The aquaporins (AQPs) are a family of intrinsic membrane proteins that function as water-selective channels in the plasma membranes of the cells of many water-transporting tissues (1, 2). The first AQP to be identified, CHIP28 (later called AQP-CHIP), was purified by Agre and his colleagues from human erythrocytes in the late 1980s and its cDNA sequence was reported in 1991 (reviewed in ref. 2). In 1992, the functional identification of AQP-CHIP as a water channel protein was established by complementary RNA expression studies in Xenopus oocytes (3) and by functional reconstitution of water transport activity in liposomes after the incorporation of the purified protein (4). These findings sparked a veritable explosion of work with an impact in several longstanding areas of investigation, such as the biophysics of water permeation across cell membranes, the physiology of fluid transport in the kidney and other organs, and the pathophysiological basis of inherited and acquired disorders of water balance. Significant progress has also been made in the analysis of the structural basis of selective water permeation through the AQP-CHIP channel. The identification of AQP-CHIP as a molecular water channel also has spawned the identification of three related AQP water channels (Table 1), which are expressed in mammalian tissues and one of which (AQP3) is reported in this issue by Ishibashi and colleagues (7).

**Functional Role of Water Channels.** In general, the plasma membranes of all animal cells (even those without water channels) are at least moderately permeable to water and can maintain osmotic equilibrium in static external environments (8). Studies in artificial lipid membranes have demonstrated osmotic water permeability (Pf) values in excess of 50 \( \mu m/s \) in pure phospholipid membranes (9). Addition of cholesterol, an important component of natural plasma membranes, lowers Pf. For example, in artificial lipid membranes with a 1:1 ratio of cholesterol/phospholipid, the Pf falls to the range 10–20 \( \mu m/s \), a low level but nonetheless theoretically sufficient to maintain osmotic equilibrium in most cells without the aid of water channels. However, the water permeabilities of many cell types have been found to exceed values that can be explained by lipid-phase water permeation alone. For example, the initial portion of the descending limb of Henle’s loop in the chinchilla kidney has an epithelial Pf value of 1500–2500 \( \mu m/s \) due to the fact that the plasma membranes of this epithelium contain large amounts of AQP-CHIP (10). In contrast, the distal portion of the descending limb in the chinchilla has a much lower Pf value (68 \( \mu m/s \)), corresponding with an absence of detectable AQP-CHIP (10). The extremely high water permeabilities that result from water channel expression in a variety of tissues subserve a number of physiological roles. (i) Water channels can raise plasma membrane water permeability to the levels required for efficient coupling between NaCl transport and water transport in epithelia that carry out isosmotic fluid transport (e.g., AQP-CHIP in renal proximal tubule and choroid plexus). (ii) Water channels allow rapid osmotically driven water transport in epithelial and endothelial cells of the renal medulla, which is critical for the countercurrent processes that concentrate the urine. (iii) Water channels provide a target for regulation of water transport [e.g., AQP-CD, the target for vasopressin-mediated regulation of water reabsorption in the renal collecting duct (CD)]

**Biophysical Basis of the Water-Selective Channel Concept.** The concept of a water pore or channel was originated by Koefoed-Johnsen and Ussing in 1953 (11) to explain the observation that osmotic water permeability is higher than diffusional water permeability in frog skins. The increase in Pf brought about by antidiuretic hormone exposure in this epithelium was explained as a widening of pore diameter, an event that was assumed to explain antidiuretic hormone-induced solute fluxes. Subsequently, numerous other biophysical investigations solidified the water pore concept but raised doubts about the ability of small solutes to penetrate water pores. An important step forward was made by Macey and Farmer (12), who demonstrated that water (molecular radius, 1.5 Å) and the small solute urea (molecular radius, 2.0 Å) penetrate the plasma membrane of erythrocytes by independent pathways.

