Aquaporin CHIP: the archetypal molecular water channel

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Agre, Peter, Gregory M. Preston, Barbara L. Smith, Jin Sup Jung, Surabhi Raina, Chulso Moon, William B. Guggino, and Søren Nielsen. Aquaporin CHIP: the archetypal molecular water channel. Am. J. Physiol. 265 (Renal Fluid Electrolyte Physiol. 34): F463-F476, 1993. —Despite longstanding interest by nephrologists and physiologists, the molecular identities of membrane water channels remained elusive until recognition of CHIP, a 28-kDa channel-forming integral membrane protein from human red blood cells originally referred to as “CHIP28.” CHIP functions as an osmotically driven, water-selective pore; 1) expression of CHIP conferred *Xenopus* oocytes with markedly increased osmotic water permeability but did not allow transmembrane passage of ions or other small molecules; 2) reconstitution of highly purified CHIP into proteoliposomes permitted determination of the unit water permeability, i.e., 3.9 × 10^-9 water molecules·channel subunit·s^-1. Although CHIP exists as a homotetramer in the native red blood cell membrane, site-directed mutagenesis studies suggested that each subunit contains an individually functional pore that may be reversibly occluded by mercurial inhibitors reacting with cysteine-189. CHIP is a major component of both apical and basolateral membranes of water-permeable segments of the nephron, where it facilitates transcellular water flow during reabsorption of glomerular filtrate. CHIP is also abundant in certain other absorptive or secretory epithelia, including choroid plexus, ciliary body of the eye, hepatobiliary ductules, gall bladder, and capillary endothelia. Distinct patterns of CHIP expression occur at these sites during fetal development and maturity. Similar proteins from other mammalian tissues and plants were later shown to transport water, and the group is now referred to as the “aquaporins.” Recognition of CHIP has provided molecular insight into the biological phenomenon of osmotic water movement, and it is hoped that pharmacological modulation of CHIP function may provide novel treatments of renal failure and other clinical problems.

channel-forming integral membrane proteins; water channels; fetal development; cysteine; channel-mediated water transport; osmotic water permeability

**WATER IS THE MAJOR COMPONENT** of all living cells as well as the surrounding extracellular spaces. Transport of water into and out of cells occurs during digestion, respiration, circulation, regulation of body temperature, elimination of toxins, neural homeostasis, and during many other essential body functions. Water crosses cellular plasma membranes by two fundamentally distinct pathways: 1) simple diffusion through the lipid bilayer; and 2) channel-mediated water transport, a process which has been described physiologically despite a lack of molecular understanding of the channel structure (20, 26a, 55).

**Diffusional Water Permeability**

Diffusional water permeability is a characteristic of the plasma membranes of all tissues. The coefficient of diffusional permeability (P_d) is variable among tissues but is usually of relatively small magnitude. Diffusional water permeability can be measured by nuclear magnetic resonance imaging or isotopic methods under isosmotic conditions, and this is not pharmacologically inhibitable. Transport of water across pure lipid membranes occurs solely by diffusion, therefore the ratio of the coefficients of osmotic water permeability to diffusional permeability approximates unity (P_f/P_d ≈ 1). Diffusional water permeability is strongly affected by the organization of membrane lipid molecules and is characterized by high Arrhenius activation energy (E_a > 10 kcal/mol), indicating that greater water movement will occur through the lipid bilayers at higher temperatures when the lipid mobilities are increased (19).

**Membrane Water Channels**

Recognition of the existence of aqueous pores in the plasma membranes of red blood cells (RBC) emerged...
from the biophysical studies of Paganelli and Solomon (42) and Sidel and Solomon (51) in the 1950s. Channel-mediated water transport permits movement of water in the direction of greater osmolality, a recognized feature of RBC and a subset of epithelia (20).

The $P_1$ was measured spectroscopically under the driving force of osmotic gradients and was believed to represent channel-mediated water transport (53). The magnitude of channel-mediated water movement is large; therefore the ratio of $P_1/P_d >> 1$ (11). The pace of water transport is often so fast that the net fluid absorption appears to be isosmotically (6). Membrane water channels were first shown to be inhibited by mercurial sulphydryl reagents including HgCl$_2$ and p-chloromercuribenzenesulfonate (pCMBS) by Macey and Farmer (36). Unlike diffusional water permeability, channel-mediated osmotic water permeability is characterized by a relatively low Arrhenius activation energy that is close to the rate of diffusion of water in bulk solution ($E_a < 5$ kcal/mol). The pathway is relatively unaffected by reductions of membrane lipid fluidity at subphysiological temperatures (63).

Most membrane water channels are thought to be constitutively activated; apparently no gating or other regulation is needed for water to permeate the channels in the direction of an osmotic gradient. This includes the water channels in membranes of RBC and in the apical and basolateral membranes of renal proximal tubules and descending thin limbs of Henle’s loop where 80–90% of the glomerular filtrate is reabsorbed, a volume approaching 200 liters/day for an adult human (33).

