domains I–IV), each of which has a motif with six putative transmembrane segments, S1–S6 (Figure 1B). The cloned K⁺ channel was first reported by the laboratories of Jan and Jan (Tempel et al., 1987), Tanouye (Kamb et al., 1987), and Pongs (Pongs et al., 1988). It is about one fourth as large as an Na⁺ channel and contains only one copy of the S1–S6 motif. As might be expected, K⁺ channels were later shown to be tetramers of the pore-forming subunit (MacKinnon, 1991), making them structurally quite similar to Na⁺ and Ca²⁺ channels. In all of three clones, segments S1, S2, S3, S5, and S6 are quite hydrophobic, and each is long enough to span the membrane as a helix. In the S4 segments, however, every third residue is a positively charged arginine or lysine, for a total of seven positive charges in K_v1.1 and five to eight positive charges in the various S4 domains of Na⁺ and Ca²⁺ channels. The S4 segment was almost immediately recognized as a candidate for the voltage sensor—one of Hodgkin and Huxley's controlling particles. After a brief consignment to the cytoplasm, it joined the membrane-crossing segments where it could experience the electric field of the membrane as required. The great importance of another amphipathic loop between segments S5 and S6 (called P or H5 and shown in red in Figure 1A) was recognized only later. It was first assigned its present status as part of the outer vestibule and the conducting pore on the basis of thoughtful molecular modeling (Conti and Guy, 1990).

The folding topology in Figure 1B has much to support it. To mention only some of the evidence, the N terminus is known from the experiments of Aldrich and colleagues

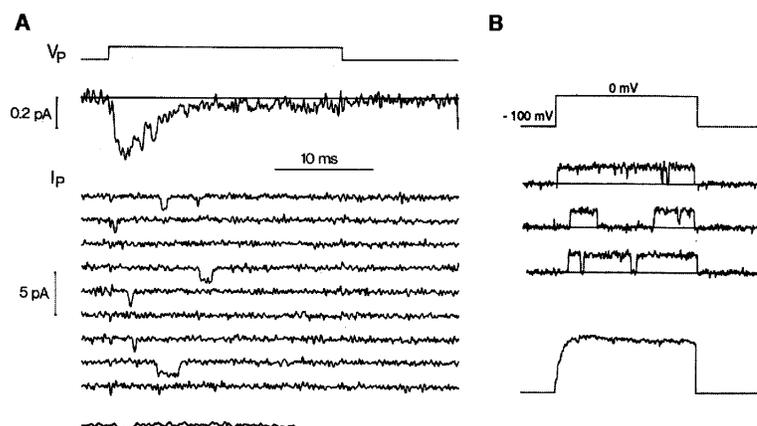


Figure 2. Ionic Currents through a Single Channel Sum to Make Classic Macroscopic Currents

(A) is a single sodium channel from Sigworth and Neher (1980), and (B) is a single potassium channel from Zagotta et al. (1988).

to be cytoplasmic, and in Shaker B (ShB) K^+ channels this terminus contains the molecular machinery for fast "N-type" inactivation gating (Hoshi et al., 1990). The sidedness of many residues of these channels has been identified by mutating an individual residue to cysteine and then testing whether one of Karlin's thiol-reactive compounds, e.g., MTSET (Akabas et al., 1992), must be applied to the extracellular or the cytoplasmic face to label the cysteine. One particularly illuminating set of experiments showed that S4 extends across the membrane: a cysteine introduced into the N-terminal end of S4 can be labeled only from the outside, and a cysteine introduced into the other end of S4 can be labeled only from the inside, both in Na^+ (Yang and Horn, 1995; Yang et al., 1996) and K^+ channels (Larsson et al., 1996). The general hairpin topology of the P region was worked out by mutating residues that altered sensitivity to pore blockers. The key was that a threonine residue in the middle of the P region is crucial for block by intracellular tetraethylammonium ion (TEA^+), whereas residues both before and after this threonine determine the sensitivity to block by extracellular TEA^+ and charybdotoxin (Yellen et al., 1991; and see Hartmann et al., 1991). Many other insightful experiments support the proposed topology (e.g., Shih and Goldin, 1997), and we are aware of no contradictions.

Conduction and Selectivity

Hodgkin and Huxley conceptually separated ion conduction from what we now call gating, but the nature of the conducting path, whether ion carrier or ion pore, lipid or protein, did not become clear for many years. Selective block and channel conductance estimates from experiments with the channel blockers tetrodotoxin (TTX^+) and TEA^+ led each of us to conclude by the early seventies that Na^+ and K^+ channels must be separate aqueous pores and that transport based on a carrier such as valinomycin was too slow to be considered. The final proof was made possible 10 years later by the patch clamp (Hamill et al., 1981), which conferred the ability to measure current through a single ion channel (Figure 2). We found this beautiful and exactly as anticipated.

What is the nature of the conducting pore? Because of its simple tetrameric structure, the K^+ channel has

been a favorite for experimentation. This channel's main task is letting K^+ ions out and keeping Na^+ ions from going in. To explain the $>50:1$ selectivity, one can invoke only the geometry of the pore and the energetics of interaction of the ions with water and with the residues of the pore. We both found by ion substitution studies in the early seventies that the pore narrows somewhere to a bore of only 3.0 Å—just accommodating Rb^+ ions. As the crystal radius of Na^+ ions is smaller than that of K^+ ions, Na^+ would fit through any hole that K^+ can. Therefore, the discrimination has to be blamed on the unfavorable energetics of stripping most of the water from the more strongly hydrated Na^+ ion in a small hole without providing much favorable interaction with the channel in return.

This constriction of the pore is now attributed to the four 20-residue P regions, which are thought to dip partway into the membrane from the extracellular side and then loop back out (red loops in Figure 1B). These residues narrow the pore, forming the lining of the outer vestibule and the narrow selectivity filter (Figure 3A) but not the inner vestibule. Within each P region is a remarkably conserved "signature sequence", -TXXTXG YG- (-Thr-X-X-Thr-X-Gly-Tyr-Gly), found in at least 50 cloned K^+ -selective channels, including the much smaller inward rectifier channels. The signature sequence is thought to be the heart of the selectivity filter. How does it work? Thus far, the most illuminating experiment has been negative. The sequence of the Shaker K^+ channel P region (TMTTGYG) contains three threonine residues whose -OH groups seemed prime candidates for complexing with and selecting K^+ ions. However, all hydroxyl groups except the one on the first threonine were found to be unnecessary for a selective channel, and evidence regarding the first was inconclusive (Heginbotham et al., 1994). As an alternate explanation, the authors speculated that each subunit contained a sharp loop (perhaps at GYG), resulting in an exposed backbone carbonyl whose oxygen served to complex the K^+ ion. Another possibility is that K^+ channels have more than one selective site, and no single mutation will completely destroy selectivity.

