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Experiments Reveal New Details of the Architecture of Ion Channels

Experiments using x-ray crystallography and electrophysiology have revealed new details about the architecture of voltage-dependent potassium channels. Researchers at the Howard Hughes Medical Institute (HHMI) at The Rockefeller University have solved the crystal structure of the cytoplasmic-facing portion of these channels, which control the flow of potassium out of the cell.

Voltage-dependent potassium ion channels are precise molecular machines that are critical in propagating electrical impulses in the brain and heart. The channels are large proteins that form a cone-shaped pore that spans the width of the cell membrane. When an electrical impulse travels along a nerve, the cell membrane depolarizes, triggering these ion channels to open and allow 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.

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— Roderick MacKinnon

The experiments by HHMI investigator Roderick MacKinnon and his colleagues at The Rockefeller University offer new structural information about the portions of the ion channels that jut inside the cell. The findings, reported in the July 7, 2000, issue of the journal *Science* represent a step toward a more complete understanding of how potassium ion channels are controlled.

In previous work, MacKinnon and his colleagues showed that the pore region of the potassium channel is constructed of four identical α subunits and bears the shape of an inverted teepee. While the researchers also subsequently solved the structure of the β subunit that extends inside the cell, there were a number of unresolved questions about that subunit's function.

"Based on the structure of the β subunit, we found that it had the capability to act as an oxidoreductase enzyme," said MacKinnon. "Understanding why cells have such an enzyme attached to voltage-dependent potassium channels has become a central goal of the β subunit research in our laboratory." MacKinnon hypothesizes that the enzyme represents part of the machinery used by the cell to control the opening and closing of the potassium channel.

In exploring the nature of the enzymatic activity, one of the team's first goals was to see how the β subunit was docked to the rest of the potassium channel. So, MacKinnon and his colleagues launched efforts to crystallize key components of the complex within the cell's cytoplasm and deduced the structure using x-ray crystallography. In this widely used analytical technique, x-rays are beamed through crystals of a protein and the patterns of diffracted x-rays are analyzed using a computer to deduce the protein's structure.

In addition to the β subunit, the potassium channel's cytoplasmic structure also contains a protein segment known as the T1 domain. The structure of the T1 had been solved previously by Senyon Choe of The Salk Institute.

"Based on the hypothesis that the T1 domain protein must somehow attach to the β subunit, we co-expressed both of these proteins in cells and were able to purify and to crystallize a complex in which the two were linked," said MacKinnon. Since both the T1 protein and the β subunit had been shown to exist as tetramers, four units joined together, "it stood to reason that somehow these would all come together with a parallel fourfold axis," said MacKinnon.

Indeed, while x-ray crystallography did reveal a tetrameric architecture for the T1- β subunit complex, key questions remained, said MacKinnon.

"Even after seeing the structure of this complex, we still didn't know which way it pointed toward the membrane," he said. "To understand that arrangement, we tested the effects of mutations on the complex and performed electrophysiology experiments, and these told us that the T1 domain is closest to the membrane, and the β subunit is furthest away.

"So, while this paper doesn't answer the central question of the role of the β subunit as an enzyme, it takes us a step closer to an answer by telling us how it is attached," said MacKinnon.

The information about the structure and attachment of the T1- β subunit complex does however answer a major puzzle about the T1 domain, he said.

"A significant conceptual problem with the structure developed by Choe—which was certainly a correct structure—was that the hole down the middle of the T1 tetramer was too narrow to allow potassium ions to pass through. It seemed as if potassium channels had a plug in them.

"So a nice outcome of our mutagenesis studies was the discovery that there appears to be openings above the T1 domain to the side, and that's how the transmembrane pore communicates with the cytoplasm," said MacKinnon.

MacKinnon's team's experiments showed that the side openings offer a route through which the inactivation gates of the potassium channel can reach the central pore. These inactivation gates are the means by which the channel's control machinery can quickly slam the channel shut when necessary to control the ion flow, for example, to alter a nerve impulse.

According to MacKinnon, the next research challenge is to understand how the activity of the β subunit enzyme couples to the potassium channel, which must occur if the enzyme plays a part in the channel's control machinery. Among the hypotheses, he said, are that some part of the channel might extend down to the enzyme to act as an on or off switch. Alternately, the state of the enzyme might somehow transmit a signal from the cell that affects the state of the channel. Further research will also focus on identifying the substrate on which the enzyme acts, which could offer vital clues to the cellular control pathway in which the enzyme participates.