The water permeability of renal collecting ducts and amphibian urinary bladder is controlled by vasopressin (antidiuretic hormone) which is released from the neurohypophysis in response to osmotic stimuli. Circulating vasopressin associates with specific membrane receptors in renal collecting duct epithelia and raises adenosine 3′,5′-cyclic monophosphate (cAMP) levels. On the basis of studies of toad urinary bladder and later of collecting duct, vasopressin-mediated regulation of apical membrane water permeability was shown to involve endocytosis and exocytosis of water channels located in the apical membrane and intracellular compartments. This process, proposed by Wade et al. (64) as the “membrane-shuttle mechanism,” was based on evidence generated by their laboratory and others (10, 12).

The first known molecular water channel was recently identified, the channel-forming integral membrane protein CHIP, a 28-kDa integral membrane protein from RBC and renal tubules (16, 45, 46, 52). At least two related proteins from mammalian and plant tissues were subsequently shown to function as water-selective pores, and this group of molecular water channels is now referred to as the “aquaporins,” abbreviated “AQP” (4). This review will concentrate upon CHIP, since it is the archetypal molecular water channel.

Search for Molecular Identity of a Water Channel

Candidate water channel proteins. Several attempts to isolate a molecular water channel have been made. These were fraught with difficulty because of the confounding existence of high baseline diffusional water permeability and the lack of specific pharmacological inhibitors needed for unambiguous, covalent labeling of water channel proteins (14, 26, 62). Over the years several candidate water channels were proposed.

Solomon et al. (54) postulated that the band 3 anion exchanger (AE1) of RBC functions as an aqueous pore permeated by water and urea. Evidence supporting this theory included the large number of copies of band 3 ($10^5$ copies/RBC) and the existence of a labile sulphydryl group in band 3 that is covalently modified by pCMBS, an organomercurial inhibitor of water channels. Inconsistencies in the band 3-water pore hypothesis were subsequently noted, including discordant inhibition of anion transport by stilbene analogues and water transport by organomercurials (36). More recent studies using the Xenopus oocyte expression system also failed to demonstrate concordance of water transport and anion transport (69).

Certain known membrane transporters have been shown to conduct some water in addition to their primary substrates. The sodium-independent glucose carrier (GLUT1) was demonstrated to permit water transport in addition to glucose by Fischbarg et al. (21, 22); GLUT1 was therefore proposed as the membrane water channel. These observations were confirmed by other laboratories, although it is now agreed that GLUT1 is not the classically defined water channel with low $E_a$ and mercurial sensitivity (15, 67, 69). Expression of the cystic fibrosis transmembrane regulator (CFTR) in Xenopus oocytes was shown by Hasegawa et al. (27) to confer a slight rise in membrane water permeability in addition to increased permeability for other ions and molecules when activated with cAMP. Estimates based on indirect observations suggested the unit water permeability may be large; however, these studies await confirmation with direct measurement of CFTR-mediated water transport. Therefore, the potential biological importance of GLUT1- and CFTR-mediated water movement remains to be explained.

A more direct approach to the identification of the molecular water channel was taken by Harris et al. (26) who isolated integral membrane proteins from intracellular vesicles of toad bladder epithelium and from the apical plasma membranes after treatment with vasopressin. These vesicles contain several integral membrane proteins of which 55- and 53-kDa proteins are the most abundant. The functions of these proteins are not yet established, but roles in the membrane targeting or function of vasopressin-regulated water channels have been proposed.

Another direct approach was taken by Tsai et al. (56) and Zhang et al. (71), who attempted to isolate water channel cDNAs by expression cloning. mRNA was isolated from water-permeable tissues and injected into Xenopus laevis oocytes for osmotic swelling measurements. When transcripts from reticulocytes and kidney were expressed, the oocytes developed a significant increase in osmotic water permeability that was sensitive to mercurial inhibition (56, 71). Osmotic water permeability was not increased in control-injected oocytes or oocytes injected with mRNA from non-water-permeable tissues. Although logical, the most promising cDNA isolated by this approach encoded a protein that conferred the oocytes with only a modest increase in water permeability and that lacked mercurial inhibition (70).
Meanwhile, van Hoek et al. used target analysis after radiation inactivation to estimate the molecular mass of water channels. Renal brush-border membrane vesicles (58) and RBC membranes (59) were subjected to electron pulses with other membrane proteins serving as calibration standards. These studies further enhanced the prevailing view that water channels are membrane proteins and provided the first evidence of a discrete functional size of $30 \pm 3$ kDa.

A Novel 28-kDa Integral Protein

The water channel protein of RBC, renal proximal tubules, and other tissues was initially isolated as a byproduct of another purification. A 28-kDa protein copurified with the 32-kDa subunit of the human RBC Rh polypeptides (3, 49). Although first regarded as a proteolytic breakdown product of the Rh polypeptide, antisera raised against CHIPs (4) enabled visualization of a distinct 28-kDa protein on immunoblots with only a discrete protein of 28 kDa and a diffuse band of 32 kDa. A 28-kDa protein copurified and provided the first evidence of a discrete functional protein of ~40-60 kDa. The 28-kDa protein failed to stain with Coomassie blue and did not correspond to any known membrane protein. Curiosity was enhanced by the apparent abundance of the protein (~150,000 copies of 28-kDa protein per RBC) and by development of a simple purification scheme that used extraction of membrane vesicles with N-lauroylsarcosine (16, 52).

