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WATER CHANNELS

The aquaporin family of water channels in kidney

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The aquaporin family of water channels in kidney. The longstanding puzzle of membrane water permeability was advanced by the discovery of channel-forming integral protein (CHIP). This protein was shown to function as a water channel when expressed in *Xenopus* oocytes or when reconstituted into synthetic membranes. Site-directed mutagenesis and electron crystallography reveal tetrameric organization of CHIP, and the two halves of CHIP are tandem repeats folded into an obversely symmetric structure which resembles an hourglass. Each tetramer is comprised of functionally independent subunits. CHIP is the archetypal member of a newly-recognized family of membrane water transporters known as the "Aquaporins" (AQPs). AQP1 (CHIP) is abundant in the apical and basolateral membranes of renal proximal tubules and descending thin limbs, and is also present in a number of extra renal tissues. In the collecting duct, AQP2 is the predominant vasopressin-sensitive water channel. AQP2 is loclized in the apical membrane and in intracellular vesicles which are targeted to the apical plasma membranes when stimulated by antidiuretic hormone. Humans are identified with mutations in AQP1 and AQP2 and exhibit contrasting clinical phenotypes. AQP3 resides in the basolateral membranes of collecting duct principal cells providing an exit pathway for water, and AQP4 is abundant in brain, where it apparently functions as the hypothalamic osmoreceptor responsible for secretion of antidiuretic hormone. Continued analysis of the aquaporins is providing detailed molecular insight into the fundamental physiological problems of water balance and water balance disorders.

Existence of membrane water channels

The plasma membranes of all mammalian cells are permeable to water, but the degree of water permeability is remarkably variable between tissues [reviewed by Finkelstein in 1]. Some tissues have only slight water permeability which is thought to represent the diffusion of water molecules through the lipid bilayer; this phenomenon is reduced at lower temperatures where lipid mobility is reduced (high Arrhenius activation energy). Other tissues are highly permeable to water which rapidly crosses the membrane in the direction of higher osmolality. Mammalian red cell, renal proximal tubules, and descending thin limbs of Henle are extraordinarily permeable to water and exhibit coefficients of osmotic water permeability (P_{fs}) in the range of 1000 μm/sec. This is a highly specialized feature of these membranes, since renal ascending thin limbs and thick ascending limbs are negligibly permeable to water, and the apical membranes of principal cells in the renal collecting ducts are also much less permeable to water unless stimulated by the antidiuretic hormone vasopressin [reviewed by Knepper and Rector in 2].

Molecular explanations were sought for the transport processes established in the mammalian kidney by classical micropuncture techniques. Over the past ten years, complementary DNAs (cDNAs) have been isolated for several membrane transporters. The existence of water selective channels in red cells, renal proximal tubules and collecting ducts were predicted to explain the large P_f, the low Arrhenius activation energy, and reversible inhibition by certain mercurial sulfhydryl-reactive agents [reviewed in 3, 4]. Despite considerable efforts, attempts to isolate a water channel protein or clone the cDNA by expression proved frustrating due to the lack of a specific inhibitor, the ubiquity of water, and the high background diffusional water permeability [reviewed in 5, 6].

Discovery of CHIP

Ironically, serendipity provided the critical step in the discovery of the first water channel [reviewed in 7, 8]. In 1988 a novel 28 kD integral membrane protein was identified in red cells and renal proximal tubules, and study of this protein was feasible due to the unusual insolubility of the protein in certain detergents, which made its purification trivial [9]. Similar to channels, this protein is a homotetramer, which resides primarily between the leaflets of the membrane bilayer [10] and was named "CHIP 28" or "CHIP" for "*c*hannel-forming *i*ntegral *p*rotein of *28* kDA" [11]. Presence of a putative channel in water permeable membranes sparked the curiosity of the late John C. Parker of U.N.C. at Chapel Hill who first predicted that CHIP is a water channel.

Demonstration of this function followed the isolation of the complementary cDNA in 1991 [11]. Expression of the complementary RNA in *Xenopus* oocytes conferred the cells with an approximate 20-fold increase in P_f and other physical behavior identical to water channels in native membranes including reversible inhibition by mercurials and low activation energy [12]. This study was directly verified by reconstitution of highly purified CHIP into proteoliposomes which permitted calculation of the unit permeability, pf $\sim 4 \times 10^9$ water molecules per subunit per second, and demonstrated the lack of permeability to urea or protons [13]. Other labs soon followed these leads and confirmed and further defined the observations [14–17].

Molecular structure and function of CHIP

The existence of a readily purified natural protein and expression of the cDNA permitted rapid progress toward the elucidation of the molecular structure [18]. The membrane topology of CHIP (Fig. 1) was first predicted by hydropathy analysis to contain six bilayer spanning domains with intracellular NH_2- and COOH-termini [11]. Internal repeats were previously recognized in

