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Structure of Biological "Transistor" Detailed in Higher Organisms

Researchers are unveiling the first detailed view of the architecture of a natural “transistor” that ensures the proper flow of potassium ions in cells. The research group, which had previously determined the structure of voltage-sensing membrane channels in primitive bacteria, has now advanced their understanding to channels in higher organisms, including mammals.

The advance, which was made possible, in part, by some clever chemistry that permitted fragile protein crystals to grow in a more “native” environment, is likely to offer new insights into how the channels function in the brain and heart. These channels control the flow of potassium ions through the cell membrane in response to voltage changes across the cell membrane.

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The researchers, led by Howard Hughes Medical Institute investigator Roderick MacKinnon, presented their findings in two papers published on July 8, 2005, in *Science Express*, the online counterpart of the journal *Science*. Co-authors Stephen Long and Ernest Campbell are members of MacKinnon's laboratory at The Rockefeller University.

Voltage-dependent potassium channels are precise molecular machines that aid in propagating electrical impulses in the brain and heart. The channels are made up of large proteins that form a pore that spans the cell membrane. The pore is extremely selective, allowing only potassium ions to pass through the channel. When an electrical impulse travels along a nerve, the charge across the cell membrane changes and the outside of the membrane becomes negatively charged relative to the inside. This change in charge triggers the potassium channels to open and allows potassium ions to flow out of the cell. The outflow of potassium returns the membrane to its resting state to prepare

for the next nerve impulse.

In previous studies, MacKinnon and his colleagues had determined the structure of the ion channel's voltage sensor by studying channels from an ancient, primitive archaeobacterium. MacKinnon said that differences exist between the channels in primitive organisms and in those of higher organisms, so it was important to progress to exploring the structure of these channels in higher organisms.

Thus, the researchers chose to study the structure of Kv1.2, a member of the *Shaker* family of voltage-dependent potassium channels found in higher organisms, including mammals. The *Shaker* family channels are particularly important to study, said MacKinnon, because they have been used in most of the functional studies of voltage-dependent potassium ion channels over the past few decades.

The researchers analyzed the structure of a Kv1.2 channel from the rat using x-ray crystallography. In this analytical technique, intense beams of x-rays are directed through crystals of proteins. The underlying atomic structure of the proteins is deduced by analyzing the pattern of diffraction of the x-rays.

The researchers had to overcome a major technical challenge to produce pure crystals of the Kv1.2 channel protein. The scientists developed a technique to crystallize the protein while maintaining it in a mixture of detergent and lipid - which more closely mimicked the oily cell membrane in which the channel exists naturally. "This is a significant technical advance that I hope will turn out to be important for crystallization of other membrane proteins," said MacKinnon.

The researchers' earlier structural studies of the bacterial channel revealed that the voltage-sensing molecular "paddles" control potassium flow by snapping open or shut. However, said MacKinnon, understanding the precise mechanism of this movement was thwarted because the voltage-sensing structure was contorted in the crystallized bacterial protein.

"We could deduce some things about how the voltage sensor worked," he said. "But identifying this voltage-sensor paddle led us to experiments that told us that this paddle moves a lot through the membrane when the channel opens. However, that channel couldn't really tell us how the paddle attached to the pore to open and close it."

The crystals of the Kv1.2 channel preserved the natural conformation of the voltage sensor. This enabled the researchers to discern that the paddle was attached by a hinge-like "linker" that is coupled to the pore through which potassium flows. "This connection was totally broken in the earlier structure, so we couldn't say anything about how the motions of the voltage sensor are coupled to the pore," said MacKinnon. "That had to be left to complete speculation."

In contrast to other membrane proteins, said MacKinnon, the voltage-dependent potassium channels have separate domains inside the

membrane, the pore and voltage sensors, that are only weakly attached to each other. “Imagine if you could grab a ‘normal’ membrane protein by its edge and pick it up - it would stay together as a rigid unit,” he said. “But pick up a voltage-dependent channel by its voltage sensor, and it would kind of tip over, since its attachment to the pore is so tenuous. It's the way nature has evolved this little voltage sensor, like a little molecular voltmeter floating in the membrane, to open and close the pore.”

By analyzing where the amino acid arginines are positioned within the voltage sensor, the researchers gleaned additional insights into the mechanism by which the voltage-sensing paddles open and close the pore. These electrically charged arginine molecules play a key role in the function of the voltage sensor to control the opening and closing of the channel pore.

In the current study, the protein of the Kv1.2 channel was crystallized with the potassium pore in the open position. MacKinnon's lab is now focused on producing crystals of the channel protein with the pore in a closed conformation.

“We can now see the switch when it is open,” said MacKinnon. “It will be extremely useful to see what this switch looks like closed. It will be tricky to trap the channel in the closed state, but we have several ideas on how to do it.”

Basic studies of the function of voltage-sensing ion channels could ultimately lead to new ideas that may aid in designing drugs to control the channels' function in a precise way. Given the ubiquity of voltage-gated channels in the brain, heart and muscle, such drugs could prove useful in treating a broad array of disorders. “However, while we believe the pharmaceutical industry will be very interested in the structure we have deduced, these basic findings are many steps away from application,” MacKinnon emphasized.