Initial characterization revealed that this is an integral membrane protein, existing in membrane vesicles extracted of all peripheral proteins. The 40- to 60-kDa protein represented a similar or identical 28-kDa core protein with a complex carbohydrate attached by asparagine linkage. A highly related or identical protein was also isolated from renal proximal convoluted tubules and descending thin limbs of Henle's loop. It was speculated that the protein functions as a transporter or provides a secondary linkage of the membrane skeleton to the lipid bilayer (16).

Biochemical characterizations. Further studies of the 28-kDa protein suggested that it is a membrane channel (52). The protein received the descriptive name of CHIP28 for "channel-forming integral protein of 28 kDa" (45) and was rechristened "aquaporin CHIP" to conform with a group of structurally and functionally related homologues (4).

CHIP was shown to be distinct from the Rh polypeptides, and the NH$_2$-terminal amino acid sequence was determined for the first 35 residues of CHIP purified from human RBC (52). The apparent linkage of CHIP to the membrane skeleton was found to reflect an inherent lack of solubility of the protein in Triton X-100, which could be overcome at higher concentrations of detergent, rather than a high-affinity linkage with the membrane skeleton (52). Selective proteolytic digestions and immunoblotting with antibodies specific for the 4-kDa COOH terminal domain of CHIP indicated that the COOH terminus is located intracellularly. Despite initial confusion that resulted from a contaminating antibody, CHIP contains no known extracellular proteolytic cleavage sites.

CHIP exists as a tetramer within the native membrane (52). Several studies including lectin affinity chromatography, glutaraldehyde cross-linking, and gel filtration yielded evidence of oligomerization. Velocity sedimentation of the pure CHIP or detergent solubilized membranes in H$_2$O or deuterated H$_2$O revealed the protein to be a noncovalently associated homotetramer, probably comprised of three nonglycosylated subunits and one bearing a large polylactosaminoglycan which may be removed by N-glycanase (Table 1; Fig. 1). The biological explanation for this apparent nonstoichiometric glycosylation of CHIP subunits remains to be established, but this feature of the protein is also found in nonerythroid tissues and in RBC from other mammalian species (40).

Isolation of aquaporin CHIP cDNA. The original cloning of the CHIP cDNA was achieved by use of a polymerase chain reaction (PCR)-based strategy, since antibody screening of expression libraries failed to provide an insert with recognized features of the CHIP protein (45). The NH$_2$-terminal amino acid sequence obtained from the purified CHIP protein permitted design of degenerate oligonucleotide primers for amplification of erythroid cDNA templates from a human fetal liver cDNA

\begin{table}
\centering
\caption{Physical properties of aquaporin CHIP protein}
\begin{tabular}{|l|l|}
\hline
Subunits, apparent mol mass & 28 kDa (SDS-PAGE) \\
GlyCHIP (N glycosylated) & 40-60 kDa (SDS PAGE) \\
CHIP-glyCHIP & \sim 3:1 \\
CHIP membrane oligomer & \\
Stokes radius & 61 Å \\
Sedimentation coefficient & 5.7 S \\
Partial specific volume & 0.795 ml/g \\
Triton X-100 bound & 0.41 mg/mg protein \\
Calculated mass of detergent-protein complex & 190 kDa \\
Calculated mass of protein & 135 kDa \\
Fraction of total membrane protein & \\
Human red blood cells & 2.4% \\
Rat renal cortex & \\
Total membranes & 0.9% \\
BBMV & 3.8% \\
\hline
\end{tabular}
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{CHIP.png}
\caption{Schematic model representing CHIP integral membrane protein within the membrane lipid bilayer. Notable features include 1) homotetrameric complex with 1 subunit bearing a polylactosaminoglycan, 2) minimal polypeptide mass extending above or below the lipid bilayer, and 3) possible individual water pore within each subunit.}
\end{figure}
library. Size-selected amplification products were subcloned, sequenced, and used to design nondegenerate oligonucleotide primers that were used together with lambda primers to amplify by PCR the 5' and 3' ends of the cDNA. These products were then used to isolate a nearly full-length 2.9-kb cDNA from a human bone marrow library. Northern blots of RNA from human bone marrow, mouse spleen, and kidney contained single major transcripts of 3 kb.