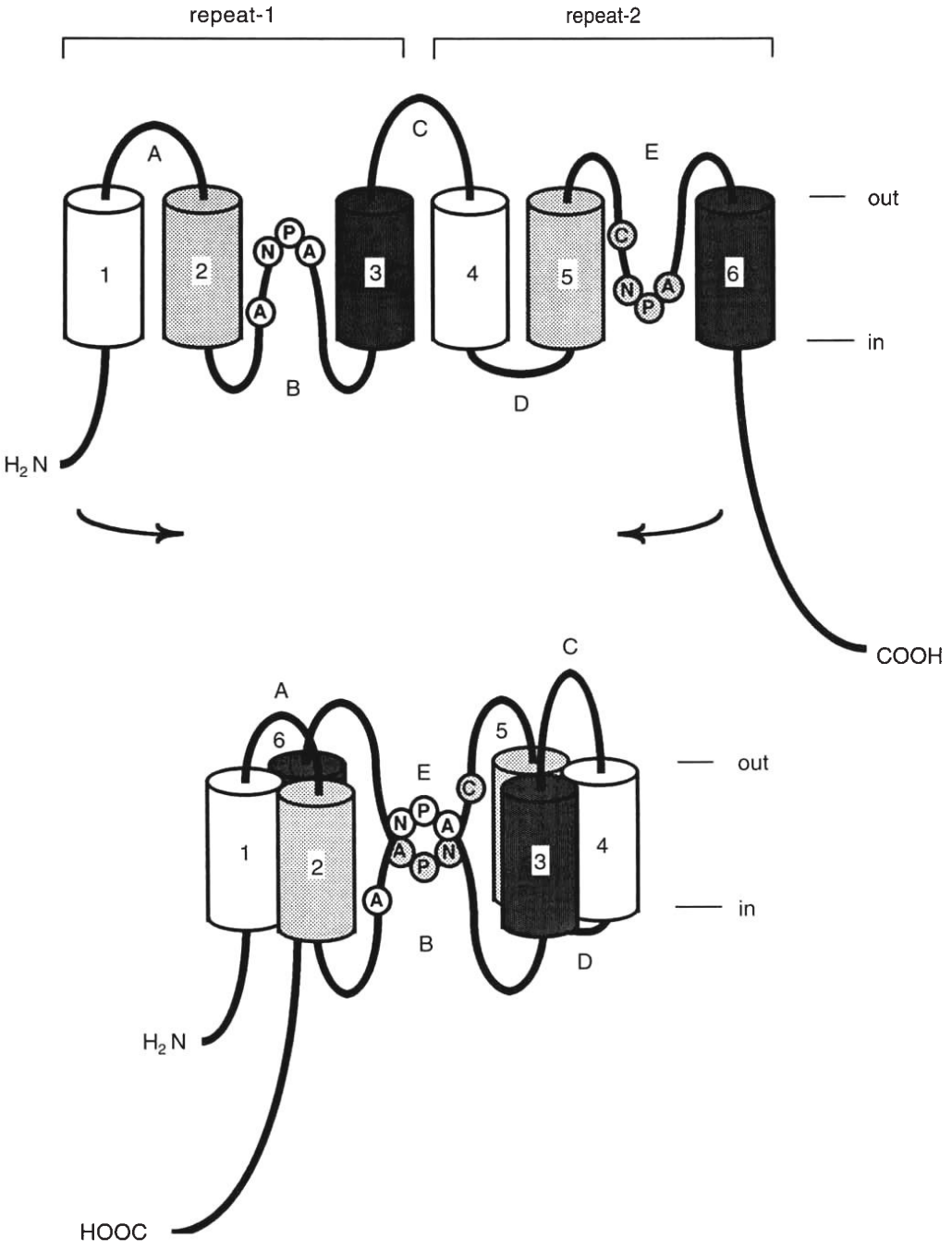

Fig. 1. *Hourglass model of Aquaporin structure.* Original six-bilayer spanning model of a single CHIP subunit highlighting the internal tandem duplication (repeat-1 = bilayer-spanning domains 1, 2 and 3; repeat-2 = bilayer-spanning domains 4, 5 and 6) and selected residues in loops B and E which form a single pathway when juxtaposed.

homologous proteins in which the NH_2- and COOH-terminal halves of the molecule are ~ 20% identical, presumably the result of an ancient gene duplication [19, 20]. The most highly conserved domains in the tandem repeats are two hydrophobic connecting loops, B on the cytoplasmic face and E on the extracellular face, and each contains a structural motif Asn-Pro-Ala (NPA). The mercury sensitivity of CHIP was demonstrated to occur through Cys189 which is adjacent to the second NPA motif [21]. The corresponding position in the first NPA motif when mutagenized to a cysteine (A73C) can also function as a mercury-sensitive site [22]. This topology is consistent with an obverse symmetry for the two halves; the structure was confirmed by construction of biologically-active CHIP recombinants with epitope tags. Recent studies indicate that the loops B and E dip into the lipid bilayer, forming hemichannels which connect between the leaflets of the lipid bilayer to form a single aqueous pathway within a structure deemed "the hourglass model" (Fig. 1) [22].

CHIP is a multisubunit oligomer predicted by sedimentation and filtration studies to be a tetrameric assembly of four identical polypeptide subunits with a large glycan attached to only one [10].

Table 1. Aquaporins relevant to kidney

Genome database designation and symbol (trivial names)	Mechanism of regulation special characteristics	Tissue and membrane distribution	Mutant phenotype
Aquaporin-1 AQP1 (CHIP, CHIP 28)	Constitutively-active Hg-sensitive	Renal proximal tubules descending thin limbs Red cells, multiple tissues Apical and basolateral membranes	Normal
Aquaporin-2 AQP2 (AQP-CD, WCH-CD)	ADH-regulated Hg-sensitive	Renal collecting duct principal cells Apical membrane and intracellular vesicles	Nephrogenic DI
Aquaporin-3 AQP3	Glycerol permeable Hg-sensitive	Renal medullary collecting duct Colon Basolateral membrane	Unknown
Aquaporin-4 AQP4 (MIWC)	Brain osmoreceptor Hg-insensitive	Hypothalamus-supraoptic and paraventricular nuclei Ependymal, granular, and Purkinjie cells	Unknown

Subsequent studies have confirmed this organization. Freeze fracture of CHIP proteoliposomes revealed tetrameric images by rotary shadowing [23] or morphometry [24]. Despite existence as a noncovalently assembled tetramer, the water permeability of CHIP is functionally determined by the individual subunits, consistent with the mass of the water channel activity which was measured by radiation inactivation to be ~ 30 kD [25]. These conclusions were established by functional studies of mutagenized CHIP molecules [22]: (i) the mercury-sensitivity and -resistance of individual subunits were retained when covalently linked mixed-dimers were expressed in oocytes; (ii) full complementation was achieved after coinjection of the cRNA encoding a nonfunctional CHIP molecule with a mutation in the loop E hemichannel and the cRNA of nonfunctional CHIP molecule lacking the COOH-terminal cytoplasmic domain. Curiously, the CHIP protein is comprised of functionally independent subunits but exists exclusively as a tetramer (Fig. 1).

A structural explanation for this paradox emerged when the highly purified CHIP protein was reconstituted into synthetic lipid bilayers at high protein to lipid concentrations. These reconstitutions yielded large 3 μm diameter vesicles comprised of crystalline arrays of the protein, and osmotic water permeability measurements indicated that each CHIP was fully functional in the membrane crystalline state, implying that neither lateral nor rotational mobility are essential for function [26]. Image analysis of the electron diffraction pattern to a resolution of 16 Å revealed tetrameric assemblies surrounding central cavities extending from the extracellular and cytoplasmic faces deep into the molecule [26]. Although still unresolved, it is believed that a thin shelf is created by overlapping loops B and E from each subunit with each containing a narrow aqueous pore at the bottoms of the central depressions, thereby providing an aqueous pathway which is much shorter than the 40 Å width of the lipid bilayer. Tilt studies have revealed an asymmetric three dimensional structure with a flat external surface and protuberant cytoplasmic domain [27]. Thus the CHIP subunit must achieve the vertical symmetry needed for residence within the lipid bilayer by oligomerization, so that the highly asymmetric loop B-loop E shelf may exist inside the center of the tetramer [22].

