23. U. Ray and M. F. Jarrold, *J. Chem. Phys.*, in press.
24. H. Ibach, H. Wagner, D. Bruchmann, *Solid State Commun.* **42**, 457 (1982).
25. B. G. Koehler, C. H. Mak, S. M. George, *Surf. Sci.* **221**, 565 (1989).
26. P. Gupta, C. H. Mak, P. A. Coon, S. M. George, in preparation.
27. J. A. Schaefer, J. Anderson, G. J. Lapeyre, *J. Vac. Sci. Technol. A* **3**, 1443 (1985); D. Schmeisser and J. E. Demuth, *Phys. Rev. B* **33**, 4233 (1986); M. Nishijima, K. Edamoto, Y. Kubota, S. Tanaka, M. Onchi, *J. Chem. Phys.* **84**, 6458 (1986); K. Fujiwara, *Surf. Sci.* **108**, 124 (1981); R. A. Rosenberg, P. J. Love, V. Rehn, *J. Vac. Sci. Technol. A* **4**, 1451 (1986); Y. J. Chabal, *Phys. Rev. B* **29**, 3677 (1984); W. Ranke and Y. R. Xing, *Surf. Sci.* **157**, 339 (1985).
28. J. R. Chelikowsky and J. C. Phillips, *Phys. Rev. B* **41**, 5735 (1990).
29. O. Echt, K. Sattler, E. Recknagel, *Phys. Rev. Lett.* **47**, 1121 (1981).
30. T. Su and M. T. Bowers, in *Gas Phase Ion Chemistry*, M. T. Bowers, Ed. (Academic Press, New York, 1979), vol. 1.
31. U. Ray and M. F. Jarrold, in preparation.
32. M. F. Jarrold and J. E. Bower, *J. Am. Chem. Soc.* **111**, 1979 (1989).
33. U. Ray and M. F. Jarrold, *J. Chem. Phys.* **93**, 5709 (1990).
34. B. G. Koehler, P. A. Coon, S. M. George, *J. Vac. Sci. Technol. B* **7**, 1303 (1989).
35. R. Wolkow and Ph. Avouris, *Phys. Rev. Lett.* **60**, 1049 (1988); Ph. Avouris, F. Bozso, R. J. Hamers, *J. Vac. Sci. Technol. B* **5**, 1387 (1987); R. J. Hamers, Ph. Avouris, F. Bozso, *Phys. Rev. Lett.* **59**, 2071 (1987).
36. F. Bozso and Ph. Avouris, *Phys. Rev. B* **38**, 3937 (1988); *Phys. Rev. Lett.* **57**, 1185 (1986).
37. K. Raghavachari, J. Chandrasekhar, M. S. Gordon, K. J. Dykema, *J. Am. Chem. Soc.* **106**, 5853 (1984).
38. J. L. Elkind, J. M. Alford, F. D. Weiss, R. T. Laaksonen, R. E. Smalley, *J. Chem. Phys.* **87**, 2397 (1987); J. M. Alford and R. E. Smalley, *Mat. Res. Soc. Symp. Proc.* **131**, 3 (1989).
39. E. K. Parks, K. Liu, S. C. Richtsmeier, L. G. Pobo, S. J. Riley, *J. Chem. Phys.* **82**, 5470 (1985); T. D. Klots, B. J. Winter, E. K. Parks, S. J. Riley, *ibid.* **92**, 2110 (1990).
40. R. E. Leuchtner, A. C. Harms, A. W. Castleman, *ibid.*, p. 6527.
41. M. R. Zakin, R. O. Brickman, D. M. Cox, A. Kaldor, *ibid.* **88**, 3555 (1988); M. R. Zakin, R. O. Brickman, D. M. Cox, A Kaldor, *ibid.*, p. 6605.
42. M. T. Yin and M. L. Cohen, *Phys. Rev. B* **26**, 5668 (1982).
43. P. Badziag and W. S. Verwoerd, *ibid.* **40**, 1023 (1989).
44. The work described in this article was performed in collaboration with J. E. Bower, K. M. Creegan, U. Ray, and Y. Ijiri.