Many ingenious experiments have explored the pore region of K^+ channels, beginning in Miller's lab with the use of charybdotoxin to probe the geometry of the pore mouth (MacKinnon and Miller, 1989). Other experiments

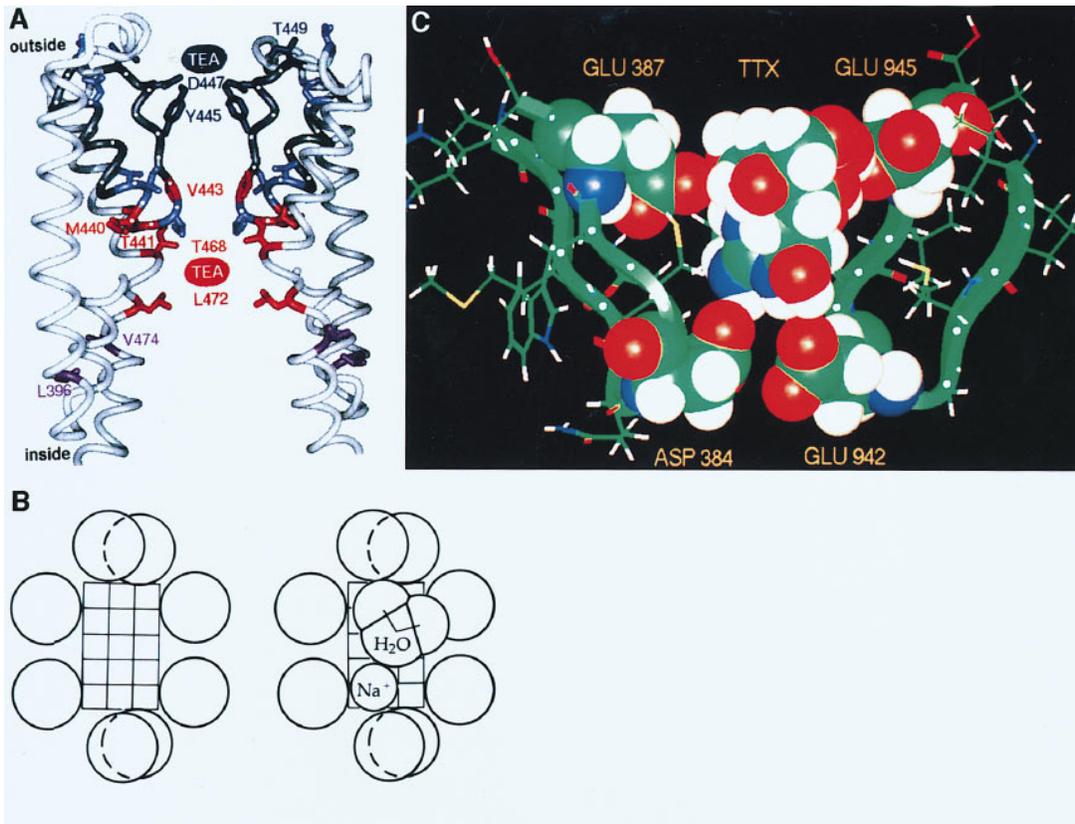


Figure 3. Pore Regions at Varying Degrees of Abstraction

(A) Cross section of the P region, S5 (containing L396), and S6 (containing V474) of a K^+ channel, as visualized by Durell and Guy (1996). White dots mark the P loops.

(B) The Na^+ channel selectivity filter, from Hille (1971). At left, the $3 \times 5 \text{ \AA}$ pore is surrounded by carbonyl and carboxyl oxygens, which, at right, bind a partially dehydrated Na^+ ion sufficiently well to allow permeation.

(C) A molecular model of the Na^+ channel P region, from Lipkind and Fozzard (1994). Two charges from the outer (Glu 387 and 945) and inner (Asp 384 and Glu 942) charged rings are shown, and a TTX molecule occupies the outer vestibule. The selectivity filter is at the inner ring. White dots mark the backbone of the P loop.

have involved replacement of selected residues by cysteine, followed by oxidation to produce disulfide bonds or reaction with Karlin's thiol reagents or Ag^+ (Yellen et al., 1994; Lu and Miller, 1995; Kurz et al., 1995; Krovetz et al., 1997). Thus far, these studies are in a general way compatible with the picture shown in Figures 1 and 3A. The outer part of the P region is a funnel that reaches its narrowest part near the signature sequence. External TEA^+ blocks the wider part of the funnel, and its binding is strongly enhanced by a mutation in the outer pore region (T449Y; Figure 3A). The part of the pore internal to the selectivity filter is formed by the S5 and S6 segments (Choi et al., 1993; Kirsch et al., 1993; Lopez et al., 1994; Holmgren et al., 1997). Ions in the outer vestibule already feel strong selectivity (Neyton and Miller, 1988). It seems likely that the outer vestibule selects on the basis of hydrated radius, whereas the narrow selectivity filter selects on the basis of the energy of stripping the ion down to its crystal radius (Hille, 1973). Unlike a K^+ ion, which can shed some surrounding water molecules, TEA^+ cannot shed its covalently linked ethyl groups. Thus TEA^+ , which is about the size of a hydrated K^+

ion, can occupy the outer and inner vestibules but cannot enter the selectivity filter.