Analysis of the cDNAs confirmed their authenticity (45). The sequences of the PCR products from the 5' end of the cDNA contained an initiating methionine with a strong translational initiation consensus prior to the deduced amino acid sequence corresponding exactly to the NH2-terminus of the protein. An 807-bp open reading frame encoding a protein of 28.5 kDa was followed by ~2 kb of 3' untranslated sequence ending with a polyadenylation signal. Authenticity of the 3' end of the coding sequence was confirmed by expression in bacteria and immunoblot analysis with an antibody specific for the COOII-terminal 4 kDa of CHIP. Hydropathy analysis of the deduced amino acid sequence revealed six potential bilayer-spanning domains and five connecting loops. Loops B and E are significantly hydrophobic, and loops A and E contain potential N-glycosylation sites (Fig. 2). The lack of a hydrophobic leader peptide, distribution of charged amino acids, and immunoelectron microscopic studies (41) all indicate that the NH2 terminus is intracellular (Fig. 2, bottom). The cDNAs encoding the mouse (34) and the rat homologues (13) were subsequently identified and are ~94% identical to human CHIP.

Homology with the major intrinsic protein of mammalian lens, MIP26. The full-length coding sequence firmly established the homology with the family of proteins related to MIP26, the major intrinsic protein of lens (24). When their NH2 terminals are aligned, CHIP and MIP26 are 42% identical overall, with the greatest levels of homology existing in loops B and E, which are > 60% identical (Fig. 2, bottom). The cDNAs encoding several sequence-related proteins of 26-29 kDa had been isolated from diverse organisms including mammals, insects, plants, and microbes (43). Lesser homologies were found between CHIP and the other MIP26 homologues. Although the functions of most of these proteins are unknown, it is thought that they form membrane channels.

An internal homology of the NH2-terminal half and the COOH-terminal half of the MIP26 molecule was identified (43, 66). CHIP and the other members of this group of proteins have a three amino acid motif, Asn-Pro-Ala (NPA) located at corresponding points in loops B and E of the tandem repeats. The proposed orientation of the tandem repeats is unprecedented, since the first and second repeats would be oriented at 180° angular to each other (Fig. 2, middle). Initial topographical studies suggest that the proposed topology is correct.

The secondary structure of MIP26 was previously characterized by near and far ultraviolet circular dichroism of octylglucoside-solubilized MIP26 and detergent-free membrane fragments (31); it was concluded that the α-helix content is ~50%, and the β-sheet content is ~20%, and this proportion was not altered when the COOH-terminal 5-kDa domain was proteolytically removed. Preliminary studies of CHIP revealed a similar composition (61). Although apparently not glycosylated,

![Diagram of membrane topology of CHIP](http://ajprenal.physiology.org/)
the hydrodynamic behavior of the homologous protein MIP26 had previously been characterized and was also found to exist as a tetramer (2). It is assumed that the membrane topologies and three-dimensional structures of most of the related proteins will be similar, and ultimate crystallization of one member of this group of proteins may provide structural predictions for all.

The aquaporin CHIP structural gene. CHIP transcripts of 3.1 kb are expressed in most tissues (13, 34, 45). Lung and small intestine also contain transcripts of 4.2 kb, whereas skeletal muscle contains only a 1.4-kb transcript (39). Because of the possibility of multiple CHIP genes, cDNAs were isolated from human kidney libraries by nonamplification methods, and the nucleotide sequences were identical to that of erythroid CHIP.

The structural CHIP gene was isolated from a human genomic library (39). The 17-kb DNA contains four exons and a TATA consensus sequence in the 5' untranslated region. The 1st exon corresponds to the NH2-terminal half of the molecule and is followed by a 9.6-kb intron. The COOH-terminal half of the molecule is encoded by the 2nd-4th exons, which are separated by small introns. Only a single CHIP gene was identified by genomic Southern analyses (39). The locations of the intron-exon boundaries are identical to the MIP26 gene (44), confirming evolutionary divergence of these genes. The human CHIP locus is on chromosome 7 at p14 (39). This location in the human genome is not coincident with any likely disease phenotypes. It remains to be established whether homozygous expression of mutant forms of CHIP would be lethal to embryos or whether tissue-specific mutations or acquired defects may exist.

The existence of a single CHIP gene (39) and the published sequences of murine CHIP cDNAs (13, 34) conflict with the sequence reported for a putative rat kidney homologue referred to as "CHIP28k," which apparently corresponds to the rat CHIP homologue with human CHIP cDNA at the 5' end of the coding sequence (72).

Aquaporin CHIP Transports Water

Several clues together strongly suggested that the CHIP protein is a molecular water channel. The total number of copies of glycosylated and nonglycosylated subunits, ~2 x 10^5 copies/RBC (16, 52), approaches the predicted number of water channels, 2.7 x 10^5 (54). The 28.5-kDa subunit size (45) is in close agreement with the predicted size determined by radiation inactivation of native water channels (58, 59). The distribution in RBC and renal proximal tubular epithelia (16) are consistent with the known distribution of constitutively active water channels but not the vasopressin-regulated water channels (33). Like water channels in native membranes (8), CHIP resists proteolytic degradation while within intact membranes. Most surprisingly, expression of one homologue of the MIP26 protein family was induced in the roots of pea plants grown under drought conditions (25).

