RESEARCH: NEUROSCIENCE

The Vision of the Pore

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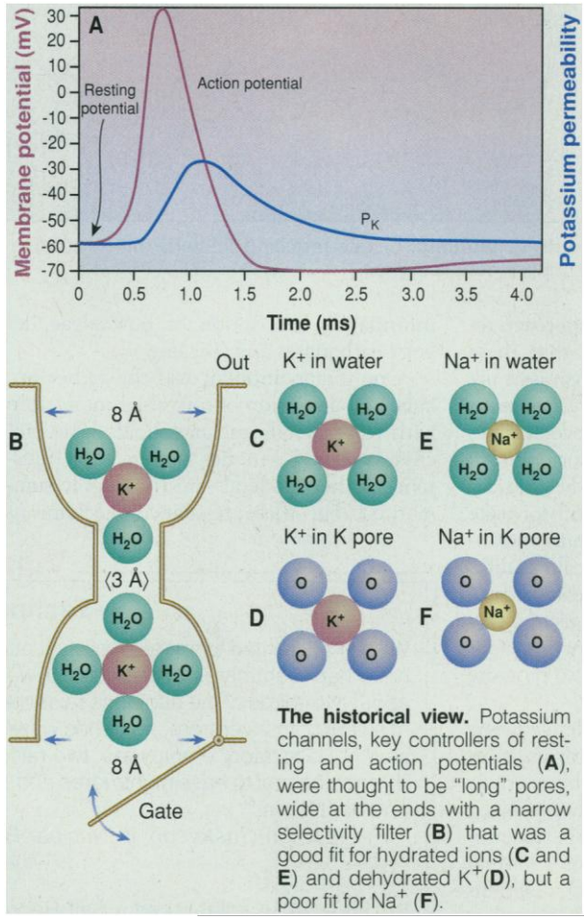
The crystal structure of the potassium ion (K^+) channel presented on pages 69 and 106 by MacKinnon and his laboratory (*1*, *2*) is a dream come true for biophysicists. Almost 50 years ago Hodgkin and Huxley (*3*) demonstrated that electrical signaling by nerve cells depends on the ability of their membranes to selectively regulate the passage of ions. Since then, the questions of how membranes select between ions—particularly sodium, potassium, and calcium ions—and control their movements have been intensely fascinating to researchers in many fields.

Although K^+ channels are best known for their role in neuronal signaling, they are found in almost all biological organisms. Early in life's evolution, the combination of K^+ channels and an internal negative charge provided animal cells with a means to remain osmotically stable. Later adapted for signaling purposes, K^+ channels are a requisite for all electrical activity in the nervous system.

The study of ion channels was greatly facilitated by the magnificent accomplishments of the Numa laboratory in which the sodium channel was first cloned (*4*) and by the Jans (*5*), who first sequenced the Shaker K^+ channel from the fruit fly *Drosophila*. But even with the sequence available, the location of the pore through which the ions passed was not clear until MacKinnon, with Yellen (*6*), discovered the P (pore) region (*7*) and later the K^+ channel signature sequence. Further work showed that side chains in the signature sequence, even hydrophilic ones, had little influence on selectivity. This led to a postulated role for backbone carbonyls in selectivity (now confirmed crystallographically). Mutagenesis had revealed most of what it could about pore structure, although mutational analysis as intelligently modeled by Durell and Guy (*8*) gave reasonable pictures of some aspects of the pore. Further insight required crystallization. Fortunately, knowledge of the P region facilitated the cloning of K^+ channels, including those of the two-transmembrane variety, and in due time the K^+ channel from *Streptomyces lividans* (KcsA). This made possible the synthesis of the large quantities of protein required for the crystallization and x-ray analysis of KcsA. A remarkable channel. A remarkable accomplishment.