Similar studies have been done with Na^+ channels. To generate the upstroke of the action potential, these channels have three selectivity tasks: letting Na^+ go in, keeping K^+ from going out, and preventing Ca^{2+} ions from getting stuck in the pore and interfering with Na^+ permeation. Early studies defined an outer vestibule as the receptor for potent block by extracellular tetrodotoxin and an inner vestibule as the receptor for block by local anesthetics. These studies also provided clear arguments for an acid group—a negative charge—within the pore that could interact with permeant cations and with tetrodotoxin. The initial evidence was that the conductance of Na^+ channels drops sharply when the bathing pH is lowered, as if an essential acid group (Hille, 1968) located quite near the extracellular end of the pore (Woodhull, 1973) becomes neutralized, preventing cations from passing. The narrow part of the pore admits cations up to the size of the aminoguanidinium ion (requiring at least a $3 \times 5 \text{ \AA}$ rectangular hole; Figure 3B), yet K^+ ions permeate only 1/12 as well as Na^+ (Hille,

1971, 1972). Why don't K^+ ions (2.7 Å diameter) glide through such an aperture as easily? Hille's working hypothesis was that as ions passing through the aperture are partially dehydrated, they are stabilized by compensatory direct interaction with the negative charge in the selectivity filter. In this view, small ions like Li^+ and Na^+ are well stabilized by the negative charge because they can get close to it, and larger ions like K^+ and Rb^+ cannot. Subsequent cloning showed that acid groups are indeed present in the P regions of all Na^+ channels (Figure 3C), and mutagenesis proved that they contribute significantly to conductance, selectivity, and tetrodotoxin binding (Terlau et al., 1991; Heinemann et al., 1992). Nevertheless, thus far we don't know how to make quantitative tests of the selectivity hypothesis.

The P regions of Na^+ and Ca^{2+} channels have similar sequences, and illuminating experiments have been performed to determine the basis for their Na^+ or Ca^{2+} preference. Both channels have two rings of charge encircling the pore, each ring containing four residues, one from each homologous domain (Figure 3C). The outer ring is entirely negative and is thought to be relatively distant from the pore axis. The inner ring, two to three residues deeper into the pore, is composed of DEKA (Asp Glu Lys Ala) in the Na^+ channel and EEEE (Glu Glu Glu Glu) in the Ca^{2+} channel. In general, it seems appropriate that the channel conducting doubly charged Ca^{2+} should have more negative charge. With this in mind, Heinemann et al. (1992) succeeded in converting a Na^+ channel into a Ca^{2+} -preferring channel by point mutations that increased the negativity in the inner ring. From their experiments, the importance of the inner ring was immediately apparent. The mutated channels lost Na^+/K^+ discrimination (see also Favre et al., 1996) and became quite Ca^{2+} permeable. In addition, Ca^{2+} interference with monovalent permeability became severe, because Ca^{2+} binds tightly to the added negative charge. The importance of the EEEE ring in Ca^{2+} channels was examined in detail by Tsien and colleagues (Ellinor et al., 1995), yielding a detailed mechanism for Ca^{2+}/Na^+ discrimination by calcium channels. Divalent/monovalent selection thus seems well understood, whereas monovalent/monovalent selectivity is at best understood in principle.

Activation Gating

Voltage-gated channels are exquisitely sensitive to small changes in membrane potential. Hodgkin and Huxley realized that Na^+ and K^+ channel opening or activation must result from movement of charges within the membrane. Their work predicted "gating current," a small charge movement generated (in more recent terms) by the voltage-driven conformational changes that open the channels. Theoretically speaking, there was no alternative, and in due course the expected current was detected, first in connection with excitation-contraction coupling in muscle (Schneider and Chandler, 1973) and subsequently in nerve membranes (Figure 4A; Armstrong and Bezanilla, 1973; Keynes and Rojas, 1974). By 1980, it seemed probable that the channels were composed of protein, leading one of us to propose

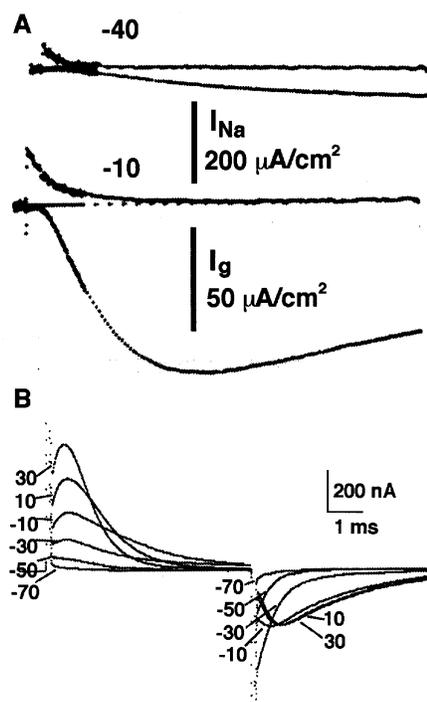


Figure 4. Gating Current Gives Evidence for Slow Steps during Activation-Deactivation

(A) The upper trace of each pair is I_g , and I_{Na} is below. At -10 mV, I_g has a slow component that parallels the activation of channels (as monitored by I_{Na}). From Armstrong and Gilly (1979).

(B) For a K^+ channel, gating currents associated with deactivation (downward tails at pulse end) are much slower for large depolarizations that open many channels. From Stefani et al. (1994).

that the gating particles of Hodgkin and Huxley were in fact charged membrane helices—specifically, a negatively charged helix that moved inward relative to a positively charged helix, yielding a lot of charge movement, and hence voltage sensitivity, with relatively little physical motion (Armstrong, 1981). Each of four or five proposed subunits would contain such a pair of helices. When the Na^+ channel was cloned a few years later, the positively charged helix—the S4 segment—was immediately visible. The expected negatively charged helix does not exist, and it is still not entirely clear how the necessary counter charges for stabilizing the amphipathic S4 helix in the membrane are provided.

The likelihood that S4 was the long-sought voltage sensor was immediately apparent, but experimental proof has come more slowly. A first strategy was to create mutant channels with neutral residues replacing some of the positive charges in S4 (Stühmer et al., 1989). These experiments provided suggestive support, but they were complicated by the failure of channels with many neutralized residues to express and by drastic and often unanticipated changes in gating properties when this sensitive helix was altered in any way. Recently, cysteine mutagenesis and cysteine labeling with thiol-reactive compounds has provided good evidence that the S4 segments in both Na^+ and K^+ channels move as expected following voltage changes (Yang and Horn,

1995; Larsson et al., 1996; Yang et al., 1996). Cysteine residues introduced at certain points in S4 show state-dependent labeling; i.e., a given cysteine may be labeled only from the outside when the channel is open and only from the inside when it is closed. The labeling patterns of both Na⁺ and K⁺ channels are consistent with outward movement of S4 in response to a positive change of membrane potential.