1990: Annus Mirabilis of Potassium Channels

CHRISTOPHER MILLER

Voltage-gated potassium channels make up a large molecular family of integral membrane proteins that are fundamentally involved in the generation of bioelectric signals such as nerve impulses. These proteins span the cell membrane, forming potassium-selective pores that are rapidly switched open or closed by changes in membrane voltage. After the cloning of the first potassium channel over 3 years ago, recombinant DNA manipulation of potassium channel genes is now leading to a molecular understanding of potassium channel behavior. During the past year, functional domains responsible for channel gating and potassium selectivity have been identified, and detailed structural pictures underlying these functions are beginning to emerge.

WE DO NOT UNDERSTAND HOW WE THINK, BUT WE DO know that the electrical signals passed around the thinking brain are generated by a single class of protein: the ion channels. In contrast to electrical signals in computers, which are carried by electrons flowing longitudinally along wires, bioelectric impulses are generated by charges flowing transversely across the thin membranes covering cells. In nerve cells, the charges are carried by the biologically abundant inorganic ions, Na^+, K^+, Ca^{2+}, and Cl^-; the ion channel proteins catalyze this transmembrane flow of ionic charge. They do this in a simple way: by forming narrow, hydrophilic pores through which ions can diffuse passively (*1*).

To operate sensibly, ion channels must perform two essential tasks. First, they must open or close rapidly in response to biological signals. A term for this process, reflecting the early influence of electrical engineers, is "gating"; biochemists likewise describe channels as proteins able to switch among conducting and nonconducting conformations. Second, the open pore must faithfully choose which ions will permeate and which will not. Extreme cases are known in which the preferred substrate, K^+, permeates the pore at a rate 10^4-fold higher than the rate for Na^+, which is only 0.4 Å smaller in crystal radius. This is an impressive trick because these ions do not possess the geometrically elaborate structures that allow organic substrates to be recognized so specifically by enzymes. A long-held aim of ion channel research is to understand these two crucial functions—gating and ionic selectivity—in terms of the molecular structures of the channel proteins. In spite of the proliferation of methods for detecting currents through single ion channels, the lack of general approaches for crystallizing membrane proteins has prevented a direct view of the structural underpinnings of their workings.

During the past year, however, a frisson has rippled through the field because for the first time a physical picture underlying basic channel behaviors is beginning to emerge in tantalizing snatches. Much of this excitement surrounds a newcomer to the collection of channels attackable at the molecular level: the family of voltage-gated K^+ channels. These results represent the early fruits of recombinant DNA manipulation, which is just now finding wide application to ion channel genes. These approaches have provided structural conclusions about K^+ channels on three issues about which classical electrophysiology has been silent: the precise nature of the conformational changes underlying voltage-dependent gating, the molecular makeup of the ion conduction pathway, and the oligomeric state of the functional channel. Each of these experimental efforts has opened a qualitatively new window on the molecular black box of K^+ channels and of voltage-dependent ion channels in general.

Shaker: The Big Break

The new results on K^+ channels were made possible by the molecular cloning of the *shaker* gene of *Drosophila* (*2*). This gene codes for a voltage-dependent K^+ channel, or rather for a multiplic-

The author is in the Howard Hughes Medical Institute, Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254.

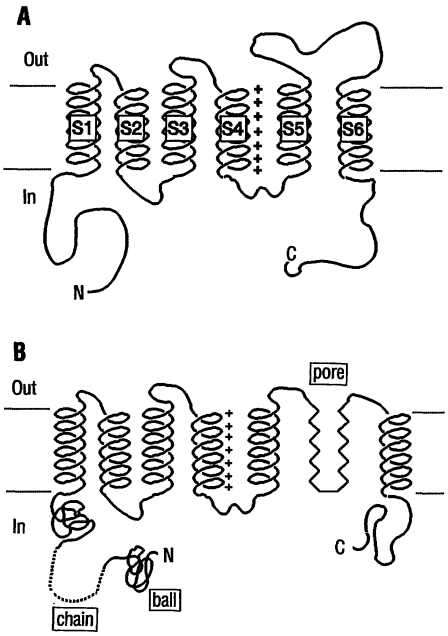


Fig. 1. Functional domains of voltage-gated K^+ channels. (**A**) A widely accepted transmembrane folding model for voltage-gated K^+ channels, as of 1989 (*3*). (**B**) Current view of functional domains in voltage-gated K^+ channels, which arose as a result of experiments discussed here. The regions involved in specific functions are the ball (residues 1 to 20 of Shaker B), the chain (23 to 40), S4 (358 to 382), the S5-S6 linker (418 to 456), and the conduction pore (431 to 449).

ity of these channels, through promiscuous alternative splicing to form a family of *shaker* isochannels from polypeptides of about 70 kD. Soon after this breakthrough, a multitude (over a score at this writing, and climbing rapidly) of voltage-dependent K^+ channels were cloned from other sources, including mammalian nervous tissue. It is now clear that these voltage-dependent K^+ channels make up a large molecular family, the members of which are identical in essential aspects of structure and mechanism. This family comprises many molecular and functional variants that can be exploited to elucidate physical pictures of how these molecules work.

