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Shaping Better Understanding of Potassium Channels

Studies by researchers at the Howard Hughes Medical Institute have identified how potassium channels open to allow potassium ions to flow through the cell membrane — a process that is crucial in many biological processes, including the rhythmic beating of the heart and the generation of nerve impulses.

Alterations in potassium channel function have also been linked to diabetes, heart disease, asthma and other diseases. Understanding fundamental details of how these channels operate may eventually help in the design of new drugs to treat many different disorders.

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The research team, led by Roderick MacKinnon, a Howard Hughes Medical Institute investigator at The Rockefeller University, published two articles in the May 30, 2002, issue of the journal *Nature* that show how the "pore" of the potassium channel opens to allow potassium to flow through the cell membrane, and how calcium ions inside the cell induce the pore to open.

Over the last four years, MacKinnon and his colleagues have made impressive gains in understanding the structure and function of potassium and chloride channels. Using purified ion channels from bacteria, the researchers showed that potassium channels feature an "inverted teepee-shaped" pore that protrudes through the cell membrane and is highly selective for potassium. Depending on the type of potassium channel, there is usually a mechanism for "gating," that is, opening the channel pore in response to a chemical ligand such as calcium, or a voltage change across the cell membrane.

"Although in previous work we had solved the structure of the potassium channel, we still did not understand this gating mechanism — how it opens and closes," said MacKinnon. In one of the *Nature* articles, MacKinnon and

his colleagues sought to understand how the pore opens by comparing the structures of two different potassium channels. Starting with two channels — one closed and one open — the researchers used a technique called x-ray crystallography to obtain molecular snapshots of the structures of the two different channels. In these studies, x-rays were beamed through purified protein crystals of the channels, and the patterns of diffracted x-rays were analyzed using a computer to deduce the structures of the channels.

Comparison of the structures of the two channels — called KcsA and MthK — revealed how the teepee-shaped pore opens. "These pores both have helical-shaped alpha-helices that line their inner surfaces," said MacKinnon. "We could see that the equivalent helices in KcsA ran straight, while those in MthK were bent outward by a little more than 30 degrees. So this difference allowed us to recognize that we were looking at a hinge."

According to MacKinnon, the studies revealed that almost all potassium channels have the amino acid glycine in the critical hinge region. The flexibility of the glycine amino acid enables the hinge to work. "All potassium channels in nature have this canonical pore, which is composed of four identical subunits," he said. "And there is a high degree of sequence conservation among these channel proteins, so we know that this mechanism is going to be similar throughout potassium channels."

In the accompanying *Nature article*, the researchers tackled the question of how calcium acts to open the pore. "The opening and closing of these channels is controlled, but we did not understand the gating mechanism," said MacKinnon. The researchers studied the calcium-gated MthK channel because that channel seemed to have a large extra piece of protein, called an RCK domain, jutting inside the cell. RCK stands for "regulates conduction of K⁺ ions."

"We knew that if we could solve the structure of the potassium channel with the RCK domains on it, that would tell us a lot about how the RCK domain can possibly bind a ligand and open the pore, said MacKinnon. "As in much of structural biology, the molecule is like a little machine with moving parts, and if you know its structure, you can figure out how these parts work." The researchers used x-ray crystallography to solve the structure of the MthK channel in the presence of calcium.

"It turned out better than we had hoped," said MacKinnon. "We saw in the structure that not only was calcium bound to the RCK domain, but the pore was splayed open." And the structure also revealed an unexpected feature.

"Since the pore structure is a tetramer (composed of four subunits), we thought that each potassium channel would have four RCK domains," said MacKinnon. "However, our studies showed surprisingly that the expression of the gene results in each subunit having two RCK domains. What's more, it was a very big surprise when our x-ray crystallography revealed that when these four subunits assemble, they form an eight-unit 'gating ring,'" he noted.

The scientists also found that the gating ring possesses both "fixed" and "flexible" interfaces between the RCK domains, and that the flexible interfaces form pockets. Calcium ions can plug into these pockets to alter the gating ring's conformation to pry open the ion channel's attached pore.

MacKinnon emphasized that the findings from his laboratory are very basic observations that help to explain how potassium channels work. "The findings help us to understand how the channel changes its shape when it opens," said MacKinnon. "So ultimately, we hope that understanding such conformational changes might suggest new drug molecules that could bind to such surfaces, affecting the function of the potassium channel."