They observed that the organomercurial sulfhydryl-reactive reagent p-chloromercuribenzenesulfonate (pCMBS) markedly decreased erythrocyte Pf (from \( \approx 200 \mu m/s \) to \( \approx 20 \mu m/s \), consistent with the elimination of an aqueous channel. pCMBS inhibited urea and glycerol permeability in addition to water permeability. However, another agent, phloretin [\( \beta-(p-hydroxyphenyl) \)chloropropionophenone], strongly inhibited urea and glycerol transport without affecting water permeability. From these and numerous other biophysical studies in erythrocytes and other cell types, the view emerged that water and small non-electrolytes can traverse plasma membranes via specialized transporters (presumably proteinaceous in nature) whose selectivity is based not on molecular size alone but also on other physical properties of the transported substrates. Thus, based on knowledge gained from biophysical studies, it was predictable that independent selective transporters for water and urea would be identified. The prediction has come to fruition in the past 2 years with the identification of four mammalian water channels (all expressed in the renal medulla) and a renal medullary urea transporter (Table 1).

**Tissue Distribution and Functional Roles of AQPs.** Table 1 summarizes several key characteristics of the known AQPs and compares them to those of the vasopressin-regulated urea transporter UT-2. AQP-CHIP is the major water channel of the erythrocyte plasma membrane and is heavily expressed at sites of constitutively rapid water transport in the kidney (proximal tubule and the descending limb of Henle’s loop) (13, 14). In addition, AQP-CHIP is heavily expressed in epithelia and endothelia thought to be involved in fluid transport at diverse sites throughout the body (15–17). AQP-CD (5) is the vasopressin-regulated water channel of the renal CD and thus has a critical function in the regulation of water excretion. It does not appear to be expressed outside the kidney. Mercurial-insensitive water channel (MfWc) is expressed in the renal medulla (perhaps in the medullary vasculature) and in the lung (6). Its physiological role is as yet undefined. AQP3, reported in this issue (7), is expressed in the CD of...
the kidney as well as in the gastrointestinal tract. Unlike AQP-CD, which is found predominantly in the apical plasma membrane, AQP3 is present predominantly on the basolateral plasma membrane of CD cells, a site at which water permeability is believed to be unregulated. AQP3 has the additional interesting characteristic that it is permeable to urea, whose transport across the inner medullary CD cells is critical to the urinary concentrating mechanism.

UT-2. AQP3, however, is unlikely to be the major urea transporter found in the medullary CD. Physiological studies (19) have identified a phloretin-sensitive transporter that is regulated by the antidiuretic hormone vasopressin and is apparently present in both the apical and basolateral plasma membranes. The cDNA for this transporter (termed UT-2) has been cloned, revealing a primary structure that is apparently unrelated to AQP3 or the other AQPs (18) (Table 1). Injection of UT-2 complementary RNA into Xenopus oocytes induced rapid phloretin-sensitive urea transport. The ability of UT-2 to conduct water molecules across the plasma membrane has not been tested. However, the observation that phloretin markedly inhibits urea permeability in the inner medullary CD without affecting water permeability suggests that the urea transporter does not permit a physiologically significant rate of water permeation.

**Water Selectivity of AQPs.** Each of the first three AQPs identified (AQP-CHIP, AQP-CD, and MIWC) has been found to be permeable to water but not to small solutes or ions. Thus, up until the description of AQP3 in this issue (7), the concept of separate selective pathways for water and small nonelectrolyte transport (see above) has been supported by the results of expression studies of the cloned AQPs. In contrast, AQP3 was found to be capable of transporting either water or the small solutes urea and glycerol (7). Like the urea transport seen in erythrocytes and in the CD, the urea transport mediated by AQP3 (expressed in Xenopus oocytes) appears to be inhibited by phloretin. However, phloretin also was found to inhibit AQP3-mediated osmotic water transport, a finding that contrasts the apparent phloretin-insensitivity of water transport in erythrocytes (12) and in renal CD (20). Furthermore, reflection coefficient measurements have demonstrated that both transepithelial urea transport (21) and basolateral urea transport (22) in the inner medullary CD occurs via a pathway separate from the major water-transporting pathway. Thus, as suggested by Ishibashi et al. (7), the physiological significance of AQP3 as a urea-transporting pathway in the renal CD is uncertain. Nevertheless, their discovery that the AQP3 pore is relatively nonselective may be of considerable importance since a comparison of its sequence to that of the water-selective AQPs may provide important clues regarding the structural basis of water selectivity.