The cloning of *shaker* provided the amino acid sequence of the channel protein and a means of manipulating it. This sequence of 656 residues, fed into the mindless mill of hydrophobicity analysis, yielded a prediction for those stretches of polypeptide likely to form α-helices spanning the cell membrane. As of 2 years ago (*3*), a consensus based on this analysis had emerged envisioning six membrane-spanning α-helices (Fig. 1A); the absence of a cleavable signal sequence places the NH_2-terminus in the cytoplasm and thus forces the COOH-terminus to the same side of the membrane. Although it is easy to dismiss such cartoons as idle sequence-gazing, in fact, hydrophobicity analysis of integral membrane proteins is by far the best predictive algorithm for locating protein secondary structures (*4*), and the membrane topology suggested by the sequencing of *shaker* forms a reasonable basis for defining regions of the protein responsible for the channel's functions.

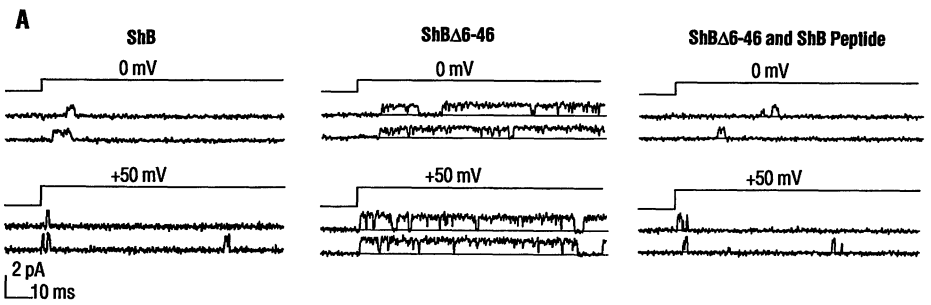
Three obvious questions arise. Are there localized parts of the sequence—true domains—that are responsible for channel gating, or are the opening and closing of the channel due to a global change in conformation involving many parts of the protein? Which transmembrane stretches line the K^+ conduction pore and thus determine its ion selectivity? How many polypeptide subunits are needed to make up a functional channel? In reviewing a few key experiments, we will see that the *shaker* model of 2 years ago must be modified substantially (Fig. 1B). There are strong indications that a small NH_2-terminal domain is directly responsible for one type of channel closing. Moreover, the pore-forming region of the channel has now been found, and it is not located in any of the six predicted helices but in two unusual (and probably nonhelical) membrane-spanning stretches between S5 and S6. Finally, the idea that the functional channel is built from four of these polypeptides, a widely held view from speculative analogy to other voltage-dependent channels (*3*), has now been confirmed experimentally.

A Physical Picture of Gating

During an action potential, electrically excitable membranes undergo voltage swings on the order of 0.1 V, equivalent to more than 10^6 V m^{-1} across the thin plasma membrane. Electric fields this high exert large forces on charged residues within membranes, and the voltage-gated ion channels have taken advantage of this abundant energy source; the membrane potential is the signal used to drive the channel molecules between open and closed conformations. In particular, the *shaker* K^+ channel can exist in three types of conformation, an open state through which K^+ ions flow and two nonconducting states, resting and inactivated. Channel gating can be crudely understood in terms of a simple sequential scheme (*5*):

$$\text{Resting} \rightleftarrows \text{Open} \rightleftarrows \text{Inactivated} \quad (1)$$

Membrane voltage strongly influences only the first of these steps, with the resting state becoming favored as membrane voltage is made increasingly negative on the inside of the cell. The single-channel records of Fig. 2A illustrate this voltage-dependent gating for single molecules. The traces show single-channel responses to a sudden change of membrane voltage from a highly negative value (at which the channel is virtually always in the resting state) to more positive values that favor channel opening. The wild-type *shaker* channel opens shortly after the voltage step and then inactivates (Fig. 2A, left). Membrane voltage has its most dramatic influence on the opening rate. At zero voltage, the time at which the channel first opens is long—a few milliseconds after the switch to the test voltage. In contrast, at +50 mV, this first latency time is short—much less than a millisecond. The amount of time the channel stays open



