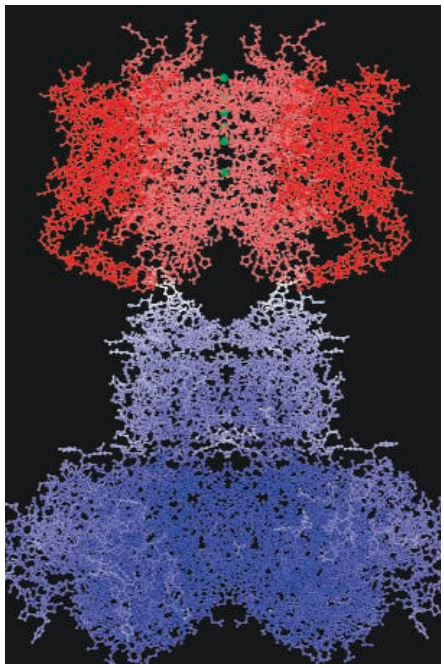
A New Portrait Puts Potassium Pore in a Fresh Light

An atomic structure of a potassium channel, which is central to the firing of neurons, may settle some debates over how this critical protein operates

Two years ago, Roderick MacKinnon and colleagues at Rockefeller University in New York City unveiled an atomic map of an ion-channel protein that rocked the small community of researchers working to unravel the details of how nerves and other cells conduct electrical impulses. Solving that structure was a tour de force of chemistry. It required getting copies of the protein, which is normally embedded in the bacterial cell membrane, to arrange in an ordered crystal. But the map showed parts of the molecule to be in positions that were not what other experts had come to expect. Now, in a pair of papers on pages 897 and 903, MacKinnon and colleagues reveal the structure of a closely related eukaryotic ion channel protein that has other experts breathing much easier. "This is a terrific structure," says Richard Horn, a physiologist at Jefferson Medical College in Philadelphia, Pennsylvania.

Both the new and earlier structures offer close-ups of proteins called potassium voltage gated ion channels. When a neuron fires, potassium ions build up inside the cell. These positively charged ions create an electrical voltage across the cell membrane that triggers the potassium channel to open, allowing the excess potassium ions to spill out and restoring the cell to its resting state, ready to fire again. Virtually all potassium channels consist of four identical subunits, each made up of six linked helical segments. Two of those helical segments from each subunit assemble to form the central pore through which ions flow, while the other segments form a cloverleaf pattern of four voltage sensors that detect voltage changes across the cell membrane and move like a lever to open and close the pore.

Getting structures of such complex proteins is no simple task. Researchers must first coax billions of copies of a protein to stack in a perfectly ordered crystal. They then fire a tight beam of x-rays at the crystal and track how those x-rays ricochet off the atoms in the crystal to work out the precise positions of each atom. That task is especially challenging with potassium ion channels, MacKinnon says, as the voltage sensors are barely connected to the pore regions and therefore are floppy and difficult to stabilize in a crystal. Two years ago, MacKinnon's group attached antibody fragments to copies of the bacterial protein to help stabilize it. But when the structure was published in *Nature* in 2003, the voltage sensors looked to many experts to be tilted on their sides from what they expected (*Science*, 27 June 2003, p. 2020). Many of the measurements that had been done on the channels over the years didn't seem to mesh with the new structure, Horn says. MacKinnon and colleagues themselves noted in the *Nature* paper that portions of the protein were in unexpected positions, possibly as a result of the technique used to crystallize the protein.



Hot shot. Potassium channel (red) is seen together with T1 and β domains (blue) for the first time.

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For their new structure, MacKinnon's team was able to do away with the antibodies. The eukaryotic channels are nearly identical to those in bacteria, but there's a key difference: Eukaryotic potassium channels contain an additional protein domain, known as T1, and another associated protein, known as β, that sit outside the cell membrane in the cytoplasm. With the help of some novel crystallization techniques that used lipids to crystallize the entire complex, MacKinnon's team found that T1 and β helped stabilize the channel protein during crystallization without requiring support from antibodies. Whether or not getting rid of the antibody fragments made the difference, the voltage sensors in the new structure are rotated upright, where other lines of evidence suggested they should be. "It was comforting to see [the position of the voltage sensors] was much more like everyone thought," says Francisco Bezanilla, an ion channel researcher at the University of California, Los Angeles (UCLA).

Like its predecessor, the new structure offers fresh insights into how the channel works. For one, Horn says, helices that form each subunit's voltage sensor aren't adjacent to those that help make up the pore. Rather, those domains from the different subunits interlace around one another. Gary Yellen, a neuroscientist at Harvard University adds that the new structure shows for the first time how the voltage sensor links to the pore, which, he says, "is a pretty neat thing to see."

Controversies remain. For example, ion-channel experts have long known that four positively charged arginine amino acids sit atop each of the voltage sensors that surround the pore. These charged arginines move in response to changes in the voltage across the cell membrane, pressing up and down on the lever that opens and closes the pore. But just how this movement takes place remains at issue.

MacKinnon's team has suggested that two of the helices that help make up the voltage sensor are part of a "paddle" that moves through the membrane and pushes on the lever. That view, he suggests, was supported by a study 2 years ago that showed that positively charged arginine amino acids that are part of this paddle move a considerable distance—15 or so angstroms—through the membrane, from the extracellular to the intracellular portion, as the pore moves from its open to its closed configuration. David Clapham of Harvard University says the new structure is consistent with this model. But not everyone is convinced. Bezanilla points out that two 1999 studies, by his group and Ehud Isacoff's group at UC Berkeley, used fluorescence tracking techniques to show that a key helix in the sensor that presses on the lever, known as S4, does not change its depth in the membrane by more than 3 angstroms.

MacKinnon points out that this debate can't be settled by the new crystal structure because it's a static view of the potassium channel in the open position. He says he and his colleagues are already working to get a structure for the protein in its closed form, which together with the current structure should reveal how the protein moves. In solving this debate, MacKinnon says, "there is nothing like data."

–ROBERT F. SERVICE

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