Aquaporin family of molecular water channel proteins

In addition to CHIP, several cDNAs from diverse animal and plant tissues encode polypeptides which are related to MIP, the major intrinsic protein of lens fiber cells [28]. The function of MIP remains undefined, although it is thought to be a membrane channel [29]; however, expression in oocytes results in only a modest rise in P_f. The internal tandem sequences have permitted isolation of cDNAs for multiple new homologues by polymerase chain amplification using degenerate primers. The cRNAs for several of these homologues have been tested in oocytes and conferred increased osmotic water permeability, similar to CHIP.

The name "Aquaporin" was proposed for water-permeable members of this protein family [30], and the gene encoding CHIP has been designated *Aquaporin-1* (abbreviated as AQP1) by the human genome database. Although the protein may be referred to as AQP1, use of the trivial epithet CHIP will probably persist for some time. Two other Aquaporin cDNAs have been isolated from renal collecting duct libraries. AQP2 encodes AQP2, a polypeptide which was shown to function as a mercurial-sensitive water channel in oocytes [31] and is thought to be regulated by antidiuretic hormone. A third Aquaporin cDNA (AQP3) was also isolated from renal collecting duct and encodes a polypeptide thought to reside in the basolateral membranes [32], thus providing an outflow water channel. A forth Aquaporin cDNA (AQP4) was found to be expressed predominantly in brain where it may play several roles including that of the hypothalamic osmoreceptor [33]. Thus four Aquaporins apparently play distinct but functionally related roles in renal physiology (Table 1).

Distribution and physiology of AQP1

AQP1 in proximal tubule and descending thin limb

Initial studies by Denker et al [9] and detailed immunolocalization studies [34] using affinity purified antibodies to N- and C-terminal regions of AQP1, both revealed that AQP1 is expressed in the proximal tubule and in the descending thin limb (Figs. 2 to 4), known to display very high osmotic water permeability. There is no expression of AQP1 in other nephron segments or in the collecting duct [34]. *In situ* hybridization studies [35, 36] and immunolocalization studies with anti-AQP1-serum [37] confirmed expression in the proximal tubule and thin descending limbs. Within the rat proximal tubule AQP1 is heavily expressed in all three segments, with the exception of the very initial portions of segment 1 proximal tubule connected to the glomerulus (Figs. 3 and 4) [34]. At the subcellular level AQP1 is almost exclusively localized in apical and basolateral plasma membranes (Fig. 4), which is logical for entrance and exit routes for transepithelial water transport. In contrast, only very limited amounts of AQP1 are localized in membranes of vesicles and vacuoles. In the basolateral plasma membranes AQP1 is localized to both basal and lateral infoldings, indicating that water may exit both into the lateral intercellular spaces or directly into the peritubular space. Immunohistochemistry reveal relatively more AQP1 protein in the brush border than in basolateral membranes

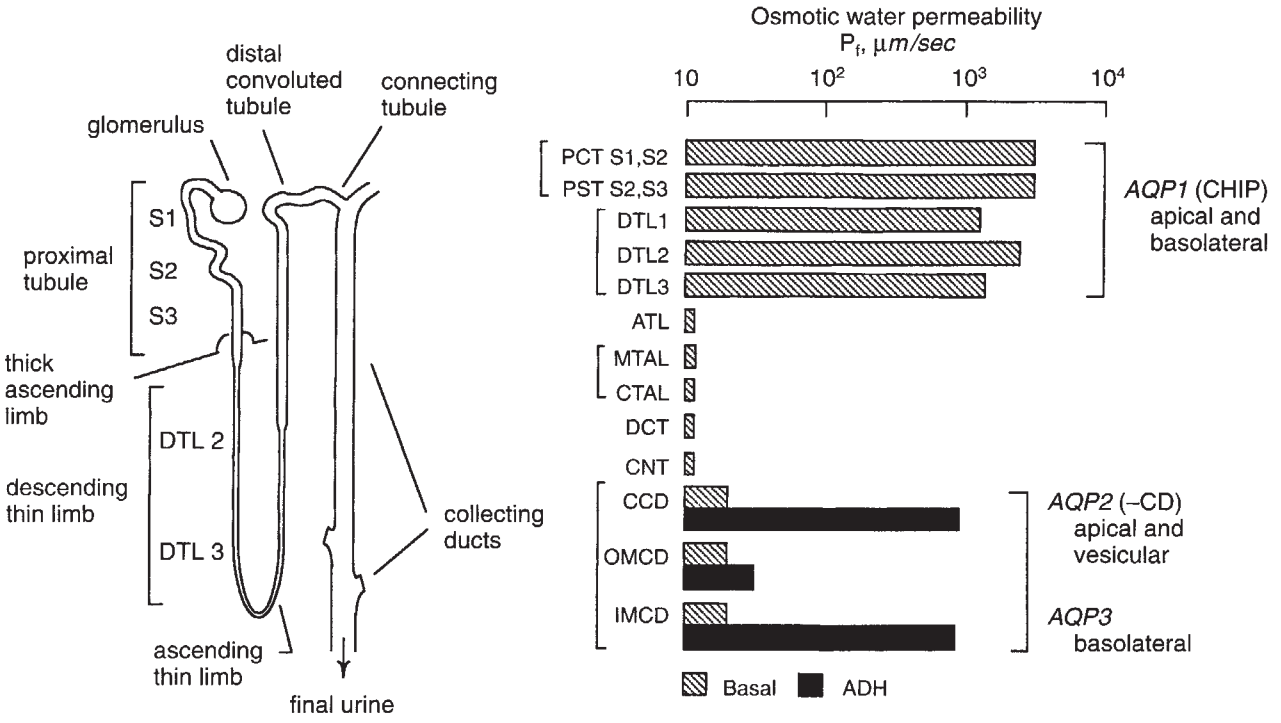

Fig. 2. *Distribution of AQP1, 2, and 3 in a model long looped nephron and compilation of published osmotic water permeability determinations of nephron segments.* (Redrawn from Nielsen et al, 1993; used with permission.)

(Fig. 3), whereas immunoelectron microscopy reveals similar labeling density of apical and basoalateral plasma membranes (Fig. 4). Higher absolute amounts in the brush border border correlate well with a higher plasma membrane area in microvilli than in basolateral membranes [for review of proximal tubule ultrastructure and function see Maunsbach and Christensen, 38]. The functional reason for higher expression in the brush border is uncertain, although it appears logical that the apical membrane should not be the rate-limiting barrier, since the driving force (presumably the Na^+/K^+-ATPase) is localized in the basolateral membranes. The water reabsorbed in the proximal tubule is returned to the circulation via fenestrated peritubular capillaries, which in the rat does not express AQP1, unlike non-fenestrated capillary endothelia cells throughout body [39].