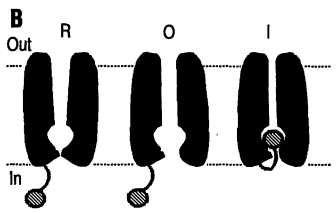


Fig. 2. Activation and inactivation of single *shaker* channels. (**A**) Single *shaker* channels in inside-out patches were held at −80 mV and stepped to either 0 or 50 mV. (**Left**) Wild-type *shaker* B channel. (**Center**) Forty-residue NH_2-terminal deletion mutant. (**Right**) Deletion mutant with 100 μM of 20-residue inactivation ball peptide present in bath solution. (**B**) Ball-and-chain model of inactivation gating. Three gating states of the channel: resting (R), open (O), and inactivated (I). The receptor for the inactivation ball becomes exposed on opening of the channel.

before inactivating is independent of voltage.

The voltage dependence of opening means that movement of charge on the channel protein must be coupled to the conformational changes leading to channel opening. The S4 sequence, in which the hydrophobic stretch is punctuated by arginine or lysine at every third or fourth residue, was recognized as unusual when it was first seen in voltage-gated Na^+ channels (*6*); the S4 motif has been seen in no protein except voltage-gated Na^+, Ca^{2+}, and K^+ channels, and it is widely thought that outwardly directed movement of S4 is required for channel opening. Detailed speculations as to how this happens have been offered (*7, 8*), but the voltage-sensing function of S4 remains unproven because experiments in which the S4 charges are systematically altered have not yet yielded rigorously interpretable alterations of voltage-dependent gating for either Na^+ (*9*) or K^+ (*10*) channels.

More telling information can be gleaned from records such as those of Fig. 2A. A few milliseconds after opening, the channel falls into the inactivated conformation of Scheme 1, and it is around this inactivation process that a delightful tale is now unfolding. Hoshi and co-workers (*11*) and Zagotta and co-workers (*12*) have shown that inactivation is driven by a small, localized domain of the *shaker* protein. The key observation leading to this proposal is shown in the center traces of Fig. 2. Here, a single channel is displayed for a Shaker variant with 41 amino acids deleted from the NH_2-terminal region. The difference from normal Shaker is striking; this deletion mutant activates normally and exhibits normal open-channel K^+ conduction, but it fails to inactivate. Numerous point and deletion mutants in the first 20 residues give this same result, a specific obliteration of the inactivation process, as does the normal *Shaker* channel treated on the cytoplasmic surface with trypsin.

This result alone tells us nothing about the physical nature of inactivation. However, inactivation can be reconstituted in an NH_2-terminal deletion mutant by adding to the internal solution a 20-residue peptide mimicking the deleted sequence (*12*), as shown in Fig. 2A (right). Now, during a test pulse, the mutant channel inactivates. We know that the inactivation is due to the added water-soluble peptide because of a crucial result: the rate of restored inactivation is linearly dependent on peptide concentration. Negative controls showed that peptides mimicking noninactivating NH_2-terminal point mutations fail to restore inactivation to the deletion mutant.

To everyone's surprise, these results support a fanciful picture of inactivation based on electrophysiological experiments performed over a decade ago: the ball-and-chain model (Fig. 2B) of Armstrong and Bezanilla (*13*). The channel, it was proposed, contains a domain (the ball) tethered to the cytoplasmic side of the protein by a protease-cleavable chain. A ball-receptor becomes exposed on opening of the channel, and, after a few milliseconds, the ball finds the receptor and physically blocks the pore. The new experiments in (*11, 12*) clearly establish the existence of the ball as a localized domain directly involved in inactivation, but these experiments do not speak to the mechanism by which the ball causes inactivation. The pore-plugging picture in the cartoon, though favored because of its immediate palpability, has not yet been seriously tested.

Further experiments suggest that a chain exists as well. Deletions of increasing length between residues 23 and 40 progressively speed up the inactivation rate, as though a ball attached with a shorter chain can find its receptor more quickly by virtue of proximity; conversely, an insertion of 40 residues in this region results in slower inactivation. These are exciting developments because they give us a detailed, testable molecular hypothesis for a conformational change involved in channel gating. In addition, they provide a natural rationale for the seemingly continuous gradation between fully inactivating, weakly inactivating, and noninactivating K^+ channels found in nature (*14*). This spectrum of inactivation behaviors may simply reflect varying binding efficiencies of the balls of different K^+ channels.

