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## Researchers Solve Structure of Key Drug Target

A 12-year effort has paid off as researchers are now unveiling the first detailed structural images of a type of protein that functions in a manner generally similar to the target of Prozac and Prilosec, two of the world's most widely prescribed drugs.

The protein belongs to a class of molecules called membrane transport proteins whose primary job is to move molecules as diverse as nutrients and neurotransmitters across the cell membrane. Membrane transport proteins play such a vital role in the cell that their disruption is thought to be involved in numerous diseases, including depression, stroke and diabetes.

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— H. Ronald Kaback

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In an article published in the August 1, 2003, issue of the journal *Science*, a research team led by Howard Hughes Medical Institute investigator H. Ronald Kaback at the University of California, Los Angeles, So Iwata and Jeff Abramson of Imperial College London report that they have solved the three-dimensional structure of the bacterial membrane transport protein lactose permease (LacY). This protein is the most studied representative of the "major facilitator superfamily" of membrane transport proteins, said Kaback. LacY uses the energy from an electrochemical proton gradient to drive accumulation of lactose, a sugar, across the cell membrane.

In their studies, the researchers were attempting to create crystals of the LacY protein to analyze using x-ray crystallography. In this widely used analytical technique, x-rays are beamed through purified crystals of a protein. The three-dimensional structure of the crystallized protein is deduced by analyzing the pattern of x-ray diffraction caused by the atoms in the protein.

Kaback said that he and his colleagues spent many frustrating years attempting to crystallize the normal, or "wild-type," LacY protein—an

excruciatingly difficult process given the complexity and “floppiness” of the molecule. Meanwhile, extensive experiments in which they studied effects of subtle mutations in the protein yielded considerable indirect evidence of how the transport protein might work. But the researchers knew that only a three-dimensional structure would yield conclusive evidence of how the protein functioned to “cotransport” protons and lactose.

Finally, the researchers identified one particularly intriguing mutant protein—in which an amino acid had been altered. This mutant binds lactose-type sugars, but isn't able to transport .

“After twelve years, I began to think that if this mutant binds and it doesn't transport, it must be favoring one conformation, when it can't move around that much,” said Kaback. Thus, he and his colleagues thought that the mutant protein might actually be stable enough to crystallize.

Sure enough, when Abramson attempted to crystallize the mutant protein, he was successful, enabling the Iwata laboratory to launch an effort to obtain a three-dimensional structure.

The result, said Kaback, was critically important for understanding how the protein works. “We needed that structure,” he said. “Without structure you can't get mechanism, although we had an approximate idea of what it looked like.”

Kaback said the resulting structure confirmed a surprising amount of information gleaned from previous indirect studies of the protein's structure and function. “It's amazing how much of it turned out to be right,” he said. “The binding and the proton translocation part of it are almost right on. I consider this to be a wonderful example of what obsessive-compulsive behavior and pure dumb luck will do for you.”

The structure revealed that LacY consists of an array of irregular helical structures that wind their way through the cell membrane and anchor the protein. “The most striking thing is the irregularity of the helices,” said Kaback. “The previous dogma was that transmembrane helices have to be rigid bodies that run perpendicular to the plane of the membrane. But we saw helices that are arched, and s-shaped, and broken.”

Kaback was also surprised by the existence of a large water-filled cavity in the middle of LacY that faces the inside of the cell and the unanticipated symmetry in the two bundles of six helical protein segments that pierce the membrane.

Most importantly, said Kaback, the LacY structure suggests how amino acids from the protein bind sugar and a proton and escort them through the membrane. The process involves an intricate choreography of interactions in which the participating amino acids perform their precise functions as the protein's water-filled cavity flips from an outward-facing conformation to an inward-facing one. And finally, after the transport through the membrane is complete, the protein returns to its “ground” state, prepared for the next

transport.

According to Kaback, solving the structure of LacY is an achievement that will likely have important implications for a broad range of studies of membrane transport proteins. “The most important thing about this structure is that we've shown it can be done, because people have shied away from attempting to structure these proteins for a long time,” he said.

“I think that this represents an important paradigm shift in the field, because these are incredibly important proteins. Thirty percent of the genome encodes membrane proteins, most of which are transport proteins. And I believe that we can expect that twenty years from now every soluble protein that can be crystallized is going to be crystallized.”