AQP1 expression in the proximal tubule continue uninterrupted into the descending thin limbs of the loop of Henle, where AQP1 is expressed in both apical and basolateral membranes (Figs. 3 and 4). The descending thin limbs of the long nephrons display a very high labeling intensity, consistent with the very high osmotic water permeability of this particular segment [40, 41]. The complex ultrastructure of this segment is characterized by extensive cellular interdigitation and elaborate basolateral infoldings all expressing AQP1 (Fig. 4). The complexity of the ultrastructural organization varies between individual descending thin limbs, and this is generally ascribed to be associated with the level at which the loops bend in the inner medulla. Importantly, all thin limbs with this type II epithelium express very high amounts of AQP1. The descending thin limb of short nephrons (with Type I epithelium) also express AQP1 [34], however, with less abundance [42], consistent with this segment displaying a lower osmotic water permeability [43]. In the inner medulla, descending thin limbs (Type III epithelium) are heavily labeled and continue abruptly into non-labeled ascending thin limbs [34], known to have very low osmotic water permeability [40, 41]. This abrupt transition is seen at all levels of the inner medulla (such as in Fig. 3D), consistent with loops bending at various levels.

AQP1 in outer medullary vascular bundles

Recent data also indicate that the non-fenestrated endothelium of descending vasa recta in vascular bundles display AQP1 labeling [42]. Moreover, the diffusional water permeability of isolated and perfused outer medullary descending vasa recta is partly inhibited with mercurials [42], consistent with the presence of functional mercurial-sensitive water channels. In addition, simultaneous measurements of water, urea and sodium permeability in isolated perfused descending vasa recta also indicate that water also traverses by a transcellular route [44]. The role of AQP1 water channels in vascular endothelium remains to be determined.

Physiological function of AQP1 in kidney

The proximal tubule reabsorbs approximately 50 to 60% of solute and water from the glomerular filtrate [45]. The discovery of the AQP1 water channel made it possible to characterize the permeation pathway at the molecular level and to address another classical question, is transepithelial water transport transcellular or paracellular? Although paracellular transport of water cannot be excluded [reviewed in 45], the abundance of AQP1 in the plasma membranes (3% of brush border membrane protein [34]) and the high single unit conductance [13] makes it likely that

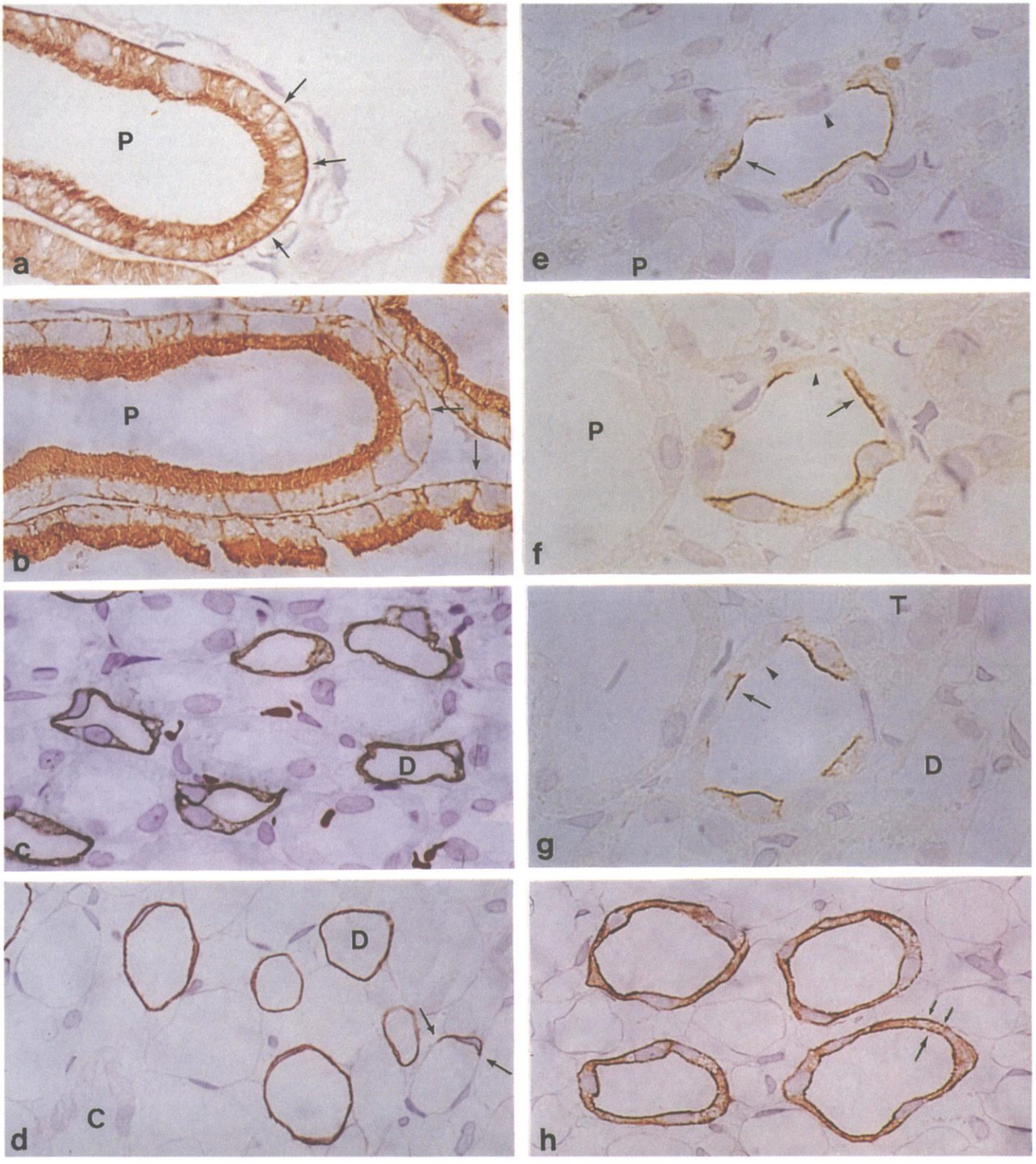

Fig. 3. *Immunohistochemical localization of AQP1* (**A-D**) *and APQ2* (**E-H**) *in kidney cortex (A and E), outer stripe outer medulla (B and F), inner stripe of the outer medulla (C and G) and inner medulla (D and H).* AQP1 is localized in proximal tubule brush border and in basolateral membranes (arrows), which are heavily interdigitated in segment 2 convoluted proximal tubules (A) and less interdigitated in segment 3 straight proximal tubules (B). Long loop descending thin limbs are heavily labeled in outer medulla (C) and in inner medulla (D). An abrupt transisitons to acending thin limbs are shown in D (arrows). AQP2 water channels are selectively localized to the apical parts of collecting duct principal cells in cortex and outer medulla (E-G). In inner medulla AQP2 expression is seen both apically and in basolateral membranes of collecting duct principal cells (H). Final magnification, × 850.