The details of how S4 moves may not be clear for some time. It is likely that all four S4 units must move in order to open the channel and that there are several nonconducting, partially activated states in which some but not all S4 units have moved. In behavior, this corresponds closely to the Hodgkin-Huxley model of the K⁺ channel with its four gating particles. But does each S4 have only two possible positions, off and on, or does it have more? This cannot yet be answered with precision. Biophysical evidence suggests that several fast steps in the activation process are followed by a slow one that is quite different. Gating current recorded from a squid axon at -40 mV decays to an undetectable level before the ionic current begins to rise (Figure 4A; Armstrong and Gilly, 1979); at -10 mV, the gating current has two distinct components, a fast one and a slower component (large enough to see at this voltage) that parallels the rise of Na⁺ inward current. From examining K⁺ channels, Aldrich and colleagues concluded that the S4 segments of the individual domains moved separately at first, followed by a "concerted" step involving all four domains (Zagotta et al., 1994). Suggestively, this last step can be separated from the early ones by a mutation at the inner end of S4 (Schoppa et al., 1992). Gating current measurements from K⁺ channels also strongly support the concept of a late, slow voltage-sensing step during activation (Stefani et al., 1994). In Figure 4B, for example, the gating current recorded as a mutant K⁺ channel closes (inward tail at pulse end) is much slower after a depolarization that opens the gates of many channels (+10 mV) than after a smaller depolarization that opens very few (-30 mV). Apparently, the gate-opening step is reversed much more slowly than the preceding transitions among closed states. The precise meaning of these tantalizing hints is a puzzle that remains to be worked out.

Movement of the S4 segments provides the impetus for conformational change, but where is the physical gate that moves to open the channel? That the gate is internal was shown long ago by experiments with TEA⁺ (Armstrong, 1971). TEA⁺ and its relatives (QA⁺) occupy the inner vestibule of the channel, but they can get to this blocking site only if the gate is open. Consistent with this, Yellen and colleagues found (1) that mutation of residues in S6, which lines the inner vestibule, strongly affects binding of internally applied TEA⁺ derivatives (Holmgren et al., 1997); and (2) that cysteines substituted into S6 can be labeled only when the gate is open (Liu et al., 1997). Thus, the gate must be inside where it can protect the inner vestibule, as in Figure 1A. Interestingly, the gate of a QA⁺-occupied channel can close rather easily, trapping QA⁺ in the vestibule. Evidently, the vestibule must be large enough to accommodate the blocker even with the gate closed (Holmgren et al., 1997).

What forms the gate is not yet clear. A reasonable

candidate would be the S4-S5 linker, the internally located 13-residue segment joining S4 to S5. Cysteines introduced into this segment are easier to label when the channels are partially or fully activated (Holmgren et al., 1996), consistent with the idea that they form flaps of the gate but perhaps falling short of proving the case. Whatever its identity, does each monomer form one-fourth of the gate? If this is the case, and the flaps are withdrawn one by one, it is hard to see why the channel does not conduct after a single one is withdrawn. There are several alternatives. One is that there are four flaps and they are withdrawn simultaneously in a single, concerted step. Another possibility is that a gate flap from one subunit is sufficient to close the channel and is overlapped by flaps from the other subunits, as in Figure 1A (a "stacked hands" model). A final possibility is that opening the channel involves both flap movement and associated changes in the P region. At least one gating change, called C-type inactivation, is associated with a clearly demonstrated constriction of the outer mouth of the pore (Liu et al., 1996). Thus far, however, there are no clear changes in the P region associated with activation gating.

Inactivation Gating

Sodium channels open in response to depolarization, admitting Na⁺ and driving the membrane voltage positive during the upstroke of the action potential. They then inactivate spontaneously (stop conducting), making it easy for the K⁺ channels to restore the membrane potential to rest. Thus, Na⁺ channels and also some K⁺ channels have a second gating factor, inactivation gating, that is mechanistically simpler and quite different from activation: after the activation gate opens, a cytoplasmically located portion of the channel peptide diffuses into the mouth of the inner vestibule of the pore and blocks conduction (Figure 5). The movement of this peptide is relatively slow, allowing the average Na⁺ channel, for example, enough conduction time to complete the rising phase of the action potential. The apparent voltage dependence of inactivation is primarily derived from the voltage dependence of the activation that precedes it rather than from highly charged voltage sensors devoted to inactivation itself.

Can it be that simple? Not quite, but let us begin simply. The picture developed in several steps. Ancient experiments showed that squid K⁺ channels could be given a rather close semblance of inactivation by putting long-chain TEA⁺ derivatives in the axoplasm (Armstrong, 1971). Conversely, Na⁺ channel inactivation could be selectively destroyed by internally perfusing a squid axon with proteolytic enzymes, suggesting that a proteinaceous inactivating particle had been removed or destroyed (Armstrong et al., 1973). The activation gate continued to work normally after this treatment. From kinetic analysis, it was deduced that each Na⁺ channel had a single inactivation gate. Other experiments made use of ionic and gating currents to follow the conformational changes of the Na⁺ channel. It was clear that (1) most channels open before they can inactivate, (2) the inactivation step has little intrinsic voltage dependence, and (3) the presence of the inactivation particle in the

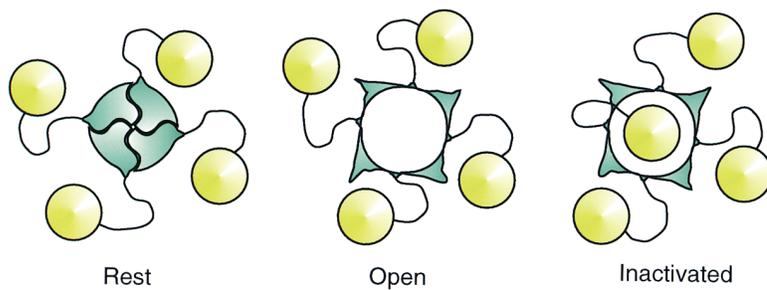


Figure 5. Ball-and-Chain Model for Inactivation of a ShB K⁺ Channel

When the channel is open (center), any one of the four inactivation balls can inactivate the channel (right). Inactivation for an Na⁺ channel is similar, but there is a single inactivation ball.

channel prevents reclosing of the activation gate, a foot-in-the-door mechanism. Cutting off the protein foot (the inactivation particle) with pronase removed this interference, making it easy to close the activation door at any time. These observations led to the ball-and-chain model (Bezanilla and Armstrong, 1977), in which an inactivation ball is attached to the inner surface of the Na⁺ channel by a peptide chain which can be cut by pronase, much like in Figure 5, but with only one inactivation ball rather than four. This model was compatible with the results obtained by Aldrich and colleagues from analysis of inactivation of single Na⁺ channels (Aldrich et al., 1983). The simple model must be complicated a bit to account for the fact that the channels do not leak Na⁺ when recovering from inactivation (e.g., Armstrong and Gilly, 1979).