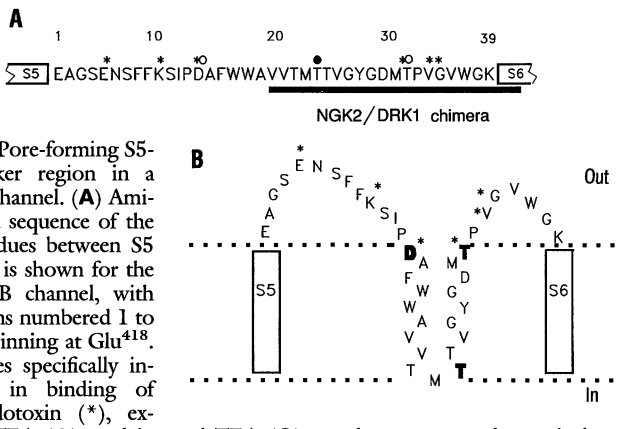


Fig. 3. Pore-forming S5-S6 linker region in a *shaker* channel. (**A**) Amino acid sequence of the 39 residues between S5 and S6 is shown for the *shaker* B channel, with positions numbered 1 to 39, beginning at Glu^{418}. Residues specifically involved in binding of charybdotoxin (*), external TEA (○), and internal TEA (●) are shown, as are the equivalent positions switched in the NGK2-DRK1 chimera (solid bar). (**B**) Topology for the conduction pore proposed on the basis of residues affecting charybdotoxin (*) or TEA (bold).

Hunting Down the Pore

Somewhere in the K^+ channel polypeptide (Fig. 1) there must be one or more transmembrane stretches of sequence that provide the lining of the ion permeation pathway. The groups presented to this watery interior form the catalytic heart of the protein wherein reside the structures allowing K^+, but not Na^+, to permeate freely. We know that the pore is physically a tight squeeze for the K^+ ion, as narrow as 3 Å in places (*15*), and it is probably formed at the central interface of four subunits acting as the staves of a barrel (see below). But which of the putative transmembrane helices in Fig. 1 is involved?

An initial clue came from a search for residues specifically influencing the inhibition of the *shaker* channel by a peptide neurotoxin from scorpion venom, charybdotoxin. This toxin and its many isoforms inhibit several types of K^+ channels by physically plugging the channels' outer mouths (*16*). A glutamate residue in Shaker influences toxin binding by a purely electrostatic, hence local, mechanism, and thus it was established that this residue is close to the external ion entryway (*17*). This residue is found in the fifth position of the 39-residue linker between S5 and S6 (Fig. 3A). MacKinnon and co-workers later showed that this region is peppered with additional sites specifically affecting toxin binding (*18*) at positions 10, 14, 32, 34, and 35, a result that locates these residues on the external side of the pore.

Peptide toxins are too large to enter the narrower parts of the pore, but tetraethylammonium (TEA) has long been known to block K^+ channels in this area (*19*). For this reason, several groups have searched for residues that specifically affect TEA blockade of voltage-dependent K^+ channels. MacKinnon and Yellen (*20*) found that mutations at a threonine residue in Shaker (position 32 in Fig. 3A) alter K^+ channel blockade by external TEA. Changing this residue to lysine, arginine, glutamine, or valine renders the channel essentially insensitive to TEA. More dramatically, substituting a tyrosine enhances blocking affinity 50-fold. These results imply that position 32 is located near the outer end of the K^+ conduction pore and that it is specifically involved in binding of TEA. An additional residue, Glu^{14}, contributing to external TEA block was also found in these studies (*20*).

The K^+ conduction pore is contained wholly within the S5-S6

linker. Joho's and Brown's groups (*21*) constructed chimeras of two mammalian K^+ channels. One of these, NGK2, has high single-channel conductance, high-affinity block by external TEA, and low-affinity block by internal TEA; the other, DRK1, has threefold lower conductance, tenfold lower sensitivity to external TEA, and about 100-fold higher sensitivity to internal TEA. The dramatic result was that substitution of a 24-residue stretch (positions 20 to 39 in Fig. 3A and four residues of S6) from the S5-S6 linker of NGK2 into the equivalent region of DRK1 produced a channel with NGK2-like open-pore behavior. Significantly, the block by both internal and external TEA and the inward and outward single-channel currents reproduce the NGK2 phenotype. This small stretch of polypeptide, therefore, spans the entire pore.

