Peter Agre, 2003 Nobel Prize Winner in Chemistry

MARK A. KNEPPER,* and SOREN NIELSEN†
National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland; and †Water and Salt Research Center, University of Aarhus, Aarhus, Denmark.

Abstract. Peter C. Agre, an American Society of Nephrology member, is the recipient of the 2003 Nobel Prize in Chemistry for his discovery of the aquaporin water channels. The function of many cells requires that water move rapidly into and out of them. There was only indirect evidence that proteinaceous channels provide this vital activity until Agre and colleagues purified aquaporin-1 from human erythrocytes and reported its cDNA sequence. They proved that aquaporin-1 is a specific water channel by cRNA expression studies in Xenopus oocytes and by functional reconstitution of transport activity in liposomes after the incorporation of the purified protein. These findings sparked a veritable explosion of work that affects several long-standing areas of investigation such as the biophysics of water permeation across cell membranes, the structural biology of integral membrane proteins, the physiology of fluid transport in the kidney and other organs, and the pathophysiological basis of inherited and acquired disorders of water balance. Agre’s discovery of the first water channel has spurred a revolution in animal and plant physiology and in medicine.

Before Agre’s work, the existence of proteinaceous water channels that mediate water transport across cell membranes was strongly suspected on the basis of studies in red blood cells and toad urinary bladders. Although artificial lipid membranes had previously been found to be moderately permeable to water, studies of water permeation through the plasma membranes of red blood cells revealed exceptionally high values (2). Studies by Macey and Farmer (3) revealed that the high water permeability of red blood cell membranes (later found to contain aquaporin-1) were highly sensitive to mercurial reagents, which interact with sulfhydryl groups of the amino acid cysteine, a component of most proteins. Studies of toad urinary bladders, which show vasopressin-stimulated increases in water permeability of red blood cell membranes revealed that the increase in water permeability was associated with the appearance of membrane particle aggregates in the apical plasma membrane. On the basis of the mosaic theory of membranes, which postulated that biologic membranes are made up of proteins floating in a sea of lipid, it appeared that the particle aggregates were likely to be proteins and were viewed as containing putative water channels.

With the advent of the molecular biology era in the late 1980s and early 1990s, during which more and more cDNAs coding for physiologically important proteins were being cloned, a number of laboratories were focused on discovering the genes that code for water channels. It is interesting that the success of Peter Agre in discovering the prototype water channel came out of a project with an entirely different aim, the identification of the protein in red cell membranes that contains the epitope responsible for Rh immunoreactivity of red cells (4). Because of the high abundance of this contaminant, which could be readily purified to homogeneity, Denker et al. (5) were spurred to characterize the contaminant biochemically. An antibody made by injecting rabbits with the purified holoprotein revealed that the protein was expressed not only in red cells, but also in the kidney proximal tubule and descending limb. This localization suggested to Agre that the protein could play some role in transport. Gregory Preston in the Agre laboratory at Johns Hopkins cloned its cDNA, demonstrating that the protein was a polytopic integral membrane protein with six likely membrane-spanning alpha helices (6). At that time, they referred to the protein as CHIP28, for CHannel-forming Integral Protein of 28 kD. However, its function remained to be defined.

Agre credits John Parker, a membrane biophysicist at the University of North Carolina, for the idea that CHIP28 could be the red blood cell’s water channel. To test the hypothesis, Preston and Agre teamed with Bill Guggino of the Johns Hopkins Department of Physiology, another ASN member, to
express CHIP28 in *Xenopus* oocytes. A few years earlier, Ernest Wright, from UCLA, had demonstrated the utility of the *Xenopus* oocyte as an expression system for functional cloning of cDNAs, and Guggino had imported this method into his laboratory. In addition, Guggino had set up a state-of-the-art video microscopy system that facilitated quantification of the swelling rates of oocytes when submersed in hypotonic solutions. When Preston injected cRNA made from his CHIP28 cDNA clone into *Xenopus* oocytes, he found a marked increase in the rate of osmotic swelling (7). Thus, expression of CHIP28 conveyed a high water permeability to *Xenopus* oocyte plasma membranes, providing the first direct evidence for the existence of water channel proteins. Nevertheless, it could be argued that CHIP28 might have been an accessory protein that activated endogenous water channels native to the oocyte. To confirm that CHIP28 was indeed a water channel itself, Agre teamed with Mark Zeidel, another ASN member, to reconstitute purified CHIP28 protein into artificial lipid membranes (8). Zeidel measured water permeability in these vesicles using a stop-flow light-scattering method that he had set up in his laboratory and found that vesicles in which CHIP28 was incorporated manifested a dramatic increase in water permeability over vesicles containing only lipid. The water permeability was demonstrated to be sensitive to mercurials just as seen in the native red blood cells. Thus, with the incontrovertible demonstration that CHIP28 is a molecular water channel, the door was opened for other investigators to clone other water channels on the basis of homology (i.e., structural similarity to CHIP28).

**Nomenclature**

To deal with confusion stemming from differing naming systems for water channels offered by various laboratories, the field rapidly adopted a consistent nomenclature based on the term aquaporin to denote a member of the water channel family followed by a numeral to identify the member (9). In this nomenclature, CHIP28 was designated aquaporin-1 or AQP1. In the remainder of this article, we use this nomenclature even though many of the original papers reporting new water channels did not employ the term aquaporin.