Where within the sequence is the inactivation gate? The answer depends upon the channel type. We have a remarkably clear picture for the "fast" inactivation gate in the ShB K⁺ channel. K⁺ channels vary with regard to inactivation, and some do not inactivate at all. ShB K⁺ channels, however, are similar in inactivation properties to Na⁺ channels, the major difference being that activation and inactivation gates of the channels are slower in opening and closing. In a series of justly famous experiments involving site-directed mutagenesis, Aldrich and colleagues (Hoshi et al., 1990) found that deletions within the first 20 residues beginning at the N terminus completely destroy fast inactivation. Deletions in the next 63 amino acids (numbers 21–83) have the remarkable effect of speeding inactivation! The interpretation is that the first 20 residues of the N terminus of each subunit form an inactivating particle that is secured by a chain composed of the next 63 residues. Shortening the chain makes it easier for the inactivation ball to find its receptor in the inner channel mouth. Aldrich and colleagues performed another set of experiments that clearly confirmed this picture. First, they made a deletion mutant with much of the N-terminal inactivation region (amino acids 6–46) missing. Channels formed with these mutant peptides showed no inactivation, but they "inactivated" almost normally when a "ball" peptide, composed of the first 20 residues of a normal channel, was added to the cytoplasm. These free-floating balls had access to their blocking site when the activation gate of the channel was open, and "inactivation" occurred at a rate proportional to their concentration in the cytoplasm.

Perfectly functional ShB K⁺ channels can be made from four identical subunits. Does this mean that any one of the four available inactivating balls can inactivate

the channel? As a test, channels can be engineered to have only one subunit with a ball (MacKinnon et al., 1993). These channels still inactivate but do so four times more slowly than normal. The conclusion is that any of the four balls can inactivate a channel, and it is simply a matter of chance which one gets there first (see also Gomez-Lagunas and Armstrong, 1995). With four present, the chances of inactivation occurring within a given short interval is four times larger than when there is a single ball.

The inactivation ball binds to a receptor in the mouth of the open K⁺ channel. Where is the receptor? This question was addressed by Isacoff, Jan, and Jan (1991). They found that inactivation was affected by mutations in the 13-residue chain linking transmembrane crossings S4 and S5. For example, changing one threonine residue to a serine almost completely removed inactivation, without much changing the function of the activation gate. Their experiments strongly suggest that the inactivation ball binds to this region to inactivate the channel.

Unlike K⁺ channels, each Na⁺ channel has a single inactivation particle, as noted above. The inactivation region of the Na⁺ channel peptide is not located at the N terminus, as K⁺ channel experience might have led us to suspect. Instead, the crucial zone is in the linking regions between homologous domains III and IV (Figure 1B). A single cut of the peptide chain in this region, performed by genetic engineering, destroys fast inactivation (Stühmer et al., 1989). Particularly important to inactivation are three successive residues, isoleucine, phenylalanine, and methionine, in the linker (West et al., 1992).

The S4 Segment As a Generalized Voltage Sensor

Shortening of a skeletal muscle fiber is initiated by a voltage change (normally an action potential) in the transverse tubular extension of the surface membrane, leading to release of stored Ca²⁺ from the sarcoplasmic (endoplasmic) reticulum. This mechanism makes use of a modified type of Ca²⁺ channel in the tubular membrane and a large channel molecule called the ryanodine receptor in the reticulum membrane. These channels have become wedged in a remarkable union, creating a mechanical linkage between the two membrane systems. The Ca²⁺ channel in this case serves mainly as a voltage sensor, and its conduction properties are less important. Depolarization of the tubule membrane causes movement of the S4 segments in the modified Ca²⁺ channel. This motion is communicated to the ryanodine receptor via the mechanical linkage, causing it to open and release calcium from the interior of the sarcoplasmic reticulum. Such mechanical interaction between proteins in

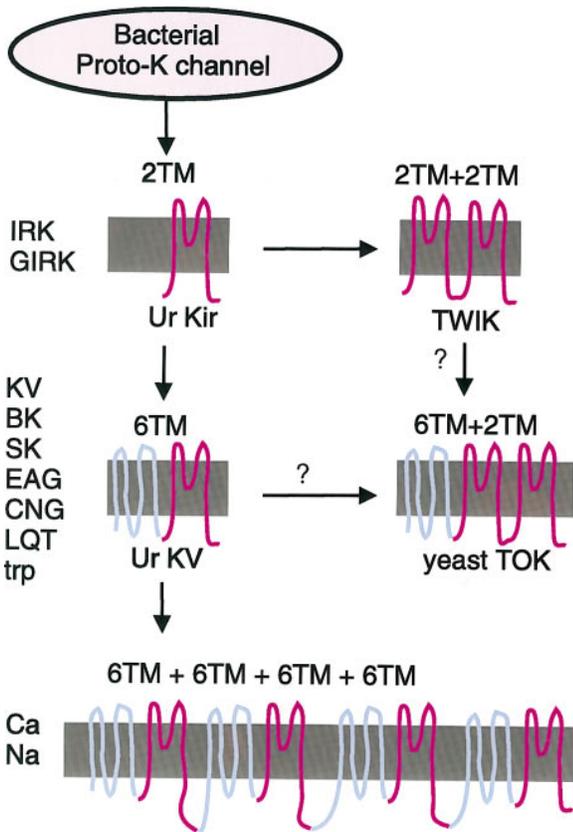


Figure 6. Evolution of the Superfamily of Voltage-Gated Channels. Names of channel families are shown next to their transmembrane folding diagrams.

completely separate membranes was first suggested by Schneider and Chandler (1973), and the role of the modified Ca^{2+} channel was first suggested (to widespread skepticism) by Rios and Brum (1987). Contraction is known to depend on the Ca^{2+} channel: dysgenic mice, lacking the channel, are paralyzed and die at birth. The mechanism of Ca^{2+} channel-ryanodine receptor interaction has been ingeniously studied by Beam and colleagues, who have provided fascinating detail on the dialog between these two proteins (Numa et al., 1990; Nakai et al., 1996).