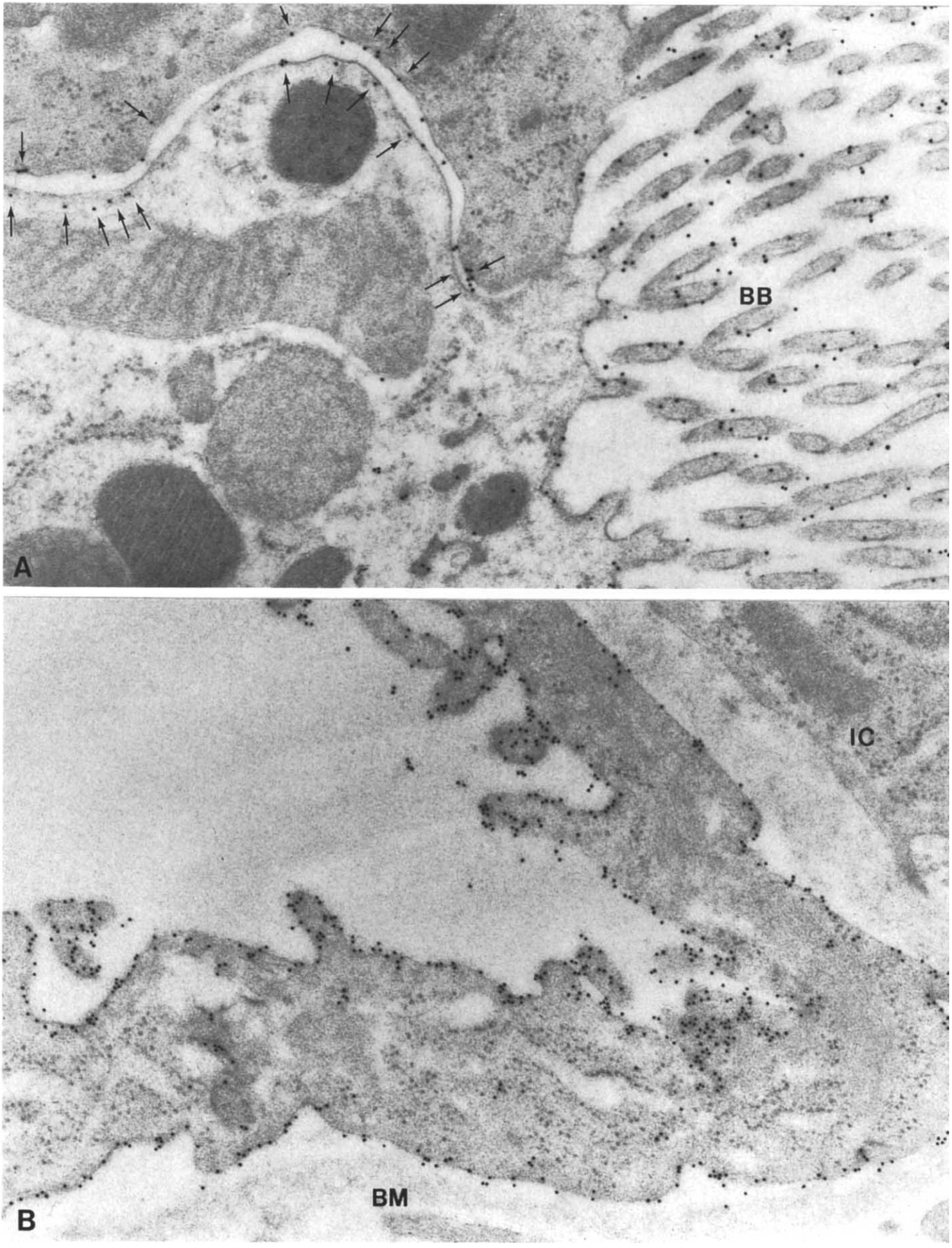
BB
A
IC
BM
B

Fig. 4. *Immunoelectronmicroscopic localization of AQP1 in kidney.* **(A)** In proximal tubules, AQP1 is localized to the apical plasma membrane AQP1 in both the microvillus membrane of the brush border (BB) and to intermicrovillar domains. Basolateral membranes are also labeled (arrows). ×60,000. **(B)** Long loop descending thin limb with Type II epithelium. Extensive labeling of apical and basolateral membranes. Interstitial cell (IC) is without labeling. ×60,000.