This conclusion is supported and refined by the identification of a residue affecting block by TEA added internally. Yellen and co-workers (*22*) found that alterations at position 24, a threonine residue in Shaker, specifically weaken internal TEA block; these replacements do not affect external TEA block, single-channel current, or gating kinetics. These results place position 24 on the internal side of the pore; in combination with the experiments on chimeric channels, they provide strong evidence that the linker between S5 and S6 spans the membrane twice to form the pore.

A third experimental line resonates with the two above. Yool and Schwarz (*23*) recently examined the ion-conduction properties of the same Shaker mutant (threonine to serine at position 24) that affected internal TEA block. This mutation profoundly altered the shapes of the open-channel current-voltage curves differently for the conducting ions, K^+, Rb^+, and NH_4^+; that is, this conservative point mutation changed the selectivity of the pore among these close K^+ analogs. This result places position 24 at a catalytically important point within the conduction pore.

These pore-probing experiments tightly constrain the architecture of the K^+ conduction pathway (Fig. 3B). Specifically, position 14 lies on the outside of the channel, position 24 on the inside, and position 32 on the outside again. Moreover, positions 24 and 32 contribute directly to the internal and external TEA binding sites, respectively; the weak voltage dependence of the blockade tells us that these sites lie near the two ends of the pore, the internal site sensing only 5% of the applied voltage, and the external site 15% (*22*). Thus, about 80% of the applied voltage falls across a span of only nine amino acid residues. If this stretch were to take up an α-helical conformation, it would be only 12 Å in length, too short to be consistent with the known properties of the channel. K^+ channels are simultaneously occupied by at least three K^+ ions lying in single file within the pore (*24*); it is unlikely that three K^+ ions could be accommodated in a space of only 12 Å without suffering excessive electrostatic destabilization. In agreement with this idea, Latorre and co-workers (*25*), using bis-quaternary ammonium blockers as molecular calipers, estimated the length of a K^+ channel at about 30 Å.

If the two stretches from positions 14 to 22 and from 24 to 32 cross the membrane twice in a fully extended rather than helical arrangement, this structure would be 25 to 30 Å in length. Given the pore properties of K^+ channels, this β hairpin hypothesis is difficult to reject. For a tetrameric channel complex, this hypothesis predicts a pore formed in the center of an eight-stranded antiparallel β barrel. Because of the close relation of K^+ channels to Na^+ and Ca^{2+} channels, these latter voltage-dependent proteins are almost certainly built along the same overall plan; a close examination of the Na^+ channel sequence alone had led Guy and Seetharamulu (*8*) to propose, iconoclastically and well in advance of any of the K^+channel results, that the sequence linking S5 and S6 dips into the membrane in an unconventional conformation to form the conduction pore.

Oligomeric State of the Channel

At the time *shaker* was cloned, its sequence motif—six putative transmembrane helices with a distinct S4 sequence—had been seen before in the voltage-gated Na^+ and Ca^{2+} channels, which are formed from polypeptides about fourfold larger than those for K^+ channels. These larger polypeptides were immediately recognized as four homologous domains repeated in tandem (*6*). By analogy, the Shaker channel complex was proposed to be constructed as a tetramer of identical subunits (*3*). This argument is powerful but circumstantial, and several groups have been motivated to adduce evidence regarding the oligomeric state of K^+ channels. Because no convenient source of K^+-channel protein is available yet, the usual approaches using protein-level biochemistry cannot be undertaken.

Instead, mixing experiments with messenger RNAs (mRNAs) of K^+ channels with different properties showed that the functional channel is a multimer of the fundamental polypeptide (*26, 28*). For instance, two K^+ channel types, RCK1 and RCK4, when expressed individually, differ greatly in inactivation kinetics and sensitivity to TEA. When mixtures of these mRNAs are expressed, a new population of channels is observed with TEA sensitivity similar to that of RCK1 and inactivation kinetics similar to those of RCK4 (*27*). These results show qualitatively that the functional K^+ channel is made of more than one subunit. Similar experiments that use tandem dimers of Shaker polypeptides imply that the channel is built with an even number of monomers (*28*).

