the preceding Precambrian era, which represents about 90% of the geological record, biostratigraphy is generally precluded by the absence of large organisms with preservable body parts, which appeared in spectacular diversity and abundance only at the end of this era. Throughout geological time, coarser grained siliciclastic rocks (rocks such as sandstones that are mostly made up of silicate rock fragments) present a particular problem because fossils are not preserved under the more destructive conditions of their deposition.

Dating of siliciclastic sedimentary sequences has, to date, been achieved mostly by the well-established stratigraphic tool of age bracketing (*2, 3*), which involves the dating of those units within a sedimentary sequence that are amenable to direct isotopic age determination (such as volcanic units, carbonate sequences, or fine-grained shales containing datable diagenetic clay minerals) or, in the Phanerozoic only, biostratigraphy. Others have studied the ages of individual detrital zircons, another uranium-rich mineral in which lead isotopes provide a chronometer (*4*). This resistant mineral survives high-energy depositional environments and is common in coarse clastic sediments. However, it can only provide a maximum date for sedimentation because the age determined from the zircon simply reflects the age of the rock from which it crystallized. For example, the Torridonian sandstones of northwest Scotland shown in the figure contain zircons that are predominantly late Archaean [about 2.7 billion years ago (Ga)] and Mesoproterozoic (about 1.8 to 1.6 Ga) (*5*), although we know that the sediment was deposited after about 1.2 Ga (*6*). Clearly, all of these methods have limitations that restrict their applicability and accuracy for dating sedimentary sequences.

McNaughton *et al.* have now dated xenotime overgrowths on zircon crystals in sandstone. They present morphological evidence from electron microscopy that the xenotimes were formed after sedimentation, because the fine, angular crystals that they observe would have been destroyed during sediment transport. They suggest that the xenotimes were formed immediately below the sediment-water interface by circulating pore fluids and are therefore truly diagenetic in origin. Xenotime is very amenable to dating with the uranium-lead radioactive decay system. It preferentially incorporates uranium in high concentrations during crystallization under exclusion of lead, and therefore all of the measured lead is radiogenic, formed from in situ decay of the uranium, greatly facilitating the dating of the xenotime formation.

McNaughton *et al.* report finding xenotime overgrowths in about half of the 25 siliciclastic sediments of all ages that they have investigated. They suggest that their method will enable diagenesis of siliciclastic sediment to be dated with reasonable accuracy throughout the geological record, although it is likely that the youngest sediments will require further development of the method to include dates based on ratios of uranium to lead because those based on lead isotopes alone become unacceptably imprecise in young rocks.

The primary application of this powerful new method will undoubtedly be the dating of major Precambrian siliciclastic sediments that have previously eluded direct dating and application of these dates to understanding the timing of local and global tectonic processes. Given the potential of this method, the limiting factor is likely to be the availability of analytical capacity on the small number of large ion microprobes, which are required for these measurement, rather than the availability of suitable rocks for dating.

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PERSPECTIVES: NEUROSCIENCE

For the Latest Information, Tune to Channel KcsA

Bojan Zagrovic and Richard Aldrich

Potassium ion (K^+) channels are transmembrane proteins that regulate K^+ ion flux across the cell membrane with remarkable selectivity and efficiency. Their activity underlies fundamental biological processes such as electrical signaling, osmotic balance, and signal transduction (*1*). Last year, MacKinnon and colleagues published the crystal structure of KcsA, the K^+ channel of the bacterium *Streptomyces lividans* (*2*), ushering the field into a new era. Building on this landmark study, Roux and MacKinnon now report on page 100 a computational analysis of the electrostatic forces that stabilize K^+ ions inside the central cavity of the KcsA ion channel (*3*). Further, Perozo *et al.* (*4*) on page 73 describe the conformational changes that take place in KcsA as it opens and closes, a process called gating that can be followed by electron paramagnetic resonance (EPR).

Although the crystal structure of the KcsA channel has yielded valuable information about permeation (that is, how the channel selectively translocates K^+ ions across the membrane), it has not offered definitive answers about how the channel opens and closes (gates), arguably the most important question in ion channel physiology. Different ion channels gate in different ways: Some are activated by changes in cell membrane voltage, others by binding of ligand. KcsA is activated by changes in extracellular pH.

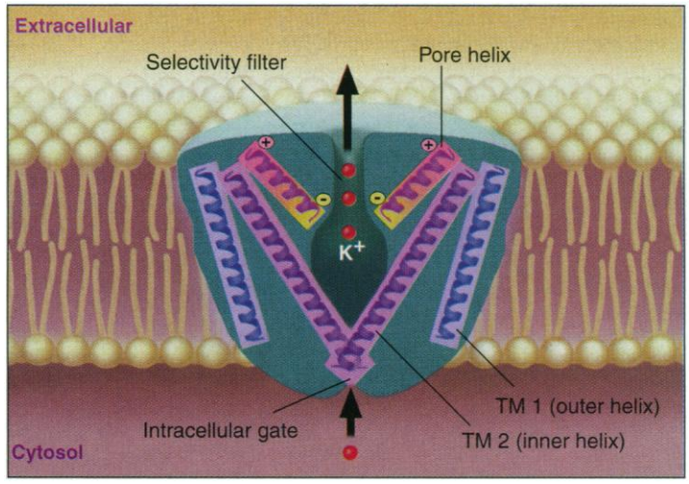
In their study, Perozo *et al.* trapped the KcsA channel in both the open and closed conformations and then analyzed the difference in the EPR signal (*5*). They introduced cysteine residues at select locations in each of the four identical subunits of KcsA—in transmembrane helices 1 and 2 (TM1 and TM2) and in the pore α helices (see the figure). They then labeled the helices with nitroxide spin labels, and analyzed the change in spin-label mobility and intersubunit spin-spin coupling as the channel gated in response to changes in pH. They found that TM1 and TM2 underwent conformational changes (rigid-body translations and counterclockwise rotations around the channel's central cavity) as the pore opened. Opening of the pore seemed to be directly coupled to the movement of the four TM2 helices: Their displacement increased the diameter of the permeation pathway at the point where the helices converge. The involvement of the four pore α helices in gating is still speculative. Although the amino terminus of each pore helix re-