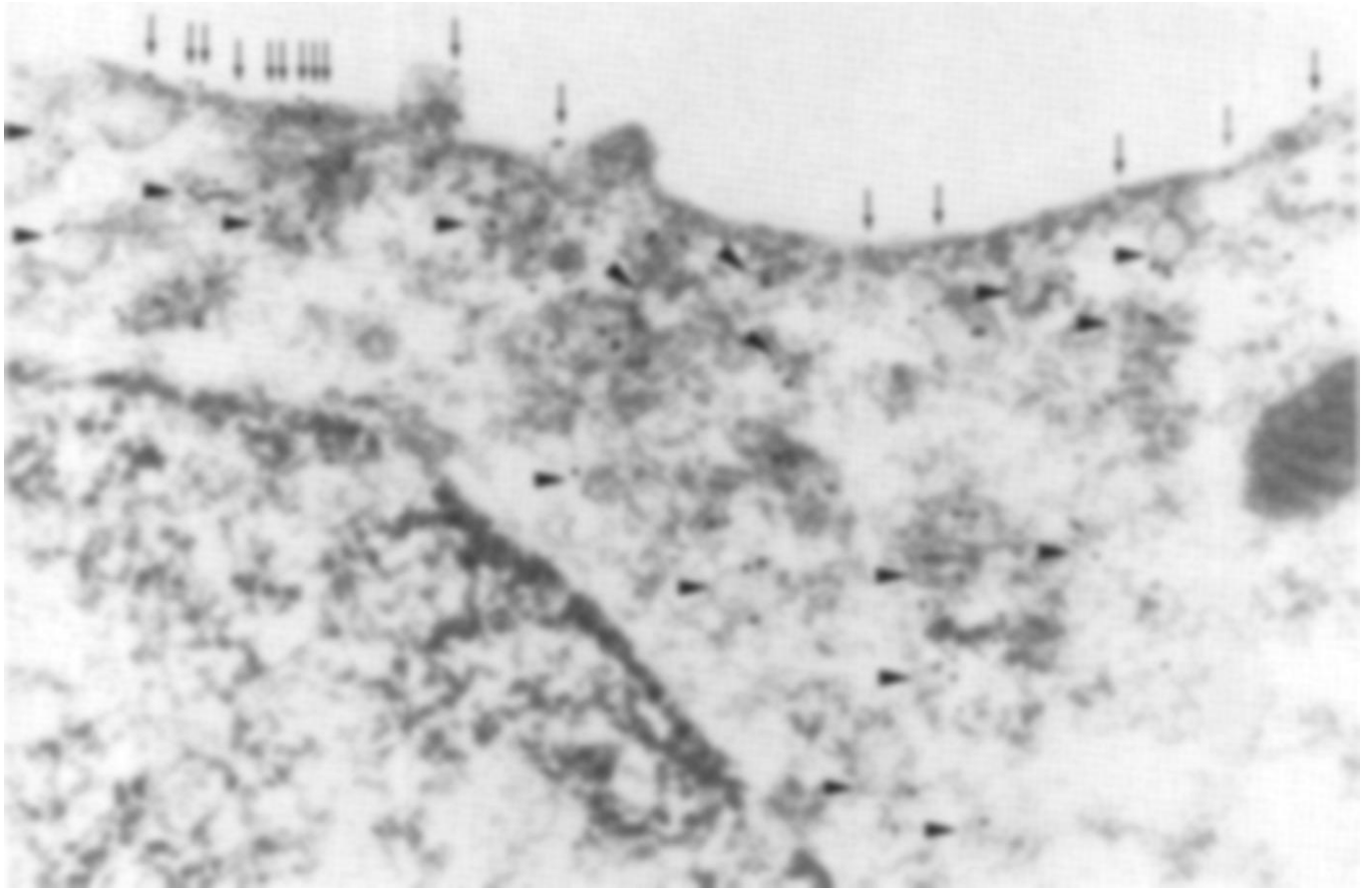

Fig. 5. *Immunoelectronmicroscopic localization of AQP2 in kidney.* AQP2 is localized in the apical plasma membrane (arrows) and subapical vesicles (arrowheads) in principal cell from inner medullary collecting duct. ×75,000.

AQP1 is the predominant pathway for water reabsorption in the proximal tubule. That CHIP is the predominant pathway has been further supported by determining the exact number of molecules of AQP1 in isolated proximal tubules, and correlations of AQP1 per mm tubule length with the unit conductance suggest that AQP1 is sufficient to account for the water permeability of the proximal tubule [46]. Thus, water most likely traverses by a transcellular route.

The architectural organization of the renal medulla is complex and consists of tubules and vascular structures organized in vascular bundles. The highly organized structural organization is believed to be intimately connected to the concentrating ability of the kidney. AQP1 is present both in descending thin limbs of short and long loops, and also in descending vasa recta. Although AQP1 content is high in the entire length of the descending thin limb, the expression decreases along the axis towards the papillary tip [34, 40, 41], which is in exact concordance with the known water permeability characteristics of the loop of Henle [40, 41]. This lends further support to the notion that AQP1 is the predominant water permeation pathway, and thereby represents the constitutive water channel of the nephron.

Expression in kidney during fetal development

The gene expression of AQP1 exhibits a differential pattern during development. AQP1 is abundant in red pulp of spleen, circulating red cells and kidney proximal tubule and descending thin limb in mature animals, whereas prior to birth the expression is much lower [35, 47]. In other tissues such as the choroid plexus [35], AQP1 is present throughout fetal development and maturity. Finally, in other tissues such as periosteum, heart endothelium, endothelium of large vessels and cornea endothelium, a mainly transient expression is observed [35]. However, in cornea endothelium a certain although lower expression continued during maturity.

AQP1 is not detected in rat red cells prior to birth, and is first identified in circulating red cells at the third postnatal day, which correlated directly with acquisition of osmotic water permeability [47]. Coincident with AQP1 expression in rat red cells after birth,

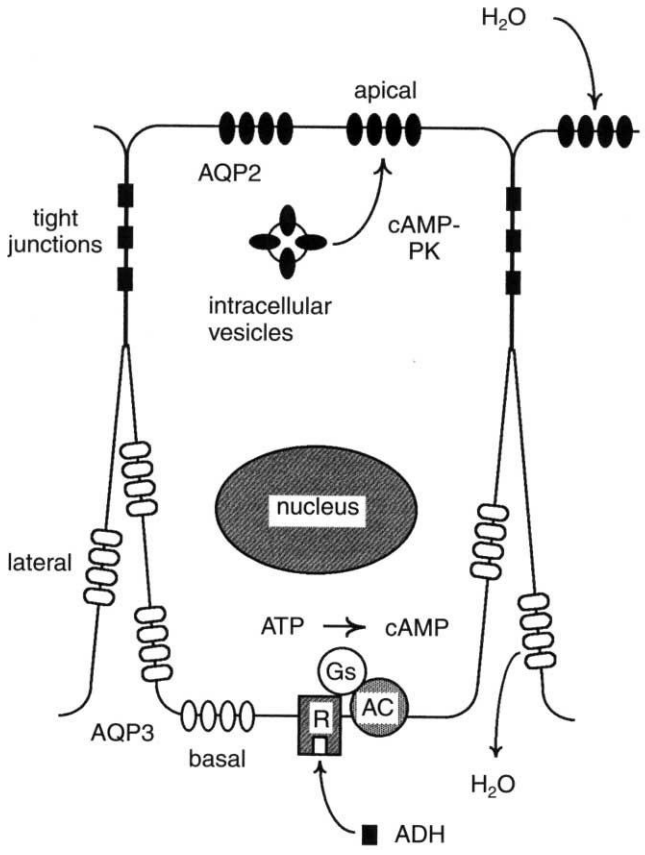

Fig. 6. *Distribution of AQP2 and 3 in collecting duct principal cells.* The antidiuretic hormone vasopressin (ADH) binds to vasopressin receptors (R), and heterotrimeric G-protein (Gs) mediate activation of adenylyl cyclase (AC) to produce cAMP which activate protein kinase A (PK). This presumeable (see text) result in externatlization of AQP2 water channels to the apical plasma membrane, thereby increasing the water permeability. AQP3 is selectively localized to the basolateral plasma membrane.

a marked increase in AQP1 expression in rat kidney proximal tubules and thin descending limbs was demonstrated [47], the time of development of renal concentrating ability. Thus, AQP1 may confer red cells with the ability to adapt to the hypertonic inner medulla of kidneys after birth. In human, lower levels of AQP1 was already observed in red cells and kidney at the second gestational trimester, and the levels increases during the later stages of fetal developement [48].