**AQP Structure.** The general structure of the AQPs, elucidated chiefly by the work of Agre and his colleagues in AQP-CHIP (2-4, 23-26), is illustrated in Fig. 1. A single polypeptide chain spans the membrane six times. The N- and C-terminal ends are both in the cell interior and there are three extracellular loops (A, C, and E) as well as two intracellular loops (B and D). The two halves of the molecule exhibit substantial sequence similarity to one another but are oriented oppositely in the membrane so that corresponding regions are found on opposite sides of the membrane. The B and E loops both contain the Asp-Pro-Ala (NPA) sequence that is characteristic of the major intrinsic protein of the lens (MIP) family of proteins, of which the AQPs are members (1).

Biochemical and structural studies have demonstrated that AQP-CHIP assembles into homotetramers in the plasma membrane (2). Nevertheless, each CHIP monomer appears to form a functional water pore. Agre and his colleagues (23, 26) have carried out extensive structure-function studies of AQP-CHIP by site-directed mutagenesis to identify amino acids that are critical to water channel function. These studies

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**Table 1. Water channels and urea transporters expressed in mammalian tissues**

<table>
<thead>
<tr>
<th></th>
<th>AQP-CHIP (2)</th>
<th>AQP-CD (5)</th>
<th>MIWC (6)</th>
<th>AQP3 (7)</th>
<th>UT-2 (18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polypeptide length (amo acids)</td>
<td>269</td>
<td>271</td>
<td>301</td>
<td>285</td>
<td>397</td>
</tr>
<tr>
<td>Putative membrane-spanning domains</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Sensitivity to mercurials</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Unknown</td>
</tr>
<tr>
<td>Sensitivity to phloretin</td>
<td>Unknown</td>
<td>No</td>
<td>Unknown</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Permeability to water</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Unknown</td>
</tr>
<tr>
<td>Permeability to small solutes</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Urea, glycerol</td>
<td>Urea</td>
</tr>
<tr>
<td>Subcellular localization in epithelia</td>
<td>Apical and basolateral</td>
<td>Predominantly apical</td>
<td>Unknown</td>
<td>Predominantly basolateral</td>
<td>Unknown</td>
</tr>
<tr>
<td>Regulation by vasopressin</td>
<td>No</td>
<td>Yes</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Yes</td>
</tr>
<tr>
<td>Regulation by water restriction</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

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*(Although structurally similar to the AQPs, MIP and other non-AQP MIP analogs have not been demonstrated to transport water (1, 2).)*
have established that the B and E loops are critical for formation of functional water-selective pores. Based on these studies and on high-resolution electron microscopic studies of two-dimensional crystals of AQP-CHIP (24), Agre and his colleagues (26) have proposed a three-dimensional model of the AQP-CHIP water pore called the "hourglass model." The B and E loops, which are intracellular and extracellular, respectively, are proposed to fold downward and overlap within the plasma membrane at the NPA sites (Fig. 1), forming a single water pore amidst the overlap. The tetrameric structure (a square array of CHIP monomers (24)) is proposed to be necessary to stabilize the position of individual monomers. Thus, the central space between the four CHIP monomers is proposed to contain four independent pores each approximately the diameter of a single water molecule (26).

A cysteine (C-189 in AQP-CHIP), located just prior to the NPA sequence in the E loop labeled by a square in Fig. 1), has been identified as the site of inhibition of water permeation by mercurials in AQP-CHIP (23). Hg2+ and organic mercurials most likely decrease water permeation by either occluding or disrupting the pore after binding to C-189. A cysteine is also present in the same relative position of AQP-CD, presumably accounting for its sensitivity to mercurials (5). However, both the mercurial-insensitive MIWC and the mercurial-sensitive AQP3 lack a cysteine in this position. Presumably, the mercurial sensitivity of AQP3 is due to a cysteine at another site, perhaps C-78, which is present in the B loop near the NPA motif of AQP3 but is not present at the analogous site in the three other AQPs.