How Did Voltage-Gated Na^+ , K^+ , and Ca^{2+} Channels Evolve?

Cloning and the compilations of the nucleotide sequences of entire genomes have opened our eyes to an unanticipated abundance and diversity of ion channels. Many seem to be homologous with the classical Na^+ , K^+ , and Ca^{2+} channels (Figure 6; Wei et al., 1996). In Figure 1, we described a motif of six transmembrane segments (6TM) that is present in one copy in voltage-gated K^+ channels and in four tandem copies (6TM + 6TM + 6TM + 6TM) in Na^+ and Ca^{2+} channels. The 6TM design has spawned at least 55 mammalian genes in several families of voltage-sensitive channels (K_v , EAG, LQT), Ca^{2+} -sensitive channels (BK, SK), cyclic nucleotide-gated channels (CNG), and possibly also the TRP family of Ca^{2+} -influx channels. Potassium-selective

channels of the 6TM design are present in animals, plants, fungi, and protozoa, so we should anticipate that they were present in the first eukaryotes. A couple of bacterial genomes also contain genes with this characteristic design. It remains to be seen if these may be examples of genes passed to these bacteria by lateral transfer from eukaryotes or if they signify that the 6TM design was already perfected in prokaryotes ancestral to the eukaryotes.

The inward rectifier K^+ channels (IRK and GIRK families) have an even simpler architecture, having only two transmembrane segments (2TM) with a P loop (signature sequence) between them in each subunit. This seems to be the core design of a K^+ -selective pore, and the two transmembrane segments are the precursors of S5 and S6 in 6TM channels. 2TM sequences are also found in many bacterial genomes, but we know neither their properties nor their functions. Presumably the proto- K^+ channel from which the large gene superfamily of Figure 6 arose was a tetramer of 2TM subunits that originated several billion years ago in the prokaryotes (Figure 6). The 6TM channel families may have arisen by adding to this core a 4TM portion that included a segment with several positively charged residues, the primitive voltage sensor. This charged portion could have derived, for example, from clusters of basic residues that give strong voltage dependence to the import of proteins destined to be pulled from the cytoplasm into mitochondria.

The more complex Na^+ and Ca^{2+} channels would have arisen from a voltage-gated version of 6TM channels by two rounds of tandem gene duplication. Although we don't have good information yet from the genome sequences, electrophysiological evidence suggests that the original tandem duplication event gave rise to Ca^{2+} channels in unicellular eukaryotes, where they were used for action potentials and Ca^{2+} signaling. Apparently, the Na^+ channels arose by radiation from some Ca^{2+} channel at the origin of the multicellular animals, where they permitted the specialization of rapidly conducting axons and the development of nervous systems.

In addition to these channel families of the 2TM, 6TM, and 6TM + 6TM + 6TM + 6TM designs, Figure 6 shows K^+ channel variations with 2TM + 2TM and 6TM + 2TM structures. The former may be very common, and the latter is known only in yeast thus far. Perhaps there will be even more interesting combinations in the future.

Emerging Themes

How far have we come after 60 years with voltage-gated channels? Hodgkin and Huxley's program to describe electrical excitability in terms of separable ionic conductances, each with its own kinetic properties, has been outstandingly successful and seems nearly completed. Even difficult examples with rich repertoires of ion channels such as cardiac action potentials or firing of neurons with attached dendrites are being plausibly modeled. With the consummation of many genome projects, we are also near the end of enumerating the extraordinary number of genes for these channels, although we certainly do not know the functional properties of many of them. In addition, the channels have kept up with every

advance in intracellular signaling and posttranslational modification. They are regulated by phosphorylation of serine, threonine, and tyrosine residues, and they interact directly with second messengers and signaling molecules such as cyclic nucleotides, Ca^{2+} , and the α and β subunits of GTP binding proteins. All of these regulatory signals allow electrical excitability to be tuned to changing physiological needs. Channels also enter into networks of protein-protein interaction. Of course, they combine with their homologous subunits and their auxiliary subunits, but they also become colocalized with other signaling molecules through recognition sites on their intracellular loops and N and C termini. We may imagine nearly stoichiometric combinations of channels, transport proteins, pumps, and enzymes gathered in major signaling complexes (Hsueh et al., 1997)—functional modules like the chips or circuit cards of a computer.

Despite great progress in other areas, the fundamental problems of channel biophysics—how channel gating and ion selectivity work—are still wide open, because we lack true three-dimensional information. We have some stimulating broad strokes but no precision. Happily, this situation will begin to change dramatically, perhaps even this year, as X-ray and NMR structures of significant parts of channels are published. As three-dimensional structures come on line, we can expect for channels the same kind of major leap forward that was experienced for enzymes a couple of decades ago. This will be a tremendous thrill and a moment of truth for many of us who have speculated so long on structure using electrophysiology.

Sometimes, as we work on these detailed and intricate questions of channel mechanism, we wonder if perhaps we have lost our way. At such times it is reassuring to remember that ion channels are relevant to health and many opportunities exist for improved therapy and understanding. For some time, we have recognized that channels of the voltage-gated superfamily are the target of drugs widely used against pain, epilepsy, cardiac arrhythmias, cardiac failure, hypertension, hyperglycemia, and more. Cloning of genes for the relevant target channels has opened the way to more rational drug design and screening and has also revealed numerous natural human mutations in the ion channels, which account for inherited conditions such as familial arrhythmias and paralyses.

From the point of view of normal function, it is evident that large questions lie ahead. Channel behavior is obviously involved in perception, learning, and memory, and there are countless subtle possibilities of how this might happen. Paraphrasing a wise saying of Steven Kuffler, asking small questions can lead to big answers.

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References

Akabas, M.H., Stauffer, D.A., Xu, M., and Karlin A. (1992). Acetylcholine receptor channel structure probed in cysteine-substitution mutants. *Science* 258, 307–310.

Aldrich, R.W., Corey, D.P., and Stevens, C.F. (1983). A reinterpretation of mammalian sodium channel gating based on single channel recording. *Nature* 306, 436–441.

Armstrong, C.M. (1971). Interaction of tetraethylammonium ion derivatives with the potassium channels of giant axons. *J. Gen. Physiol.* 58, 413–437.