CHIP-mediated water channel activity was first demonstrated with the X. laevis oocyte expression system. This system had previously been used in the search for molecular water channels (21, 71), because of the low diffusional water permeability exhibited by the oocytes that survive in freshwater ponds. An expression construct was made by inserting the CHIP coding sequence between 5' and 3' untranslated sequences from X. laevis β-globin cDNA. In vitro transcribed RNA in 50 nl of water was microinjected into defolliculated oocytes; control oocytes were injected with 50 nl of water without RNA. CHIP protein expression was assessed by immunoblot and found to be maximum after 72 h of incubation (46). The Pf was determined by transferring the oocytes from 200 to 70 mosmol/kgH2O Barth's buffer with measurement of oocyte swelling by video microscopy. Oocytes expressing CHIP invariably ruptured after increasing in volume by 30-50% (Fig. 3A). On-line computer integrations of surface tracings were generated throughout the swelling experiments and permitted determination of Pf (Fig. 3B).

Several experiments indicated that CHIP expressed in oocytes behaved in a manner characteristic of the water channel in native membranes (46). When measured at 22°C, the CHIP oocytes exhibited an increase in Pf (>200
was coexpressed in oocytes along with wild-type CHIP, van Hoek et al. (58) predicted that each CHIP subunit trafficking (47).

Tryptophan, unpublished observations). Moreover, two-electrode voltage-clamp experiments failed to detect an increase in conductance in the CHIP oocytes until the point of membrane rupture during swelling experiments (46).

CHIP permeability is selective for water (46). When placed in an osmotic gradient, oocytes expressing CHIP absorbed $^3$H$_2$O, but failed to absorb urea or glucose (Preston, unpublished observations). Moreover, two-electrode voltage-clamp experiments failed to detect an increase in conductance in the CHIP oocytes until the point of membrane rupture during swelling experiments (46).

Structure of Water Pathway

Site-directed mutagenesis. The X. lacvis oocyte system has been employed to probe the functional importance of domains within the CHIP structure by site-directed mutagenesis. The known mercury sensitivity was evaluated by individually replacing each of the four cysteine residues with a serine, a residue with similar structure (47). Each of the mutagenized proteins functioned like the wild-type CHIP in the oocyte swelling assay (Fig. 4A).

When the assay was performed after incubation in HgCl$_2$, only C189S was insensitive to the inhibitor at all concentrations tested (0.1-3 mM). When other amino acid substitutions were made at residue 189, the $P_f$ of the oocytes coincided inversely with the size of the residue (Fig. 4B). Replacement of residue 189 with an alanine (smaller than cysteine) conferred wild-type $P_f$, whereas replacement with a valine (larger than cysteine) caused a reduction in $P_f$. Replacement of residue 189 with still larger residues, methionine, tryptophan, or tyrosine, reduced the $P_f$ to the level of control oocytes. It was initially thought that the greater size of the amino acids substituted at residue 189 may have produced simple occlusion of the channel pore, but it was demonstrated that the functionally inactive CHIP mutants had incompletely formed high-mannose glycans, which may have led to altered membrane trafficking (47).

It is unknown whether the individual subunits within the CHIP tetramer each have an internal pore, analogous to the bacterial porins, or whether the tetrameric complex forms a single channel, analogous to the Shaker potassium channels (32). The radiation inactivation data of van Hoek et al. (58, 59) predicted that each CHIP subunit will function as an individual water pore. Coexpression of any of several distinct functionally inactive mutants with wild-type CHIP demonstrated no diminution of the $P_f$. This lack of "squelching" suggested a lack of functional cooperativity among the subunits when coexisting in mixed tetramers. The mercury insensitive mutant C189S was coexpressed in oocytes along with wild-type CHIP, and their activities approached the anticipated sum of these proteins when measured without mercury, but the activity was reduced to the level of the mercury-insensitive mutant alone when measured after incubation in 3 mM HgCl$_2$ (47). These observations support the hypothesis that individual subunits each bear a water pore, raising the question of why the protein exists exclusively as a tetrameric oligomer.

Reconstitution of purified aquaporin CHIP into proteoliposomes. Although the results were clear, the oocyte studies could not rule out the existence of other water channels within the CHIP tetramer. Therefore highly purified CHIP protein was reconstituted into proteoliposomes for direct demonstration of osmotic water permeability (68). This was made possible by the remarkably simple purification system that had already been developed (52), the abundance of CHIP in human RBC that are available in liter quantities, and the development of efficient reconstitution systems (5). Pure CHIP protein in octyl glucoside and purified Escherichia coli phospholipid (70% phosphatidylethanolamine, 15% phosphatidylglycerol, 15% cardiolipin) were rapidly diluted in the presence of carboxyfluorescein. The
spontaneously forming proteoliposomes were purified by ultracentrifugation (Fig. 5A). Control liposomes were prepared identically, except CHIP protein was omitted.