In a surprisingly short time, MacKinnon and his laboratory solved the crystal structure to a resolution of 3.4 Å, detailed enough to answer most of the traditional questions. Like all K^+ channels, the KcsA channel is a tetramer. Each subunit has two transmembrane crossings and a P region, which joins the outer-surface ends of the two crossing segments. The two crossings in each subunit are α helices, with a peripheral and an inner helix that run almost in parallel through the membrane. The inner helix, which corresponds to S6 in the well-characterized Shaker K^+ channel from *Drosophila*, forms the lining of the inner part of the pore. The four helix pairs are like the support poles for an inverted teepee (vertex inside), widely separated near the outer membrane surface and converging toward a narrow zone (about 6 Å in diameter) near the inner surface. The P region folds in from the outside to fill most of the space in the outer part of the teepee, and it forms the selectivity filter, which discriminates between K^+ and Na^+ ions.



The historical view. Potassium channels, key controllers of resting and action potentials (**A**), were thought to be "long" pores, wide at the ends with a narrow selectivity filter (**B**) that was a good fit for hydrated ions (**C** and **E**) and dehydrated K^+ (**D**), but a poor fit for Na^+ (**F**).

The amino-terminal portion of the P region is also α-helical, slanting in toward the pore axis from the outside. The helix is followed by a strand composed largely of the previously recognized "signature sequence" (*8*, *9*). Five amino acids in this zone form the lining of the selectivity filter, turning their main chain carbonyls in toward the pore axis and their side chains outward. Perhaps most astounding is the visualization of K^+ ions within the selectivity filter. From electron density changes that are apparent on transferring crystals from a solution containing K^+ to one containing Rb^+, two K^+ ions are seen to occupy the short selectivity filter. These ions are separated from each other by 8 Å, are almost completely dehydrated, and are presumably coordinated by the backbone carbonyls. *Mirabile dictu!* Internal to the selectivity filter is a cavity of complex shape, roughly 10 Å in diameter, and floating in its center, presumably surrounded by water molecules, is a third ion (again visualized thanks to difference maps). A surprising feature is that the pore narrows somewhat (to about 6 Å) as it runs inward and is quite long. Its inner half is lined by predominantly hydrophobic amino acid side chains. At the inner mouth of the pore are four negative residues that help exclude anions.

The outer mouth of the channel is the subject of the MacKinnon *et al.* report (*2*), which models the structure of the pore bound to a molecule of scorpion toxin. The outer mouth is a flat area, surrounded at a distance by four turrets, rather like a prison yard. The toxin molecule extends at a diagonal across the pore mouth, from one turret to another, much as expected from previous experiments (*10*, *11*). The outer mouth of KcsA is very similar to that of eukaryotic K^+ channels such as Shaker, a point of great importance to drug research because of the easy availability of KcsA channels.

How does this structure compare to the K^+ channel of previous imaginings? Hodgkin and Huxley (*3*) ascribed to K^+ permeability

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the important functions of setting the resting potential of the cell and repolarizing the membrane after an action potential (see figure, part A). With no structural or chemical evidence to guide them, they said little about the nature of the conducting path. Subsequently, Hodgkin and Keynes (*12*) described a "long pore" effect for K^+ channels, which suggested that two or more K^+ ions at a time permeate in single file. This general idea fit well with results from experiments with the cation tetraethylammonium (TEA^+) and its derivatives (*13*). TEA^+—a central nitrogen surrounded by four ethyl arms—is about 8 Å in diameter, approximately the size of K^+ with one hydration shell. TEA^+ applied to the intracellular side of the pore blocks K^+ channels, and the internal receptor is protected by a gate that must be open for TEA^+ to enter. A simple picture is that both TEA^+ and a hydrated K^+ ion (see figure, B) can occupy the channel's inner vestibule, but only the K^+ ion can pass through a narrow part, by partially dehydrating. TEA^+, with covalently linked ethyls, remains stuck in the vestibule, blocking the pore. The narrow part of the pore was measured by Hille with ion substitution experiments (*14*) and is about 3 Å in diameter. Experiments with TEA^+ derivatives (for example, C_9^+, which has seven carbonyls added to one arm) indicated the presence of a hydrophobic region, now clearly seen in the crystal structure, in the inner vestibule.