Armstrong, C.M. (1981). Sodium channels and gating currents. *Physiol. Rev.* 61, 644–683.

Armstrong, C.M., and Bezanilla, F. (1973). Currents related to movement of the gating particles of the sodium channels. *Nature* 242, 459–461.

Armstrong, C.M., and Bezanilla, F. (1977). Inactivation of the sodium channel. II. Gating current experiments. *J. Gen. Physiol.* 70, 567–590.

Armstrong, C.M., and Gilly, W.F. (1979). Fast and slow steps in the activation of sodium channels. *J. Gen. Physiol.* 74, 691–711.

Armstrong, C.M., Bezanilla, F., and Rojas, E. (1973). Destruction of sodium conductance inactivation in squid axons perfused with pronase. *J. Gen. Physiol.* 62, 375–391.

Bezanilla, F., and Armstrong, C.M. (1977). Inactivation of the sodium channel. I. Sodium current experiments. *J. Gen. Physiol.* 70, 549–566.

Choi, K.L., Mossman, C., Aube, J., and Yellen, G. (1993). The internal quaternary ammonium receptor site of Shaker potassium channels. *Neuron* 10, 533–541.

Cole, K.S., and Curtis, H.J. (1939). Electrical impedance of the squid giant axon during activity. *J. Gen. Physiol.* 22, 649–670.

Durell, S.R., and Guy, H.R. (1996). Structural model of the outer vestibule and selectivity filter of the Shaker voltage-gated K^+ channel. *Neuropharmacology* 35, 761–773.

Ellinor, P.T., Yang, J., Sather, W.A., Zhang, J.F., and Tsien, R.W. (1995). Ca^{2+} channel selectivity at a single locus for high-affinity Ca^{2+} interactions. *Neuron* 15, 1121–1132.

Favre, I., Moczydlowski, E., and Schild, L. (1996). On the structural basis for ionic selectivity among Na^+ , K^+ , and Ca^{2+} in the voltage-gated sodium channel. *Biophys. J.* 71, 3110–3125.

Gomez-Lagunas, F., and Armstrong, C.M. (1995). Inactivation in ShakerB K^+ channels: a test for the number of inactivating particles on each channel. *Biophys. J.* 68, 89–95.

Guy, H.R., and Conti, F. (1990). Pursuing the structure and function of voltage-gated channels. *Trends Neurosci.* 13, 201–206.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F.J. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch./Eur. J. Physiol.* 391, 85–100.

Hartmann, H.A., Kirsch, G.E., Drewe, J.A., Tagliatela, M., Joho, R.H., and Brown, A.M. (1991). Exchange of conduction pathways between two related K^+ channels. *Science* 215, 942–945.

Heginbotham, L., Lu, Z., Abramson, T., and MacKinnon, R. (1994). Mutations in the K^+ channel signature sequence. *Biophys. J.* 66, 1061–1067.

Heinemann, S.H., Terlau, H., Stühmer, W., Imoto, K., and Numa, S. (1992). Calcium channel characteristics conferred on the sodium channel by single mutations. *Nature* 356, 441–443.

Hille, B. (1968). Charges and potentials at the nerve surface: divalents and pH. *J. Gen. Physiol.* 51, 221–236.

Hille, B. (1971). The permeability of the sodium channel to organic cations in myelinated nerve. *J. Gen. Physiol.* 58, 599–619.

Hille, B. (1972). The permeability of the sodium channel to metal cations in myelinated nerve. *J. Gen. Physiol.* 59, 637–658.

Hille, B. (1973). Potassium channels in myelinated nerve. Selective permeability to small cations. *J. Gen. Physiol.* 61, 669–686.

Hodgkin, A.L., and Huxley, A.F. (1939). Action potentials recorded from inside a nerve fibre. *Nature* 144, 710–711.

Hodgkin, A.L., and Katz, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (Lond.)* 108, 37–77.

Hodgkin, A.L., and Huxley, A.F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.)* 117, 500–544.