Measurements of osmotic water permeability of the CHIP proteoliposomes and control liposomes were performed by abruptly increasing the osmolality by 20% while measuring the self-quenching of carboxyfluorescein by stop-flow fluorometry (68). CHIP proteoliposomes and control liposomes both behaved as perfect osmometers, but the tracings demonstrated that equilibrium was reached up to 50-fold faster by the CHIP proteoliposomes (Fig. 5, B and C). The size of the proteoliposomes (radius = 70 nm) and average number of CHIP subunits (220 subunits/proteoliposome) permitted calculation of the unit water permeability (Table 2). Extrapolation to the CHIP-mediated water permeability of an individual RBC, 0.017 cm/s, was close to the known RBC water permeability (20). Determination of $P_f$ over a range of temperatures and in the presence of mercurial inhibitors provided estimated Arrhenius activation energies that indicated water transport in the CHIP proteoliposomes was channel mediated, whereas that of the control liposomes represented diffusional water permeability (Table 2).

The reconstitutions revealed several other features of CHIP. Specialized lipids are not required for channel function (68). The CHIP-mediated water permeability was highly selective for water and did not transport protons or urea. The actual pore within the protein must be both extremely narrow and contain structural mechanisms to prevent movement of protons that would otherwise be expected to jump along a continuous column of water in the form of hydronium ions ($\text{H}_3\text{O}^+$). Other investigators have qualitatively confirmed these observations by light scattering measurements with membrane vesicles enriched in CHIP by $N$-lauroylsarcosine extraction and with proteoliposomes reconstituted with partially purified CHIP (60). Together, the osmotic water permeability of the CHIP proteoliposomes studies reported by all investigators indicate that CHIP behaves identically to the water channels in native membranes.

**Other Aquaporins**

Since the discovery that CHIP conferred oocytes with increased osmotic water permeability, at least two other sequence-related proteins were also found to increase $P_f$ (Table 3). These proteins are a subset of the large family of membrane proteins related to MIP26 found in diverse organisms (43). To facilitate recognition of their function as water-selective pores and promote better communication among scientists, the name “aquaporins” was selected (4). Preliminary evidence from other laboratories suggests that several other sequence-related proteins are also functional water channels.

**Collecting duct aquaporin (WCH-CD).** A cDNA most likely corresponding to the vasopressin-regulated water channel was isolated by Fushimi et al. (23) from a rat kidney library by PCR amplification using degenerate oligonucleotide primers corresponding to the cDNAs of...
CHIP and MIP26. Expression of the protein conferred oocytes with increased osmotic water permeability, and the protein was localized to the apical membrane of renal collecting duct cells (hence, "WCH-CD"). Moreover, thirsting of rats enhanced expression of the WCH-CD channel in renal cortex and medulla but is not the predominant water channel in papilla.

CHIP was immunolocalized to water-permeable segments of rat nephron (Fig. 7). A polyclonal antibody was raised in rabbits to a synthetic peptide corresponding to the NH\textsubscript{2} terminus, and another was raised to the whole CHIP protein, although reactivity was restricted to the 4-kDa cytoplasmic domain (52). These antibodies were affinity purified and used in detailed immunohistochemical and immunoelectron microscopic localizations of CHIP (41). Except for the initial portion of the S1 segment, abundant immunostaining of CHIP was observed over the apical brush border and basolateral membranes of renal proximal convoluted and straight tubules (Figs. 7 and 8). The CHIP immunostaining appeared less intense over the lateral and basal membranes and more intense over the apical brush border where CHIP comprises the apical membrane. Moreover, MIP26 does not confer significantly increased Pf in the oocyte system (Preston, unpublished observations). It therefore cannot presently be resolved whether the failure of certain homologues to confer increased Pf results from problems in the expression system or whether the proteins do indeed have different functions.

Phylogenetic comparisons of the deduced amino acid sequences (Fig. 6) indicated that CHIP is intermediate between the plant proteins and WCH-CD, which is restricted to the renal collecting duct (23). Moreover, CHIP is widely distributed in many mammalian tissues (see below). These observations suggest that CHIP may be a functionally less specialized water channel that may be evolutionarily more primitive.

**Distribution and Expression**

**Aquaporin CHIP in kidney.** The original report describing CHIP in RBC also reported the identification and isolation of a highly related protein from kidney where it appeared in proximal tubules and descending thin limbs (16). Renal CHIP was subsequently shown to be the same gene product as erythroid CHIP (39). Moreover, injection of oocytes with mRNA from kidney is known to increase the Pf (71). The increased Pf obtained from cortical or medullary mRNA was neutralized when mixed with CHIP antisense oligonucleotides; the increased Pf from papillary mRNA was not neutralized (17, 72). These studies indicate that CHIP is a major water channel in renal cortex and medulla but is not the predominant water channel in papilla.

CHIP and MIP26. Expression of the protein conferred oocytes with increased osmotic water permeability, and the protein was localized to the apical membrane of renal collecting duct cells (hence, "WCH-CD"). Moreover, thirsting of rats enhanced expression of the WCH-CD channel in renal cortex and medulla but is not the predominant water channel in papilla.

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EDITORIAL REVIEW

Fig. 7. Diagram of an individual long-looped nephron (A) and summary of osmotic water permeabilities of indicated segments in rodent nephrons from multiple physiological studies (B). Sites of immunolocalization of CHIP are indicated. ADH, antidiuretic hormone. [Reprinted from Nielsen et al. (41) by copyright permission from the Rockefeller Univ. Press.]