But how does a 3-Å pore prevent the permeation of Na^+, which is only 1.9 Å in diameter? One thought was that a permeant ion had to fit closely in the pore (*16*). A K^+ ion in a pore of fixed 3-Å diameter and coordinated by carbonyl oxygens could have much the same energy as a K^+ ion in water (see figure, C and D). For a Na^+ ion, the rigid pore will not collapse to form good bonding (see figure, F), making the ion's energy higher than in water (see figure, E). Remarkably, the crystal structure vividly reveals a framework for providing such rigidity, a key point in understanding selectivity.

This work answers in some detail, sufficient for the present, the question of K^+ channel selectivity. Inactivation gating is already essentially solved (*15*). Next will come the question of voltage gating of channels, a fascinating property of the more complicated six-transmembrane voltage-gated channels.

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PALEOBIOLOGY

How Old Is the Flower and the Fly?

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The first book that Charles Darwin wrote after publication of *The Origin of Species* (1859) was *On the Various Contrivances by Which British and Foreign Orchids Are Fertilised by Insects, and on the Good Effects of Intercrossing* (1862), an intellectual forerunner to modern work on pollination biology. In this volume, Darwin applied a tradition of careful observation with principles such as reciprocal adaptation toward understanding pollination—one of the most pervasive and diverse of mutualisms known in nature. Orchids, although fascinating in their own right and the premiere group of plants renowned for their intimate and intricate coevolutionary associations with pollinating insects (*1*), nevertheless represent a relatively recent (Cenozoic) event in the geologic history of pollination. Recently, Friis (*2*) and others have produced anatomical evidence from spectacularly preserved floral structures and have elucidated the first occurrences of pollinator-associated floral features during the mid-Cretaceous. These angiosperm-centered discoveries have pinpointed some of the earliest known fossil occurrences of particular pollination syndromes. [Pollination syndromes are morphologically convergent adaptive trends exhibited by both the floral features of pollinated plants and the mouthpart structure and other flower-interactive features of their respective pollinators (*1*).] Nevertheless, the earlier Mesozoic history of insect pollination is considerably more ambiguous. At present, there are few clues regarding the pollination biology of "preangiospermous" Mesozoic insects. Most inferences come from modern associations between primitive lineages of insects and their gymnospermous seed plant hosts, especially cycads (*3*), and from fossil gut contents and coprolites of pollen-consuming insects (*4*). Diagnostic mouthpart structures (*4*, *5*) are rarely observed, which is now remedied by the discoveries reported by Ren on page 85 of this issue (*6*). This impressive documentation now places three lineages of lower brachyceran flies [see figure, panel (**B**)] as pollinators in China during the Upper Jurassic. However, as explored below, the group of plants that these external fluid feeders were pollinating is as intriguing as the presence of the pollination itself.

Pollen consumption (pollinivory) has generally been the evolutionary precursor to pollination. Pollinivory can become a mutualism (that is, pollination) if the pollinivore can deliver unconsumed pollen to the female reproductive organs of its host plant more efficiently than alternative dispersal by wind, splashing rain, or gravity. Pollination mutualisms require a plant to sacrifice pollen for improved access and efficiency in the fertilization of conspecific ovules. Even pollinivory is a derived feeding strategy, because it is temporally preceded by spore consumption (sporivory) in the fossil record. The earliest terrestrial sporivory occurs in Late Silurian to Early Devonian terrestrial ecosystems, indicated by distinctive coprolites, produced probably by myriapods or insects, with abundant to occasional spore contents from primitive land plants (*7*) [see assemblage 1 in panel (**A**) of the figure]. During the Carboniferous, a younger assemblage has been documented [assemblage 2 in panel (**A**) of figure], represented by insects. By the end of the period, pollen consumption was established, evidenced both by well-preserved, dispersed coprolites [panels (**D**) and (**E**) of figure] and gut contents of hemipteroid and ortho-

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