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## Channel Structure Improves Understanding of Protein Transport

Researchers have determined the first high-resolution structure of a type of channel that transports proteins across and integrates proteins into membranes. According to the researchers, the structure of the channel is providing new information about protein transport.

In the cells of higher organisms, channels transport newly synthesized proteins across the convoluted membranes of the endoplasmic reticulum. Proteins are ferried from their site of synthesis to locations in the cell where they can be made ready for distribution. Channels are also needed to integrate certain proteins into membranes, where they function as receptors and other cellular components. Similar channels allow the secretion and membrane integration of proteins in bacteria and archaea.

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— **Stephen C. Harrison**

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The researchers, led by Howard Hughes Medical Institute investigators Tom A. Rapoport and Stephen C. Harrison, both at Harvard Medical School, published their studies in an advance online publication in the journal *Nature* on December 3, 2003.

"The structure determined by the Rapoport and Harrison labs makes a major contribution to understanding protein-translocation systems," said HHMI investigator Jonathan Goldberg, who is at Memorial Sloan-Kettering Cancer Center. "It illuminates in detail how a single trimeric complex could form the functional channel for protein conduction across or into a membrane."

"While biochemical and mutational studies in other laboratories, particularly Tom Rapoport's, had revealed a great deal generally about the various subunits of this protein channel and their roles, very little was known about how they were organized three-dimensionally," said Harrison.

Previously, low-resolution data were obtained on the channel's three-dimensional structure from electron microscopy of two-dimensional crystals of the channel protein from the bacterium *Escherichia coli*. To obtain better crystals for x-ray studies, the researchers isolated channel proteins from numerous species of bacteria. They determined that the channel protein from the methane-consuming bacterium *Methanococcus jannaschii* yielded the best crystals under the harsh conditions required for purification.

In a demanding process that took some five years, the researchers produced pure crystals of the *M. jannaschii* channel protein. They then used x-ray crystallography to analyze the crystal structure at a resolution of 3.2 angstroms. In this widely used analytical technique, x-ray beams are directed through crystals of a protein. The resulting diffraction pattern, which is created when x-rays bounce off atoms in the crystal, is then analyzed to determine a protein's three-dimensional structure.

said, the The images of the protein channel structure revealed a surprise to Rapoport and his colleagues. "The major unexpected result is that it looks as if the pore of the channel is formed from one copy of the complex," said Rapoport. "We had all assumed that the channel pore would be formed at the interface of several copies of this complex."

The researchers' analysis of the structure also revealed that the ring around the pore itself was a flexible ring of amino acids. Thus, the pore could seal itself around a protein in passage, preventing leakage of other molecules through the relatively large pore. Previous theories had held that the pore was sealed by the massive protein complex of the ribosome—the cell's protein-making factory—as the ribosome docked with the channel to extrude newly synthesized proteins through the endoplasmic reticulum membrane.

"We already had data showing that the ribosome membrane junction isn't as tight as what's claimed," Rapoport said. "So there was a sizeable gap, but that raised the question, how do you maintain the membrane barrier, if there is a gap? Now this gasket-like pore ring provides an answer."

According to Harrison, the researchers in Rapoport's laboratory arrived at a model in which the channel resembles a clamshell inserted "sideways" into the membrane, with its hinge running from one side of the membrane to the other. "In this model, a protein that will pass through the membrane carries a 'signal peptide' that targets it to the channel," said Harrison. "The initial interaction between this signal peptide and the channel in essence pries open the clamshell, forming a pore that is sealed at the open end of the clamshell by the signal peptide itself. This opening also causes a plug-like structure in the pore to pop out like a cork on a string. Then, the loop of the protein to be transported is fed through the opening of the clamshell and continues until the protein is completely through."

From Rapoport's perspective, the model offers a good starting point for further studies that will yield more information about how the channel is constructed. "In the short term, we need to test the speculations that we have made on the basis of this structure," he said. "We want to know exactly

where the pore is, whether the plug moves the way we think it does, and whether the channel is formed from a single copy of the complex.”

“One valuable experiment will be to try to trap the channel with a segment of translocating polypeptide in its jaws, propping it in an open state,” said Harrison. “Obtaining structures of this state would give us a snapshot of another stage of the process. And subsequently, we would like to add other of the protein components of the translocation process—such as the enzyme that shoves the protein through—to arrive at a complete picture of how it all works.”