The authors are in the Department of Molecular and Cellular Physiology and Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA. E-mail: zagrovic@leland.stanford.edu; raldrich@leland.stanford.edu

mains immobile during gating, there does appear to be a small movement at the carboxyl terminus. Several other proteins with transmembrane helices, including the acetylcholine receptor (*6*) and rhodopsin (*7*), are also activated by a change in conformation in the absence of any change in secondary structure.

The Perozo study is a good example of the fruitful melding of structural information from KcsA (a prokaryotic ion channel) and functional information from eukaryotic channels such as the fruit fly K^+ channel (Shaker), for which no high-resolution structure exists. For example, chemical modification of cysteine residues introduced into S6—a putative transmembrane segment of the Shaker channel homologous to TM2 in KcsA—showed that the gating of Shaker is accompanied by a conformational change in the carboxyl terminus of S6 akin to the displacement seen in TM2 (*8*). The findings in KcsA and Shaker were interpreted as evidence for the existence of a gate in the intracellular region of the pore that controls entry of K^+ into the pore from the cell cytosol. This intracellular gate seems to be distinct from the narrow section of the pore that acts as a selectivity filter, allowing only K^+ ions (and not Na^+ ions) to enter the pore. A series of ion-trapping experiments initiated by Armstrong (*9*) also supports this view. The extracellular region surrounding the selectivity filter appears not to be involved in opening and closing of the pore because binding of scorpion toxin to the extracellular region of the Ca^{2+}-activated K^+ channel (another type of K^+ channel with a homologous pore) has almost no effect on gating (*10*). This fits nicely with the Perozo results, which show no movement of this region during KcsA gating. Together these observations suggest that conformational changes during gating are highly conserved in K^+ channels from many different organisms. The agreement between findings in ion channels from very different organisms exemplifies the usefulness of prokaryotes for understanding eukaryote, and ultimately mammalian, ion channel biology.

Each subunit of KcsA has two transmembrane helices, whereas most of the eukaryotic channels studied so far (for example, voltage-activated channels) are far more complex. If the conformational changes observed by Perozo *et al.* occur in eukaryotic channels, then how are the voltage sensors in voltage-activated channels coupled to the displacement of the transmembrane helices? The answer will probably have to await analysis of a high-resolution structure of a eukaryotic voltage-activated channel. Together with what we already know about voltage-activated ion channels from functional measurements, this may lead to an unprecedented degree of understanding of a biological macromolecule.



K^+ ions on the move. Depicted are two of the four subunits of the bacterial K^+ channel, KcsA. Each subunit is composed of two transmembrane helices, an outer helix (TM1) and an inner helix (TM2), and an interior fold called the pore α helix. The central cavity and the four pore α helices help to preferentially select monovalent over divalent cations and to stabilize the ion as it passes through the membrane. Movement of the four TM2 helices opens and closes the pore (a process called gating), allowing K^+ ions to exit the cell cytosol. TM1 corresponds to S5 in the fruit fly K^+ channel (Shaker) and TM2 to S6.

In a complementary report, Roux and MacKinnon present an incisive computational analysis of the electrostatic profile of the KcsA pore. By solving the finite difference Poisson equation they dissect the electrostatic influence of the central cavity and the four pore α helices on the stability of the K^+ ion at the pore's center (see the figure). The low electrical polarization of the membrane hydrocarbons creates a high energy barrier to the passage of ions, a key problem for the pore to overcome (*11*). As expected, the investigators found that the water-filled central cavity of the pore was the key stabilizing element, but the pore helices also revealed some interesting properties. The authors estimate that as much as 80% of the stabilization of K^+ ions by protein arises from the amide-carbonyl dipoles of the first 13 amino acids of the pore α helices. Consequently, K^+ ion stability in the pore's central cavity is critically dependent on the exact orientation of the helices with respect to the central cavity and their rearrangement during the course of gating. The most surprising but satisfying finding is that the electrostatic effects of the ion-binding site in the cavity are tuned to preferentially accommodate a monovalent cation. It will be interesting to see whether the same principles hold for other monovalent and divalent cation-selective channels.

The conformational change in the carboxyl terminus of the pore α helices observed by Perozo *et al.*, combined with the contribution of these helices to the stability of the K^+ ions in the pore determined by Roux and MacKinnon, suggests a possible explanation for a long-standing puzzle about the origin of subconductance levels in different ion channels (*12, 13*). (Sometimes channel molecules conduct less than the maximum level of current, and this is referred to as subconductance.) On the basis of these two studies, we speculate that movements of the pore α helices may alter the stability of the ion in the central cavity, thereby affecting the amount of current passing through the channel. In Shaker channels, subconductance states are traversed as the pore opens and closes (*12*). It is possible that this is caused by the pore α helices occupying a transient intermediate state, creating a different affinity binding site for a K^+ ion during gating. The support for this hypothesis will have to come from further experiments and computational analyses.

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