

JANUARY 17, 2002

## Images Reveal How Body Regulates Salt Uptake in Cells

Using x-ray crystallography, a team of scientists led by Howard Hughes Medical Institute investigator Roderick MacKinnon at The Rockefeller University has determined the three-dimensional structure of the chloride ion channel. The images, which were reported in the January 17, 2002, issue of the journal *Nature*, reveal an entirely new type of protein architecture designed to be an efficient conductor of chloride ions across the membrane of cells.

The results, called a “spectacular breakthrough” by Thomas Jentsch of the Center for Molecular Neurobiology in Hamburg, Germany, in an accompanying *News and Views* article, resolve a confusing series of biochemical studies that failed to explain how the channel works.

“It is a complicated structure,” said MacKinnon. “Scientists did an excellent job deducing many aspects of the chloride ion channel. For example, its dimer architecture was predicted 20 years ago by HHMI investigator Christopher Miller at Brandeis University, and firmly established more recently by his laboratory using biochemical methods and electron microscopy. But to understand the physical principles of anion selectivity, an atomic structure is needed. Although the structure is complicated, it conveys a simple message of how nature arranged the protein to stabilize an anion such as chloride inside the membrane.”

Electrically charged ions are used by living organisms for many types of signaling, including controlling the heart rhythm, generating nerve impulses and secreting hormones. Cells use ions to signal by creating an electrical charge difference between the inside and outside of the cell.

The channels solve an important conundrum, said MacKinnon. “Being charged, ions would rather be in water than in an oily membrane. Nature has to have a mechanism to get the ion across the cell membrane.” It accomplishes the feat through ion channels, which are essentially pores in the cell membrane that can distinguish one type of ion from another and only admit those that pass the selection process.

Chloride ion channels are found throughout the animal kingdom. In humans, nine different ClC channels regulate processes as diverse as salt reabsorption in the kidneys and muscle contractility. Several human disorders, including

diseases of the kidney and muscle, have been linked to chloride ion channel mutations. People with Thomsen's disease, for example, have chronic muscle stiffness and delayed ability to release a handgrip. Mutations in another type of chloride ion channel, the cystic fibrosis transmembrane regulator, cause the most common genetic disease of people of European descent.

In 1998, MacKinnon and his colleagues solved the crystal structure of the potassium ion channel. Potassium, which carries a positive charge, is used for signaling in the nervous system, among other roles.

Once this structure was discovered, the researchers set out to solve the structure of the chloride ion channel to discern how negative ions move across the cell membrane. They chose the CIC channel because other scientists had shown previously that all the CIC channels, from bacteria to humans, had the same basic protein sequence. Being able to isolate the protein from bacteria was important because large quantities are needed to create the high purity protein crystals required for X-ray crystallography. The scientists chose to crystallize the CIC protein from both *Salmonella typhimurium* and *Escherichia coli*, two bacteria commonly studied in the laboratory.

They discovered that the chloride ion channel has a completely different structure from the potassium ion channel. While the potassium ion channel has one large single pore with a water-filled, pyramid-shaped cavity, the chloride ion channel has two pores, each shaped like an hourglass with a narrow constriction at the center. The scientists also discovered the arrangement of the protein subunits that make up the channel are arranged entirely differently in the two types of channels. In the potassium ion channel, four protein subunits contribute to a single pore. In the chloride ion channel, each protein subunit has its own pore and the two halves of the subunit have opposite orientations in what's called two-fold rotational symmetry.

"We can see where the chloride ion binds," said MacKinnon. "We can pinpoint the chemistry that holds a chloride ion and defines the selectivity filter. This is important to designing future experiments that will test how the chloride ion flows through the channel. By pinpointing the important chemical interactions, we narrow down where mutations should be made to test function."

Once the scientists saw the structure, they realized that biochemical analysis of the protein would likely never have produced a useful model structure. "It is complicated to figure out until you see it," MacKinnon said. "We needed the structure to really understand how this protein works." Without the x-ray structure it would have been impossible to understand how the various parts come together to create the pore, he added.

Now that the structure is known, said MacKinnon, it will help scientists figure out how the channel opens and closes to maintain the appropriate concentration of ions inside the cell. This process, called gating, is only beginning to be understood, he said. Future experiments in MacKinnon's laboratory will focus on determining how the ion channel accomplishes the

gating process.