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Researchers Discover Structure of Nature's "Circuit Breaker"

Researchers have answered an important question in biology by discovering the exquisite mechanism by which channels in the cell membrane sense voltage changes that trigger them to snap open or slam shut with extraordinary speed and precision.

Voltage-dependent ion channels are central to the function of nerves and muscles, and without them the brain would immediately suffer neural gridlock and the heart would seize up.

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According to the researchers, which were led by Howard Hughes Medical Institute investigator Roderick MacKinnon, the discovery may lead to a new class of drugs for neurological, heart and muscle disorders that can exert more subtle influences on the activity of ion channels.

MacKinnon and his colleagues at The Rockefeller University published their findings in two papers in the May 1, 2003, issue of the journal *Nature*. Specifically, the researchers deduced the structure and function of the voltage-sensing mechanism in a potassium channel of an archaeobacterium that thrives in the near-boiling temperatures of hot springs. However, they said, the mechanism undoubtedly applies to voltage-sensing calcium and sodium channels as well, and is present in organisms from the most ancient bacteria to humans.

Voltage-dependent potassium ion channels are precise molecular machines that are critical to propagating electrical impulses in the brain and heart. The channels are large proteins with a pore that pierces the cell membrane and is designed to allow only the passage of potassium ions. When an electrical impulse travels along a nerve, the charge on the cell membrane changes—with the outside becoming more negative—triggering these ion

channels to open and allowing potassium to flow out of the cell. This outflow of potassium allows the membrane to return to its resting state and prepare for the next impulse.

According to MacKinnon—whose past work has revealed many new details about the architecture and inner workings of potassium channels—the voltage-sensing mechanism remained a frustrating mystery. “The general principle was understood that the electric field on the membrane somehow moved ‘gating charges’ on the channel that would somehow open it to allow potassium to flow,” he said.

Figuring out the location and function of the charge-carrying structure remained a major problem, he said. “This has been a central project for me and members of my lab for a very long time,” said MacKinnon. “We’ve been working on this for almost six years.”

Indirect experiments in other laboratories suggested that the channel included some sort of voltage-sensing mechanism buried deep within the protein that slid back and forth with changing membrane charge to open or shut the channel. However, the only way to reveal the mechanism definitively, said MacKinnon, was to obtain high-resolution x-ray crystallographic images of the structure. Such images are produced by beaming x-rays through purified protein crystals of the channels and then using a computer to analyze the patterns of diffracted x-rays to deduce the structure of the channel.

Producing the crystals necessary for such studies presented a major challenge, said MacKinnon. “Membrane proteins are notoriously hard to crystallize because they are embedded in this oily membrane, and to get them out of the membrane and crystallize them, you have to use detergents. And these detergents form a sort of ‘life jacket’ around the proteins, making them mushy and not amenable to crystallizing.

“What’s more, we had another strike against us in crystallizing the potassium channel protein, because it had many moving parts, which really prevented crystallization,” he said. After years of testing many different potassium channel proteins and crystallization techniques, the researchers finally found one protein—from the thermophilic archaeobacterium *Aeropyrum pernix* that proved more rigid and stable when isolated. They also developed a technique of attaching monoclonal antibodies to the “floppy” portions of the protein, to create attachment points for crystallization of the proteins.

The first x-ray structures using the crystals revealed a startling surprise about the voltage-dependent potassium channel, said MacKinnon. Instead of being embedded deep within the protein, the voltage-sensing gating charges appeared to be incorporated in “paddles” on the outside of the protein.

Working from the x-ray structure, the researchers theorized that these positively charged paddles would flip-flop back and forth from inside to outside the membrane, according to the charge across the membrane. When the membrane became negatively charged on the outside, the paddles would be attracted and would flip toward the outside, opening the channel and

allowing potassium to flow out, restoring the membrane charge to its resting state. And when the inside of the membrane became negatively charged, the paddles would flip back, snapping the channel shut.

Indeed, in the second *Nature* paper, MacKinnon and his colleagues proved that the paddles actually functioned in the way suggested by the structure. In those biochemical experiments, they used antibodies and other chemicals to grab the paddles on one or the other side of the membrane, proving that they flopped back and forth with changing membrane charge.

“So, we've shown that the membrane voltage decides whether the channel's open, because the paddle feels the voltage and goes one way or the other,” said MacKinnon. “It's a feedback loop. The channel sets the membrane voltage, but the membrane voltage decides whether the channel's open. This is precisely the kind of feedback loop that you need in sodium and potassium channels to propagate a nerve impulse.”

MacKinnon emphasized that, although they found the paddles in potassium channels, the same voltage-sensing mechanism is likely to exist in other channels. “Voltage-dependent calcium, sodium and potassium channels are all members of the same big family and are all related evolutionarily,” he said. “And what relates them is that they have the same voltage sensor. So there is absolutely no question that this is a conserved biological mechanism.”

Discovery of the voltage-sensing mechanism may yield important clinical applications, said MacKinnon. “These paddles, I think, will be important targets for compounds that modulate ion channels,” he said. “Current drugs that block the ion pore can only inhibit the channel. However, a molecule that binds to the voltage-sensor paddle could either lock it shut or hold it open.” Given the ubiquity of ion channels throughout the body, such drugs will likely prove useful in a broad array of disorders of the nervous system, muscles or heart, MacKinnon said.