Distribution and physiology of AQP2 and AQP3 in collecting duct

AQP2 location

Recently, Fushimi et al [31] identified the collecting duct water channel AQP2. Based on the predicted amino acid sequence specific antibodies were raised to synthetic peptides, and AQP2 appear to be exclusively expressed in the kidney collecting duct [31, 49]. As shown in Figure 3, AQP2 is localized selectively to a subpopulation of cells in the collecting duct, the principal cells, whereas intercalated cells, engaged in acid-base transport, does not express AQP2. AQP2 is predominantly localized in the apical plasma membrane and subapical vesicles of principal cells (Figs. 3 and 5) [49]. However, in the inner medullary portion of the collecting duct, AQP2 is in addition localized to the basolateral plasma membranes (Fig. 3). Importantly, AQP2 is very abundant in small vesicles, mainly localized in the subapical parts of the cytoplasm (Fig. 5), and moderate immmunolabeling of multivesicular bodies was also observed [49].

Regulation of AQP2

As described in the following, several lines of evidence point to the conclusion that AQP2 is the predominant vasopressin regulated water channel in the kidney collecting duct. Thus, so far, AQP2 is the only characterized water channel, which function is regulated. Two fundamentally different mechanisms for regulation of collecting duct water permeability are currently established, a short-term mechanism and a long-term adaptive mechanism. Both appear to involve AQP2 water channels and vasopressin.

Vasopressin receptors and signaling mechanisms has recently been reviewed by Knepper et al [50]. Briefly, vasopressin binds to heterotrimeric G-protein-coupled vasopressin receptors (V2-receptors [51], which activate adenylyl cyclase to produce cAMP serving as a second messenger (Fig. 6). The cellular effects of cAMP is believed to be connected to activation of protein kinase A, which phosphorylate various proteins [52], presumably also regulatory proteins, which in turn result in an increase in the water permeability. However, the complex regulation also include inhibition of the water permeability through the phosphoinositide pathway with transient mobilization of calcium [53–55].

For short-term regulation (in the time frame of minutes), vasopressin increases the apical plasma membrane water permeability, which is the rate limiting barrier for transpeithleial transport [56]. Isolated perfused collecting ducts has been instrumental in dissecting the vasopressin responses, since concentration of vasopressin and exact phases of vasopressin stimulation and withdrawal can be tightly controlled. The water permeability increases within 30 to 40 seconds [57, 58], with a rapid increase over 10 minutes followed by a slower secondary increase [57–59]. The decrease in water permeability after vasopressin removal follows a similar time course [59]. The cellular mechanism by which the water permeability increases and decreases has until recently been undefined.

Three general mechanisms for short-term regulation has been formulated. (1) The number of functional water channel in the plasma membrane may increase for example by exocytosis of water channels from an intracellular reservoir to the plasma membrane. (2) The unit conductance of individual water channels constitutively residing in the plasma membrane may be regulated. (3) The two former mechanisms may act in parallel, with chemical modification (activation) of water channels after externalization of those to the plasma membrane. Studies of *in situ* activation of water channels revealed conflicting results with some or no increase in water permeability in *Xenopus* oocytes after expression of AQP2 [17, 60]. Regulation of water permeability by membrane trafficking, originally proposed by Wade, Stetson and Lewis [61], was based on observations that clusters of intramembrane particles appear in the apical plasma membrane of toad urinary bladder and collecting duct cells, in parallel with the vasopressin-induced increase in water permeability [62–64; recent review in 65]. Direct evidence, however, for trafficking of water channels was first made possible with the molecular characterization of the vasopressin-sensitive water channel AQP2.

For this purpose high-resolution immunoelectron microscopy of single isolated perfused collecting ducts was applied on tubules, in which water permeability was determined during different phases of vasopressin stimulation and withdrawal. This allows direct quantitative comparisons between the magnitude of water permeability response and the morphometrically-determined water channel distribution in the same collecting ducts [66]. Inner medullary collecting ducts were perfused in three sequential periods: no vasopressin, 40 minutes 100 pM vasopressin in the peritubular bath, and washout of vasopressin. The osmotic water permeability was measured, and at the end of the periods, the tubules were fixed and prepared for immunoelectron microscopy. Preliminary results reveal, that vasopressin induces a shift in localization of water channels from an intracellular reservoir in vesicles, to the plasma membrane. After vasopressin stimulation, the ratio of AQP2 labeling of the apical plasma membrane as fraction of the labeling of intracellular vesicles increased about fourfold. Furthermore, the labeling density of the apical plasma membrane also increased fourfold after vasopressin stimulation. Conversely, after washout of vasopressin, the water channel distribution ratio, the apical plasma membrane labeling density, and the water permeability returns to basal levels. Thus, the results demonstrate a large *reversible* vasopressin-induced shift of AQP2 water channel localization to the apical plasma membrane from vesicles, in parallel with the increase in water permeability. These data therefore provide direct evidence for the Shuttle hypothesis.

The second regulatory mechanism involve long-term adaptive mechanisms, where urinary concentration is dependent on other factors than the short-term actions of vasopressin [reviewed in 67]. Inner medullary collecting ducts display a basal water permeability [57, 59, 68] even in the absence of vasopressin (Fig. 2). This basal water permeability of collecting ducts has been shown to increase after thirsting of the rats for 48 hours, with a concomitant increase in the incidence of intra membrane particle clusters in the apical plasma membrane [68, 69]. Recent studies indicate that the thirsting-induced increase in basal water permeability may be related to AQP2 expression, since dehydration of rats induce a marked increase in expression of AQP2 in kidney inner medulla, as revealed by immunoblotting [49]. The marked increase in AQP2 expression was observed both in subapical vesicles and, importantly, in the apical plasma membrane [49].

To analyze the mechanisms for expression of water channels further, the lack of vasopressin secretion in Brattleboro rats, which manifest central diabetes insipidus, were exploited [70]. First, Brattleboro rat kidney inner medulla display a very low expression of AQP2, correlating with the lack of concentrating ability. Second, vasopressin infusion s.c. in osmotic minipumps for five days in Brattleboro rats, caused a threefold increase in AQP2 expression in the inner medulla. AQP2 was expressed both in the apical plasma membrane and vesicles. Third, isolated perfused inner medullary collecting ducts dissected from vasopressin-treated Brattleboro rats, displayed a threefold higher vasopressin-induced water permeability as compared to tubules dissected from vehicle treated rats. Thus, long-term vasopressin infusion regulate the expression of AQP2 water channels (directly or indirectly), and the data also demonstrate that AQP2 is the predominant vasopressin-regulated water channel both for short- and long-term regulation.