**AQP1 Mutations in Humans**

Agre’s previous work with red blood cell antigens led him to the recognition that the polymorphism responsible for the discrimination of Colton blood groups occurs in the coding sequence of AQP1 (10). He and his colleagues recently published a paper in the New England Journal of Medicine describing patients that are Colton-null due to mutations in the AQP1 gene (11). These patients fail to express functional AQP1 and fail to concentrate their urine, demonstrating the importance of AQP1 in normal renal function.

**Cloning and Characterization of Other Members of the Aquaporin Family**

On the basis of the primary structure of the AQP1 gene and its mRNA, investigators worldwide have succeeded in identifying many other aquaporins by homology cloning, many of which demonstrated clinical importance. From the perspective of renal medicine, none is more important than aquaporin-2 (AQP2), the vasopressin-regulated water channel of the collecting duct, cloned by Fushimi et al. (12). Deen, van Os, and colleagues demonstrated that human individuals with mutations in AQP2 suffer from severe nephrogenic diabetes insipidus (NDI), underscoring that aquaporin-2 is essential for urinary concentration. Soon after the discovery of AQP2, we were able to demonstrate that vasopressin regulates collecting duct water permeability, and hence body water balance, by regulating trafficking of AQP2-laden vesicles to and from the apical plasma membrane (13), validating the shuttle hypothesis of Wade et al. (14). Further studies identified another mode of regulation of AQP2 that was unanticipated; long-term elevations of circulating vasopressin was shown to markedly increase the abundance of AQP2 in the collecting duct (15,16).

Extensive studies of the pathophysiology of water balance disorders in rats (too extensive to review here) have revealed a central role of AQP2 dysregulation in many clinical important syndromes, including congestive heart failure, cirrhosis, nephrotic syndrome, and acquired NDI due to lithium administration (1). As referred to above, mutations in AQP2 have been also shown to be cause of 20% of the rare hereditary NDI with autosomal recessive inheritance. Most of the remaining cases are caused by mutations in the vasopressin-V2 receptor, leading to inability to regulate AQP2 and hence severe NDI. Thus, Peter Agre’s discovery of the aquaporins has led to an understanding of the pathophysiology of water balance abnormalities that affect millions of patients, and it is leading us toward better therapies for these disorders.

Peter Agre and co-workers also cloned an additional member of the water channel family, AQP4, which has been found...
to be expressed in a polarized fashion in astrocytes in the brain, on the aspect of the cells that face the vasculature (17). Functional studies have provided evidence that AQ4 is an important element of the blood brain barrier and may be an important molecular target in treatment of post-traumatic brain edema (18).

Aside from progress in the physiology and pathophysiology of water transport in the animal tissues, the discovery of aquaporins by Agre has spurred important new work in plant physiology (19). Many aquaporin genes are expressed in different parts of plants and are responsible for water transport in plant tissues. For example, when a fruit grows, it accumulates large amounts of water that are transported through aquaporins in the stem. Given the importance of aquaporins in plant physiology, it appears likely that plants can be engineered for better growth in arid environments through manipulation of aquaporin gene expression.

Biochemical Characterization and Structural Analysis of AQP1

Aside from his initial discovery of the aquaporins and the cloning of many of the important animal aquaporins, Agre has played a central role in understanding the relationship between structure and function of the aquaporins. His initial work demonstrated that AQ1 assemble as homotetramers. By use of a variety of biochemical techniques, studies of recombinants demonstrated that AQ1 assemble as homotretramers. By use of various biochemical techniques, studies of recombinants demonstrated that AQ1 assemble as homotetramers. By use of various biochemical techniques, studies of recombinants demonstrated that AQ1 assemble as homotetramers. By use of various biochemical techniques, studies of recombinants demonstrated that AQ1 assemble as homotetramers. By use of various biochemical techniques, studies of recombinants demonstrated that AQ1 assemble as homotetramers. By use of various biochemical techniques, studies of recombinants demonstrated that AQ1 assemble as homotetramers. By use of various biochemical techniques, studies of recombinants demonstrated that AQ1 assemble as homotetramers. By use of various biochemical techniques, studies of recombinants demonstrated that AQ1 assemble as homotetramers.

The ability of the Agre laboratory to purify human red cell AQ1 protein in milligram amounts made it a candidate for structural determination. Collaborating with Andreas Engel and his group in Basel, purified aquaporin-1 protein was reconstituted into membrane crystals and structure was determined at 16-Angstrom resolution in a biologically active form (21,22). This structure was advanced to 3.8-Angstrom resolution in collaboration with Yoshi Fujiyoshi and his group in Kyoto (23). This work confirmed the hourglass model and explained how AQ1 is freely permeated by water molecules but is impermeable to protons, an important issue because our kidneys reabsorb up to 180 L of fluid each day without reabsorbing excreted acid. Moreover, this revealed for the first time the structure of a human channel protein solved from human tissue and sparked the interest of other research groups who further resolved the structure by electron crystallography and x-ray crystallography.

References