Paracellular water permeability may also occur (20, 55, 65), but the abundance, distribution, and unit water permeability of CHIP strongly suggest that it is the major or exclusive constitutively activated water channel of renal proximal tubules and thin descending limbs ofHenle’s loop. Therefore, CHIP provides a molecular explanation for transcellular water flow that is known to occur at these locations.

Cortical, medullary, and papillary collecting ducts have low basal water permeabilities, but stimulation with vasopressin produces a large increase in water permeability (33). Collecting tubule epithelia from control and thirsted rats were not immunolabeled with affinity-purified antibodies specific for CHIP (Fig. 8) (41). The cDNA encoding WCH-CD, the probable vasopressin-regulated water channel, was recently isolated and is 42% identical to CHIP (23). The immunogenic COOH-terminal domains of these proteins share only 6 of 36 residues (23, 45), explaining the lack of cross-reactivity. Taken together, these studies indicate that CHIP is neither the vasopressin-regulated water channel nor the basolateral water channel of renal collecting ducts.

Aquaporin CHIP in other tissues. Water-permeable epithelia exist in several tissues in addition to kidney. Although kidneys function to conserve water by reabsorption of glomerular fluid, several other tissues function to secrete water during generation of cerebrospinal fluid,

nearly 4% of the total membrane protein. The functional significance of this apparent imbalance is uncertain. Heavy, uninterrupted CHIP immunostaining was also observed over apical and basolateral membranes of descending thin limbs of both short- and long-loops of Henle (Figs. 7 and 8). Very little CHIP immunolabeling was observed over intracellular vesicles. The ascending thin limbs, thick ascending limbs, and distal tubules are known to exhibit negligible transepithelial water permeability and these structures were also not immunolabeled. These studies were supported by those of Sabolic et al. (48), who performed immunofluorescence localizations with whole antiserum raised to CHIP protein. Although of lower resolution, in situ hybridizations with CHIP antisense probes were interpreted similarly (9, 28, 72).

For transcellular water flow that is known to occur at these locations.

Cortical, medullary, and papillary collecting ducts have low basal water permeabilities, but stimulation with vasopressin produces a large increase in water permeability (33). Collecting tubule epithelia from control and thirsted rats were not immunolabeled with affinity-purified antibodies specific for CHIP (Fig. 8) (41). The cDNA encoding WCH-CD, the probable vasopressin-regulated water channel, was recently isolated and is 42% identical to CHIP (23). The immunogenic COOH-terminal domains of these proteins share only 6 of 36 residues (23, 45), explaining the lack of cross-reactivity. Taken together, these studies indicate that CHIP is neither the vasopressin-regulated water channel nor the basolateral water channel of renal collecting ducts.

Aquaporin CHIP in other tissues. Water-permeable epithelia exist in several tissues in addition to kidney. Although kidneys function to conserve water by reabsorption of glomerular fluid, several other tissues function to secrete water during generation of cerebrospinal fluid,
aqueous humor, saliva, sweat, bile, semen, and pulmonary secretions. The existence of water channels has been predicted, although the physiological studies are less well developed, in tissues other than kidney. It is not known whether a single set of channels could explain these diverse processes, since water moves in one direction during absorption and in the opposite direction during secretion. The tandem repeats within CHIP (Fig. 2, middle) suggest that it may function in either direction (46). Northern analyses demonstrated that it is widely expressed (13, 34, 41).
45). Therefore CHIP may be a general water channel.

The affinity-purified antibody specific for the COOH-terminal cytoplasmic domain of CHIP was used to screen several tissues (40). CHIP was detected by immunoblot in most tissues. Therefore immunohistochemical and immunoelectron microscopic analyses of several tissues were undertaken (Table 3). Only one structure in the central nervous system was found to contain immunoreactive CHIP; the apical microvillar surface of the choroid plexus contains abundant CHIP, implying a role in secretion of the aqueous component of cerebrospinal fluid. Several structures in the anterior chamber of the eye contained immunoreactive CHIP in both apical and basolateral membranes. The ciliary epithelium is rich in CHIP and is the site of aqueous humor secretion. The corneal endothelium is known to contain functional water channels and also contains abundant CHIP protein. CHIP is present in lens epithelium, whereas lens fiber cells lack CHIP but are rich in MIP26. Eccrine sweat gland epithelia are also abundant in CHIP (G. Anhalt and P. Agre, unpublished observations). The endothelia of nonfenestrated capillaries and venules at many sites throughout the body are also rich in CHIP, including the endothelium of descending vasa recta (S. Nielsen and A. B. Maunsbach, unpublished observations). This suggests that movement of water through these water channels will balance the hydrostatic and oncotic pressures which control interstitial fluid and vascular volumes. In contrast, CHIP was not observed in fenestrated capillaries.

