Solving the mystery of "leaky" membranes

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Some cells, such as red blood cells, readily transport water, whereas the water permeability of other cells, such as neurons, is nearly undetectable. For years, researchers wondered why water transport seems to occur in some tissues but not in others. Was there an ion-transport channel doing double-duty as a water channel, or did proteins expressed in certain membranes make them leaky to water? The mystery remained until 1992, when, for the first time, Peter Agre and colleagues identified a dedicated water channel protein, aquaporin-1 (AQP-1)—work that earned Agre the Nobel Prize in 2003.

Agre was trained as a blood specialist, and, in 1988, had a small laboratory at Johns Hopkins University (JHU) that studied red cell membrane proteins, hoping to identify the D antigen of the Rh blood group. He and his team had identified a unique 32-kDa RhD protein, and when they purified it, to their surprise, a 28-kDa protein was copurified. "Our interest in this 28-kDa protein, which we now know is AQP-1, was really because it was a contaminant that initially we misunderstood as a breakdown product of the 32-kDa RhD polypeptide," Agre says.

At the time, they had no idea that this protein was the water channel. "In the beginning, we stumbled upon something we didn't expect to see," Agre says. "It certainly was not genius. It was probably a little bit of observation." Hundreds of laboratories were studying red cell membrane proteins. They would separate them with SDS-PAGE, stain the gels with Coomassie, and get a series of bands. In the 28- to 32-kDa region, nothing seemed to stain well, which had allowed the protein to go undetected.

The 28-kDa protein's peptide maps were unique. After purifying it, Agre's team calculated that it was one of the most abundant proteins in the membrane. "It took a bit of confidence building to assess that, in fact, it was a new protein," Agre says. In a 1988 paper in the *Journal of Biological Chemistry* (JBC) (1), they explained how they had identified the protein and described its basic bilayer-spanning orientation and its presence in renal proximal tubules as well as red blood cells. "We thought the 28-kDa protein might be a membrane-associated protein to bracket transport molecules," Agre recalls. "We didn't realize that it was the transport molecule itself."

Afterward, they searched for a function. The protein "appeared to be mostly living within the bilayer itself, with an extracellular glycosylation site and some small cytoplasmic domains visible at the termini," Agre says. They spoke with dozens of scientists to determine what the protein did. Agre had a discussion with John Parker, a scientist at the University of North Carolina, who had been Agre's clinical mentor, who suggested it might be a channel specific for water. Agre says, "It was really John Parker's suggestion that caused us to roll up our sleeves and collaborate" with William Guggino, an associate professor at JHU who had studied water permeability in fish eggs.

For a 1992 paper in *Science* (2), Agre, Guggino, and colleagues cloned the protein and expressed complementary RNA in frog eggs, conferring water permeability to the otherwise impermeable membranes. With the protein expressed, the eggs "swell by osmosis and explode like popcorn," Agre says. They established that the protein was a water-selective channel, described its function, and named it aquaporin-1.

"Usually with a new idea you get a lot of skepticism, and people argue that it's wrong," Agre says. But the *Science* paper was immediately greeted with enthusiasm. "We were a small lab and were astonished by how interested people were. Science is sometimes not predictable."

Over the next two years, Agre's lab published many papers on the topic, including two in JBC. A 1993 paper (3) addressed AQP-1's function, showing that AQP-1 forms a complex made up of multiple subunits and that water molecules permeate AQP-1 through a single pore in each of those subunits. The next (4), in 1994, presented an hourglass model for the way water channel proteins such as AQP-1 function during water transport (Fig. 1).

"The hourglass has a specific structure in the pore that allows water to move right up through the pore itself," Guggino says. "The water molecule fits very nicely in the pore, and thus the water channel is highly selective for water."

It has since been shown that there are many homologs in other tissues and in other organisms, including plants and even archaea. The number of aquaporins determines a tissue's permeability. "Most tissues have a very small number, although in situations where cells are stressed,"—for example, in the case of dehydration—"the renal AQP-2 levels rise, causing kidneys to maximally reabsorb water from urine," Agre says.

For discovering this essential physiological process, Agre won one-half of the Nobel Prize in Chemistry in 2003. "It is my opinion that the work in these three JBC papers forms a significant part of the basis for his Nobel Prize," says Eric Fearon, director of the University of Michigan Rogel Cancer Center and a JBC associate editor.

"It would have been nice to slow down and savor each paper a little more, but when things get going, they get going," says Agre, who now directs the JHU Malaria Research Institute. He

Eric Fearon at the University of Michigan Rogel Cancer Center nominated this paper as a Classic.

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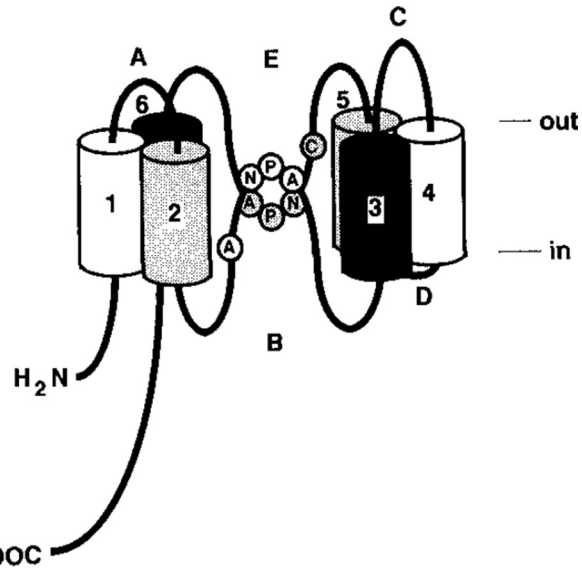

Figure 1. An illustrated model of an aquaporin-1 subunit from Ref. 4. The protein contains four of these oligomerized subunits and a central depression with four water-channel pores.

counts himself and his team lucky to have worked on a solvable problem with an elegant solution.

"I like to tell people that we didn't discover the AQP-1 protein—it discovered us," Agre says. "I wish everyone would have the opportunity to make a discovery like the aquaporins and figure out how they function."

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