Recently, MacKinnon (*29*) exploited charybdotoxin blockade of Shaker channels to argue quantitatively that the channel is a tetramer. He mixed mRNAs coding for toxin-sensitive and toxin-insensitive channels. To circumvent combinatorial confusion, only a small amount of toxin-sensitive mRNA was doped into a background of insensitive channel, so that almost no channel complexes would have more than one wild-type subunit. When only 10% of the mRNA codes for toxin-sensitive channel, 35% of the expressed channels are sensitive to toxin; if random mixing occurs, and if a single wild-type polypeptide is sufficient to confer toxin sensitivity on a tetrameric channel complex, then, at this mixing ratio, the fraction of sensitive channels should be $1 - (0.9)^4 = 0.34$. An extensive analysis supported the idea that the mRNAs were mixing randomly in these experiments and showed that the data support a tetrameric, but not dimeric or hexameric, arrangement of subunits. The results also suggest that, by virtue of a fourfold symmetry, K^+ channels offer charybdotoxin, a highly asymmetric molecule (*30*), four equivalent faces on which to bind in the outer vestibule.

The Future

The results reviewed here have been pouring out at a dizzying pace during the last year, too rapidly to anticipate any questions beyond the obvious (and obviously answerable) ones. Does the inactivation ball literally plug the inner side of the pore? The sorts of experiments that could answer this question have already been done to show that charybdotoxin is a pore blocker (*16*). It is known that internal TEA competes with the inactivation ball (*31*); a demonstration that the ball can be displaced by K^+ ions coming through the channel from the opposite side would be convincing evidence of this picture. A homotetrameric channel would have four balls, but how many are actually needed for inactivation?

Where does the channel's ion selectivity come from? What groups provide the electronegative ligands that select K^+ ions in the conduction pore? Do the well-conserved amino acids in the S5-S6 linker accomplish this selection with their side chains, or do backbone carbonyls point inward to create ion binding sites, as in

some globular proteins (*32*)? How drastically can the channel's ion selectivity be manipulated before mutagenesis becomes mutagenocide? A good probe of the deeper regions of the conduction pore will be the Ba^{2+} ion, known to act in many K^+ channels as a strongly blocking divalent K^+ analog (*33*).

Finally, the quaternary interactions needed to hold the tetramer together must be sorted out. What types of subunit contacts are involved? Is external Ca^{2+} required for intersubunit stability (*34*)? These questions will almost certainly require attack directly at the protein level because dead channels tell no tales in site-specific mutagenesis. For this attack, efficient expression systems will be required from which milligrams of functional K^+ channel can be purified; functional *shaker* channels have been expressed in both baculovirus and vaccinia virus (*35*) systems, but the practical utility of these as biochemical sources requires detailed assessment.

If this past year of K^+ channels is any guide, future mechanistic work will be busy and raucous. But, behind the inevitable excitement and clamor, an unspoken question always will be lurking: without a high-resolution molecular structure, what do we really know?