CHIP has an interesting distribution within the gastrointestinal tract and lung (40). CHIP is present in apical and basolateral membranes of epithelium in hepatic bile ductules and lining the gall bladder neck. There it may play a role in secretion and concentration of bile. Although the existence of CHIP RNA was reported in colonic crypts by in situ hybridization (28), immunoreactive CHIP does not exist in intestinal mucosa (40). The lack of CHIP in gastrointestinal epithelium explains the characteristic barrier to water absorption of these structures (57). It is probable that most intestinal water uptake is paracellular or diffusionally mediated (55). CHIP in lymphatics and submucosal capillaries may promote rapid movement of absorbed water into lymph and the vascular bed. The presence of abundant CHIP in alveolar capillary endothelia may be needed for humidification of airways; however, this may also explain the rapid and direct uptake of water into the vascular space during a freshwater drowning. The lack of CHIP in certain water-permeable epithelia such as mammary, lacrimal, and salivary glands, as well as the initial segment (S1) renal proximal tubules, suggests that other water channels may exist at those locations.

Aquaporin CHIP during development. During in situ hybridization screening of diverse tissues, it was surprising to find a divergent pattern of expression in development (9). Fetal rats have no detectable CHIP transcripts in liver or spleen, the fetal erythroid tissues (Fig. 9). Fetal rat RBC did not contain detectable CHIP by immunoblot, although the presence of other known membrane proteins was clear. Expression of CHIP is also very low in fetal kidney (Fig. 9). These patterns were found to change rapidly after birth when fetal RBC were replaced by RBC containing immunoreactive CHIP. There appears to be no fetal water channel isoform, since the water permeability of fetal RBC is low and the Arrhenius activation energy is high, suggesting that only diffusional water permeability is present (52a). Rat kidneys express CHIP protein in the first few weeks of postnatal life, coincident with the appearance of the ability to concentrate. The coincident expression of CHIP in RBC and kidneys suggests that the protein plays an adaptive role in RBC, presumably by providing them with a mechanism to rapidly rehydrate after traversing the hyperosmotic renal medulla (37). The total lack of CHIP in rat RBC before birth demonstrates that the protein is not needed for oxygen transport or circulation throughout the vascular bed (9, 52a).

Two other developmental patterns of expression were also noted (9). CHIP mRNA is constitutively highly expressed in fetal and adult rat choroid plexus, whereas the transcript was abundant in certain other fetal tissues where expression was downregulated after birth. The mesenchyme surrounding developing bone and the developing endocardium have large amounts of CHIP mRNA before birth (Fig. 9), but not in mature rats. Likewise, fetal corneal endothelia have abundant CHIP transcripts at the point in development when the tissue becomes transparent, a desiccation process thought to be mediated by CHIP. Adult corneas have lower levels of CHIP mRNA (9) and CHIP protein (40). This transient expression is reminiscent of the expression noted by Lanahan et al. (34), who identified CHIP among delayed early response genes that were transiently expressed by fibroblasts after stimulation with selected growth factors, but the physiological importance of transiently expressing CHIP protein remains unknown. All of the distributional and developmental studies underscore the hypothesis that CHIP plays a major role in transcellular water movement at numerous sites within the body.

Future Directions

Three aquaporins have been rigorously defined so far, and it is likely that many more remain to be discovered. Use of degenerate PCR oligonucleotide primers corresponding to CHIP and MIP26 nucleotide sequences is an approach that has already led to the isolation of the cDNA for WCH-CD from the renal collecting duct (23). This approach may yield other homologues, but demonstration of the function of newly identified cDNAs will be essential, since it is likely that only a subset may be water channels.

The unusual patterns of expression during development and after stimulation with selected growth factors remain to be explained at a molecular level. It is likely that specific cis regulatory sequences will be identified that lead to the expression of certain aquaporins. The transcriptional factors responsible for these events remain to be identified.

The structure of CHIP and related proteins is still poorly understood. Water rapidly permeates the channel, but protons are not conducted. This feature is consistent with the physiological need for the kidney to conserve water while excreting acid, but the structural mechanisms by which this is accomplished are entirely unknown. Creation of a mutant CHIP that is permeable to protons could permit detailed electrophysiological studies of the
channel that are not feasible with the oocyte swelling assay. The existing evidence suggests that the aquaporins have a novel structure with tandem repeats oriented 180° to each other. The three-dimensional structure remains to be established, as does the structure of the water pathway within the CHIP protein. Such information could provide structural information needed for the design of inhibitors which may be tested in either the oocyte or proteoliposome water permeability assays.

Several clinical settings exist in which therapeutic benefits may potentially be achieved by modulation of the CHIP. 1) Inhibitors of proximal nephron water reabsorption or choroid plexus secretion may be useful in congestive heart failure, renal failure, or acute hydrocephalus. 2) Topical inhibitors of aqueous humor secretion could be helpful in managing glaucoma. 3) Inducers of eccrine sweat gland water flow may permit enhanced heat exchange in threatening environmental conditions.
metic reconstitution of damaged lacrimal or salivary glands may restore tearing or saliva in patients suffering from Sjögren's syndrome or after receiving local irradiation. Continued aquaporin research may provide improved understanding and treatment of certain human diseases in addition to contributing molecular insight into the fundamental biological phenomenon of osmotic water movement.

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