- Holmgren, M., Jurman, M.E., and Yellen, G. (1996). N-type inactivation and the S4-S5 region of the Shaker K⁺ channel. *J. Gen. Physiol.* *108*, 195-206.
- Holmgren, M., Smith, P.L., and Yellen, G. (1997). Trapping of organic blockers by closing of voltage-dependent K⁺ channels: evidence for a trap door mechanism of activation gating. *J. Gen. Physiol.* *109*, 527-535.
- Hoshi, T., Zagotta, W.N., and Aldrich, R.W. (1990). Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation. *Science* *250*, 533-538.
- Hsueh, Y.P., Kim, E., and Sheng, M. (1997). Disulfide-linked head-to-head multimerization in the mechanism of ion channel clustering by PSD-95. *Neuron* *18*, 803-814.
- Isacoff, E.Y., Jan, Y.N., and Jan, L.Y. (1991). Putative receptor for the cytoplasmic inactivation gate in the *Shaker* K⁺ channel. *Nature* *353*, 86-90.
- Keynes, R.D., and Rojas, E. (1974). Kinetics and steady-state properties of the charged system controlling sodium conductance in the squid giant axon. *J. Physiol. (Lond.)* *239*, 393-434.
- Kirsch, G.E., Shieh, C.C., Drewe, J.A., Vener, D.F., and Brown, A.M. (1993). Segmental exchanges define 4-aminopyridine binding and the inner mouth of K⁺ pores. *Neuron* *11*, 503-512.
- Krovetz, H.S., VanDongen, H.M.A., and VanDongen, A.M.J. (1997). Atomic distance estimates from disulfides and high-affinity metal-binding sites in a K⁺ channel pore. *Biophys. J.* *72*, 117-126.
- Kürz, L.L., Zühlke, R.D., Zhang, H.-J., and Joho, R.H. (1995). Side-chain accessibilities in the pore of a K⁺ channel probed by sulfhydryl-specific reagents after cysteine-scanning mutagenesis. *Biophys. J.* *68*, 900-905.
- Larsson, H.P., Baker, O.S., Dhillon, D.S., and Isacoff, E.Y. (1996). Transmembrane movement of the shaker K⁺ channel S4. *Neuron* *16*, 387-397.
- Lipkind, G.M., and Fozzard, H.A. (1994). A structural model of the tetrodotoxin and saxitoxin binding site of the Na⁺ channel. *Biophys. J.* *66*, 1-13.
- Liu, Y., Jurman, M.E., and Yellen, G. (1996). Dynamic rearrangement of the outer mouth of a K⁺ channel during gating. *Neuron* *16*, 859-867.
- Liu, Y., Holmgren, M., Jurman M.E., and Yellen, G. (1997). Gated access to the pore of a voltage-dependent K⁺ channel. *Neuron* *19*, 175-184.
- Lopez, G.A., Jan, Y.N., and Jan, L.Y. (1994). Evidence that the S6 segment of the Shaker voltage-gated K⁺ channel comprises part of the pore. *Nature* *367*, 179-182.
- Lü, Q., and Miller, C. (1995). Silver as a probe of pore-forming residues in a potassium channel. *Science* *268*, 304-307.
- MacKinnon, R. (1991). Determination of the subunit stoichiometry of a voltage-activated potassium channel. *Nature* *350*, 232-235.
- MacKinnon, R., and Miller, C. (1989). Mutant potassium channels with altered binding of charybdotoxin, a pore-blocking peptide inhibitor. *Science* *245*, 1382-1385.
- MacKinnon, R., Aldrich, R.W., and Lee, A.W. (1993). Functional stoichiometry of Shaker potassium channel inactivation. *Science* *262*, 757-759.
- Nakai, J., Dirksen, R.T., Nguyen, H.T., Pessah, I.N., Beam, K.G., and Allen, P.D. (1996). Enhanced dihydropyridine receptor channel activity in the presence of ryanodine receptor. *Nature* *380*, 72-75.
- Neyton, J., and Miller, C. (1988). Discrete Ba²⁺ block as a probe of ion occupancy and pore structure in the high-conductance Ca²⁺-activated K⁺ channel. *J. Gen. Physiol.* *92*, 569-586.
- Numa, S., Tanabe, T., Takeshima, H., Mikami, A., Niidome, T., Nishimura, S., Adams, B.A., and Beam, K.G. (1990). Molecular insights into excitation-contraction coupling. *Cold Spring Harbor Symp. Quant. Biol.* *55*, 1-7.
- Pongs, O., Kecskemethy, N., Müller, R., Krahe-Jentgens, I., Baumann, A., Kiltz, H.H., Canal, I., Llamazares, S., and Ferrus, A. (1988). Shaker encodes a family of putative potassium channel proteins in the nervous system of *Drosophila*. *EMBO J.* *7*, 1087-1096.
- Rios, E., and Brum, G. (1987). Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle. *Nature* *325*, 717-720.
- Schneider, M.F., and Chandler, W.K. (1973). Voltage-dependent charge movement of skeletal muscle, a possible step in excitation-contraction coupling. *Nature* *242*, 244-246.
- Schoppa, N.E., McCormack, K., Tanouye, M.A., and Sigworth, F.J. (1992). The size of gating charge in wild-type and mutant Yellen, G., Jurman, M.E., Abramson, T., and MacKinnon, R. Shaker potassium channels. *Science* *255*, 1712-1715.
- Shih, T.M., and Goldin, A.L. (1997). Topology of the Shaker potassium channel probed with hydrophilic epitope insertions. *J. Cell Biol.* *136*, 1037-1045.
- Sigworth, F., and Neher, E. (1980). Single Na⁺ channel currents observed in cultured rat muscle cells. *Nature* *287*, 447-449.
- Stefani, E., Toro, L., Perozo, E., and Bezanilla, F. (1994). Gating of Shaker K⁺ channels. I. Ionic and gating currents. *Biophys. J.* *66*, 996-1010.
- Stühmer, W., Conti, F., Suzuki, H., Wang, X.D., Noda, M., Yahagi, N., Kubo, H., and Numa S. (1989). Structural parts involved in activation and inactivation of the sodium channel. *Nature* *339*, 597-603.
- Tempel, B.L., Papazian, D.M., Schwarz, T.L., Jan, Y.N., and Jan, L.Y. (1987). Sequence of a probable potassium channel component encoded at Shaker locus of *Drosophila*. *Science* *237*, 770-775.
- Terlau, H., Heinemann, S.H., Stühmer, W., Pusch, M., Conti, F., Imoto, K., and Numa, S. (1991). Mapping the site of block by tetrodotoxin and saxitoxin of sodium channel II. *FEBS Lett.* *293*, 93-96.
- Wei, A., Jegla, T., and Salkoff, L. (1996). Eight potassium channel families revealed by the *C. elegans* genome project. *Neuropharmacology* *35*, 805-829.
- West, J.W., Patton, D.E., Scheuer, T., Wang, Y., Goldin, A.L., and Catterall, W.A. (1992). A cluster of hydrophobic amino acid residues required for fast Na⁺-channel inactivation. *Proc. Natl. Acad. Sci. USA* *89*, 10910-10914.
- Woodhull, A.M. (1973). Ionic blockage of sodium channels in nerve. *J. Gen. Physiol.* *61*, 687-708.
- Yang, N., and Horn, R. (1995). Evidence for voltage-dependent S4 movement in sodium channels. *Neuron* *15*, 213-218.
- Yang, N., George, A.L., and Horn, R. (1996) Molecular basis of charge movement in voltage-gated sodium channels. *Neuron* *16*, 113-122.
- Yellen, G., Jurman, M.E., Abramson, T., and MacKinnon, R. (1991). Mutations affecting internal TEA blockade identify the probable pore-forming region of a K⁺ channel. *Science* *215*, 939-942.
- Yellen, G.D., Sodickson, D., Chen, T.-Y., and Jurman, M.E. (1994). An engineered cysteine in the external mouth of a K⁺ channel allows inactivation to be modulated by metal binding. *Biophys. J.* *66*, 1068-1075.
- Young, J.Z. (1936). Structure of nerve fibers and synapses in some invertebrates. *Cold Spring Harbor Symp. Quant. Biol.* *4*, 1-6.
- Zagotta, W.N., Brainard, M.S., and Aldrich, R.W. (1988). Single-channel analysis of four distinct classes of potassium channels in *Drosophila* muscle. *J. Neurosci.* *8*, 4765-4779.
- Zagotta, W.N., Hoshi, T., and Aldrich, R.W. (1994). *Shaker* potassium channel gating. III. Evaluation of kinetic models for activation. *J. Gen. Physiol.* *103*, 321-362.