AQP3 location

A new water channel, termed AQP3 [32] is, in contrast to AQP2, selectively expressed in the basolateral plasma membranes of collecting duct principal cells. Interestingly, AQP3 displays mercurial sensitive water permeability, although it lacks the cysteine in position 189, a cysteine shown to be critical for mercurial blockage of AQP1 water conductance [21]. Moreover, AQP3 has the additional characteristics that it also transports non-ionic small molecules such as urea and glycerol. It is therefore the first molecular water channel, which also transport other molecules in addition to water. Urea transport is critical for urinary concentration. However, the role of AQP3 as a urea transporter in relation to the urea transport UT2, which is expressed in the inner medullary collecting duct [71], is not yet known. It is likely that AQP3 may have an important function as a water and urea exit pathway in the basolateral membranes of principal cells.

Distribution and physiology of AQP4 in brain

A novel cDNA was recently isolated from a rat brain cDNA library [33]. The mRNA was very abundant in brain tissues but is detectable at only low levels in other tissues by RNase protection. When expressed in oocytes, the 323 amino acid polypeptide conferred a large P_f which was not sensitive to mercury even after substitution of a cysteine at the site corresponding to Cys189 of AQP1. The sequence corresponds closely to MIWC, a recently identified mercury-insentive water channel from lung and other tissues [72], although differences in the translation start site and coding region of MIWC resulted in a markedly reduced water permeability of MIWC when compared to AQP4 [33]. *In situ* hybridization of brain revealed a strong signal for AQP4 over several neural tissues, including the paraventricular and supraoptic nuclei with project axon terminals to the neurohypophysis; these neurons contain osmoreceptors responsive to very small changes in osmolality leading to the activation of mechanosensitive cation channels and release of vasopressin [73]. Therefore, AQP4 is very likely to be the osmoreceptor through which the central nervous system senses the need for antidiuresis [33].

Aquaporin mutations and clinical states

Humans have recently been identified with mutations in the coding region of AQP1 and AQP2. Identification of the patients was achieved through existing clinical databases and their naturally phenotypes have provided unique and surprising insight into the physiological importance of these molecules.

The site of the AQP1 locus on human chromosome 7p14 [74] coincided with that of the Colton blood group antigen [75]. AQP1 was selectively immunoprecipitated by anti-Co^a and anti-Co^b serum, and a surface Ala-Val polymorphism was established at residue 45 [76]. World-wide blood group referencing has led to the identification of only five kindreds with Colton null red cells. Blood and urine samples from the probands of three families were found to lack the protein, and biophysical analysis of the red cells confirmed a markedly reduced P_f which was no longer sensitive to mercurials [77]. Genomic DNA analysis confirmed that the proband from each kindred was homozygous for a different mutation in AQP1. Two individuals could be considered "knockouts", since exon I was deleted in one and a frameshift mutation was identified in the other. The third proband suffered a missense mutation

which was found to encode an unstable molecule when expressed in oocytes, and her red cells were subsequently found to have a < 1% of the normal level of AQP1. Surprisingly, none of the three probands manifested any significant clinical abnormality.

Humans with mutations in AQP2 were identified among the patients with nephrogenic diabetes insipidus, a trait which is X-linked coinciding with the locus for V2 vasopressin receptors on the X chromosome [78, 79]. Nephrogenic diabetes insipidus patients with normal V2 receptor cDNAs were examined for defects in their AQP2 cDNAs. The first human with an AQP2 defect was a compound heterozygote with two missense mutations which were shown to be dysfunctional when expressed in oocytes [80]. Unlike the AQP1 mutants, these individuals were affected with a severe renal concentration defect and secondary neurological dysfunction apparently resulting from infantile dehydration [80]. Thus, expression of AQP2 is apparently restricted to the renal collecting duct, and its absence is not completely compensated by another mechanism.

The Brattleboro rat is a well-studied model for primary antidiuretic hormone deficiency, and the resulting central diabetes insipidus was found to be related to distinct alterations in Aquaporin expression in kidney. The kidneys of Brattleboro rats were examined for expression of AQP1 and AQP2 by immunocytochemistry and immunoblotting. No changes was observed in AQP1 expression [81]; however, as described in detail above, a marked reduction in expression of AQP2 was observed as compared to expression in normal rats [70]. Thus, the low expression correlate with the lack of concentrating ability. Moreover, it was shown that vasopressin-infusion markedly stimulate AQP2 expression, demonstrating that vasopressin is critical for AQP2 water channel expression. This may play a role in clinical conditions with chronical alterations in circulating vasopressin levels or defects in urinary concentrating ability.

As documented, certain forms of nephrogenic and central diabetes insipidus are caused by changes in the AQP2 molecule or changes in AQP2 expression [70, 80]; however, these conditions are relatively rare. In contrast, acquired nephrogenic diabetes insipidus is more common, and a number of conditions and pharmacological agents causes acquired nephrogenic diabetes insipidus [82]. For example, lithium, which is very commonly used to treat manic-depressive psychosis, often causes nephrogenic diabetes insipidus. It has recently been shown that prolonged treatment of rats (10 to 35 days) with lithium within therapeutic ranges causes a very extensive decrease in AQP2 expression, concomitant with the development of severe diabetes insipidus [83]. These studies [70, 80, 83] strongly support the notion that AQP2 plays a key role for maintenance of body water balance and in body water balance disorders.

Prospects. Some initial conclusions have been established in this review, but several new questions about the biological roles of the Aquaporins have emerged. How do AQP1 mutant individuals compensate? The lack of a clear phenotype suggests a lack of major importance for this protein, although it cannot presently be concluded that it could be due to subclinical defects in renal concentration or other physiological processes. Alternatively, the role of AQP1 may be absolutely essential to the normal functions of the proximal tubule and descending thin limbs, but may be equivalently performed by induced-expression of another Aquaporin. If the latter case is correct, why are multiple Aquaporins expressed in a single organ? It is likely that specialized structures exist on each Aquaporin leading to their cellular distributions. For example, AQP1 is targeted to both apical and basolateral membranes of renal proximal tubules and several other tissues, but structures on AQP2 apparently permit targeting only to the apical membranes of collecting duct principal cells in response to vasopressin-induced stimulation of adenyl cyclase, while AQP3 is sorted independently to the basolateral membranes. Is AQP3 regulated by vasopressin like AQP2? What promoter differences lead to expression of AQP4 in brain? Of parallel interest will be the search for mutations in the APQ3 and APQ4 genes whose involvement in human disease is not yet documented. Also, can agonists and antagonists to specific Aquaporins be developed for the treatment of refractory fluid retention and other disorders, or can agents selectively induce or reduce expression of specific Aquaporins? Furthermore, the role(s) of MIP and the other homologous cDNAs which fail to behave as Aquaporins in oocytes remain to be elucidated. These and other unanswered questions portend that our studies of the Aquaporins are only beginning.

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