REFERENCES AND NOTES

1. C. M. Armstrong, *Q. Rev. Biophys.* **7**, 179 (1975); B. Hille, *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA 1984).
2. D. M. Papazian, T. L. Schwarz, B. L. Tempel, Y. N. Jan, L. Y. Jan, *Science* **237**, 749 (1987); O. Pongs *et al.*, *EMBO J* **7**, 1087 (1988); L. E. Iverson, M. A. Tanouye, H. A. Lester, N. Davidson, B. Rudy, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5723 (1988).
3. W. A. Catterall, *Science* **242**, 50 (1988); L. Y. Jan and Y. N. Jan, *Cell* **56**, 13 (1989).
4. D. M. Engelman, T. A. Steitz, A. Glodman, *Annu. Rev. Biophys. Biophys. Chem.* **15**, 321 (1986); S. H. White and R. E. Jacobs, *J. Membr. Biol.* **115**, 145 (1990).
5. W. N. Zagotta and R. W. Aldrich, *J. Gen. Physiol.* **95**, 29 (1990).
6. M. Noda *et al.*, *Nature* **321**, 121 (1984).
7. E. M. Kosower, *FEBS Lett.* **182**, 234 (1985); R. E. Greenblatt, Y. Blatt, M. Montal, *ibid.* **193**, 125 (1985); H. R. Guy, in *Monovalent Cations in Biological Systems*, C. A. Pasternak, Ed. (CRC Press, Boca Raton, FL, 1989).
8. H. R. Guy and P. Seetharamulu, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 508 (1986).
9. W. Stuhmer *et al.*, *Nature* **339**, 597 (1989).
10. D. M. Papazian, L. C. Timpe, Y. N. Jan, L. Y. Jan, *ibid.* **349**, 305 (1991).
11. T. Hoshi, W. N. Zagotta, R. W. Aldrich, *Science* **250**, 533 (1990).
12. W. N. Zagotta, T. Hoshi, R. W. Aldrich, *ibid.* p. 568.
13. C. M. Armstrong and F. Bezanilla, *J. Gen. Physiol.* **70**, 567 (1977).
14. H. Thompson and R. W. Aldrich, in *The Cell Surface and Neuronal Function*, C. W. Cotman, G. Poste, G. L. Nicolson, Eds. (Elsevier/North Holland, New York, 1980), pp. 49–85.
15. B. Hille, *J. Gen. Physiol.* **61**, 669 (1973).
16. C. Anderson, R. MacKinnon, C. Smith, C. Miller, *J. Gen. Physiol.* **91**, 317 (1988); R. MacKinnon and C. Miller, *ibid.*, p. 335; C. Miller, *Neuron* **1**, 1003 (1988); R. MacKinnon and C. Miller, *Biochemistry* **28**, 8087 (1989).
17. R. MacKinnon and C. Miller, *Science* **245**, 1382 (1989).
18. R. MacKinnon, L. Heginbotham, T. Abramson, *Neuron* **5**, 767 (1990).
19. G. Yellen, *Annu. Rev. Biophys. Bioeng.* **16**, 227 (1987).
20. R. MacKinnon and G. Yellen, *Science* **250**, 276 (1990).
21. H. A. Hartmann *et al.*, *ibid.* **251**, 942 (1991).
22. G. Yellen, M. E. Jurman, T. Abramson, R. MacKinnon, *ibid.*, p. 939.
23. A. J. Yool and T. L. Schwartz, *Nature* **349**, 700 (1991).
24. B. Hille and W. Schwarz, *J. Gen. Physiol.* **72**, 409 (1978); T. B. Begenisich and P. DeWeer, *ibid.* **76**, 83 (1980); B. C. Spalding, O. Senyk, J. G. Swift, P. Horowicz, *Am. J. Physiol.* **241**, C68 (1981); R. Latorre and C. Miller, *J. Membr. Biol.* **71**, 11 (1983); J. Neyton and C. Miller, *J. Gen. Physiol.* **92**, 569 (1988); B. Vestergaard-Bogind, P. Stampe, P. Christophersen, *J. Membr. Biol.* **88**, 67 (1985).
25. A. Villarroel, O. Alvarez, A. Oberhauser, R. Latorre, *Pfluegers Arch.* **413**, 118 (1989).
26. M. J. Christie, R. A. North, P. B. Osborne, J. Douglass, J. P. Adelman, *Neuron* **4**, 405 (1990).
27. J. P. Ruppersberg *et al.*, *Nature* **345**, 535 (1990).
28. E. Y. Isacoff, Y. N. Jan, L. Y. Jan, *ibid.*, p. 530.
29. R. MacKinnon, *ibid.* **350**, 232 (1991).
30. F. Bontems *et al.*, *Eur. J. Biochem.* **196**, 19 (1991).
31. K. L. Choi, R. W. Aldrich, G. Yellen, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
32. N. C. J. Strynadka and M. N. G. James, *Annu. Rev. Biochem.* **58**, 951 (1989).
33. C. Vergara and R. Latorre, *J. Gen. Physiol.* **82**, 543 (1983); C. D. Benham, T. B. Bolton, R. J. Lang, T. Takewaki, *Pfluegers Arch.* **403**, 120 (1985); C. Miller, R. Latorre, I. Reisin. *J. Gen. Physiol.* **90**, 427 (1987); J. Neyton and C. Miller, *ibid.* **92**, 549 (1988).
34. C. M. Armstrong and C. Miller, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7579 (1990).
35. K. Klaiber *et al.*, *Neuron* **5**, 221 (1990); R. J. Leonard *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7629 (1989).

36. I thank R. W. Aldrich and T. Hoshi for single-channel records and I. B. Levitan and C. E. Morris for critical readings of the manuscript. I acknowledge R. W. Aldrich, A. M. Brown, I. Y. Jan, R. MacKinnon, T. Schwarz, W. Stuhmer, and G. Yellen for information on work in progress.