Plasma Membrane Domains of AQPs. Another interesting feature of the AQPs is the observed variability in targeting of the protein to distinct plasma membrane domains of epithelial cells (Table 1). AQP3 is found predominantly in the basolateral membrane of CD cells (7). In contrast, AQP-CD is located predominantly in the apical membrane of the same cells (5, 27). AQP-CHIP, however, is found in very high levels in both the apical and basolateral plasma membranes of the proximal tubule and descending limb cells (13, 14). Presumably, these differences in targeting are based in part on structural differences in the AQP proteins. Clearly, the availability of cDNAs and antibodies for these three aquaporins can potentially provide valuable tools for deciphering the structural basis of selective targeting of membrane proteins.

Regulation of Water Channel Activity. Among the AQPs, AQP-CD is the only one that has been shown to be regulated physiologically (Table 1). The cellular content of AQP-CD protein has been demonstrated to increase in CDs in response to water restriction in normal rats (27) or in response to vasopressin infusion in Brattleboro rats, which lack circulating endogenous vasopressin (S. Di-Giovanni, S. Nielsen, and M.A.K., unpublished observations). Furthermore, AQP-CD mRNA content in the renal medulla (28, 29) has been demonstrated to increase with water restriction. The increase in AQP-CD expression with water restriction is presumably part of a physiological mechanism for coping with long-term water deficits. In contrast, AQP-CHIP mRNA content (29) and AQP-Chip protein content (S. Nielsen, personal communication) did not increase in the renal medulla in response to water restriction. Furthermore, renal medullary AQP3 mRNA content (30) was not altered by long-term water restriction.

In addition to the long-term regulation of AQP-CD water channel expression, AQP-CD is regulated acutely by the antidiuretic hormone vasopressin. After exposure to vasopressin, the osmotic water permeability of CDs increases to very high levels, typically from <50 μm/s to >1000 μm/s, within 5–10 min. The mechanism of this rapid water permeability increase has been a matter of considerable interest for many years. It is clear that the response involves an increase in the number of functional water channels in the apical plasma membrane of CD cells. Based on studies in toad bladders, it was proposed that this increase occurs as a result of delivery of water channels to the apical plasma membrane by vasopressin-induced exocytosis and conversely that the decrease in water permeability after vasopressin withdrawal involves endocytic retrieval of water channel-bearing plasma membrane domains. Thus, the rapid regulation of water permeability is proposed to involve redistribution of a fixed pool of water channels between an intracellular population of water channel-laden vesicles and the apical plasma membrane (the shuttle hypothesis) (31). Recent electron microscopic immunolocalization studies with antibodies to AQP-CD have demonstrated that the AQP-CD protein is present in large amounts in both the apical plasma membrane and in subapical vesicles within CD cells, a distribution compatible with the shuttle hypothesis (27). The dynamic redistribution of AQP-CD water channels between the intracellular compartment and the apical membrane in response to vasopressin has not yet been demonstrated. Nonetheless, a large amount of evidence has been accumulated that unequivocally demonstrates that vasopressin alters the rates of exocytosis and endocytosis in CD cells (32). Thus, although definitive proof of the shuttle hypothesis is lacking, it appears likely that the chief means of regulation of water permeability by vasopressin is via regulated trafficking of the AQP-CD water channel. Given the fact that AQP3 is expressed in the same CD cells as is AQP-CD (7), an important future goal will be to determine whether it too is regulated by vasopressin.

The importance of the AQP-CD water channel to the regulation of body water balance has recently been underscored by the identification of a patient with distinct mutations in the NPA domains of the AQP-CD gene and a normal type 2 vasopressin receptor (33). This patient manifested severe nephrogenic diabetes insipidus (NDI)—i.e., he was unable to concentrate his urine despite high circulating concentrations of vasopressin. This defect was presumably due to an inability of the renal CD to increase water absorption to a physiologically appropriate rate in response to vasopressin. Expression of both mutated forms of AQP-CD in Xenopus oocytes revealed a complete lack of water channel function (33). Thus, AQP-CD appears to be critical for normal CD water transport. This form of NDI is inherited in an autosomal dominant pattern consistent with the location of AQP-CD on chromosome 12 (33, 34). Consequently, NDI due to AQP-CD water channel defects may be expected to be relatively rare in comparison to NDI due to mutations in the V2 vasopressin receptor gene (X chromosome-linked NDI), assuming an approximately equal prevalence of mutations in the two genes. This is the only known example of a clinically significant defect in AQP expression, although others are likely to be reported in the future.

Commentary: Knepper
