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Aquaporin water channels: unanswered questions and unresolved controversies

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The long-standing biophysical question of how water crosses plasma membranes has been answered by the recent discovery of the aquaporins. Identification of this large family of membrane water-transport proteins has generated new questions about the physiological functions, tissue distributions, and regulatory mechanisms of individual aquaporins. The fast pace of developments in this field has also resulted in major discrepancies in published reports which warrant resolution.

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Introduction

Discovery of the aquaporins (membrane water-transport proteins) has unleashed a large number of studies in many laboratories, and the mechanism by which water crosses plasma membranes is now becoming understood at a molecular level. Extensive progress has been made in both basic and clinical arenas, and much of this has been reviewed recently [1,2]. The purpose of this review is to identify areas in mammalian biology where the next advances may be expected, and to highlight significant areas of ongoing controversy. To this end, we have liberally interpreted the charge to interject *Opinion* in the hope of sparking curiosity among the readers.

Nomenclature

> What's in a name? That which we call a rose
> By any other name would smell as sweet.
> (William Shakespeare, *Romeo and Juliet* II:2)

Despite the admonition of the Bard, the current explosion of biological information is proving that nomenclature is essential for communication among scientists. Therefore 'aquaporin' was proposed as the family name by which these genetically related membrane water transporters may be recognized [3]. Although logical, the descriptive name 'water channel family' was not proposed, as it implies that all undiscovered water channels must also contain related amino acid sequences. Moreover, although aquaporins are indeed 'water channels', so are irrigation ditches, and this ambiguity was creatively exploited by the organizers of a recent international symposium on biomembrane water transporters who successfully applied for funding from their municipality's Bureau of Sewers and Water!

'Aquaporin' (abbreviated to AQP) is now the official designation of the Human Genome Nomenclature Committee; however, the use of multiple common names continues and is potentially perplexing to scientists outside of the field (Table 1). Confusion may occur because of pre-existing common names; for example, 'MIP' (major intrinsic protein of lens; [4]) is also the acronym for macrophage inflammatory protein and other unrelated proteins. Some homologs do not transport water; for example, 'GlpF' facilitates transport of glycerol in bacteria [5]. Thus the name aquaporin is applied to only those sequence-related proteins shown to transport water.

Several aquaporins are referred to by multiple names. AQP1 was originally named CHIP28 for 'channel-forming integral protein of 28 kDa' of human red cells and renal tubules [6], and 'DER2' refers to the mouse homolog which was identified among growth factor induced delayed early response elements [7]. After the discovery that CHIP28 is a water transporter [8], a number of groups reported the cloning of species homologs with somewhat different names. Although proposed as novel kidney and ciliary isoforms, 'CHIP28k' [9] and 'CHIP29' [10] are the rat [11] and bovine homologs of CHIP28/AQP1.

The cDNAs corresponding to unique but related genes have also been isolated by homology cloning. AQP2, the vasopressin-sensitive water transporter of kidney collecting duct, was first known as 'WCH-CD' [12]. AQP3 is the water transporter in basolateral membranes of kidney collecting ducts [13•,14•], but

Abbreviations

AQP—aquaporin; **cRNA**—complementary RNA; **MIP**—major intrinsic protein of lens; **PCR**—polymerase chain reaction.

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Table 1. The mammalian aquaporins.

Official name	Common names	Functional features, mutant phenotypes
MIP (AQP0)	MIP26	Lower water permeability — other function? Mutant mice — congenital cataracts
AQP1	CHIP28, DER2, CHIP, CHIP28k, CHIP29	Widely distributed, constitutively active Human mutants — subclinical
AQP2	WCH2, WCH-CD, AQP-CD	Renal collecting duct, regulated by vasopressin Human mutants — nephrogenic diabetes insipidus
AQP3	GLIP, BLIP?	Basolateral membranes Constitutively active outflow channel
AQP4	MIWC	Predominantly found in brain, mercury-insensitive Spinal fluid reabsorption, osmoreception
AQP5		Neurohormonal regulation? Secretion of tears, saliva and sputum

the same sequence was published as 'GLIP,' a putative glycerol-selective transporter [15•]. Moreover, AQP3 may also be the membrane component referred to as 'BLIP' [16] because of staining of basolateral membranes by cross-reacting antibodies. Likewise, AQP4, the major homolog found in brain [17•] is referred to by some investigators as 'MIWC' for mercury-insensitive water channel [18•] (however, others refer to MIWC in jest as 'mostly-inactive water channel' because of an apparent sequencing error in a functionally important site). Thus, it is worthwhile that an aquaporin nomenclature committee be composed to field nominations to the Genome Committee.

Structural understandings

Little controversy surrounds the higher-order structure of aquaporins, which has been reviewed recently in detail in *Current Opinion in Structural Biology* [19]. Hydrodynamic studies of MIP (AQP0) and AQP1 similarly predicted that both proteins are homotetramers [20,21]. The tetrameric organization has been visualized by freeze fracture of AQP1 in proteoliposomes [22] and negative staining of highly purified AQP1 protein [23•]. The tetrameric organization of AQP1 was demonstrated by high resolution (~15 Å) electron microscopy of reconstituted two-dimensional membrane crystals containing biologically active AQP1 protein [24] and confirmed by other investigators [25]. A three-dimensional projection has been achieved by cryoelectron microscopy of tilted specimens [23•]. It remains to be established where the aqueous pores reside within the subunits and what mechanisms are responsible for the existence of these proteins exclusively as tetramers within the bilayer, rather than as an equilibrium of monomers and higher-order oligomers. Higher resolution electron crystallography and atomic force microscopy should provide still greater understanding of the structure of AQP1.

Workers in the water-transport field also agree about several structural features of the aquaporin subunits. Hydrophobicity analysis of MIP [4] predicted the existence of six transmembrane segments separated by five connecting loops (see Fig. 1a). The results of analyses of all known mammalian, plant, and microbial homologs are very similar, and the deduced amino acid sequences are 20–40% identical when their amino termini are aligned [26]. An internal homology was noted in which the amino- and carboxy-terminal halves of the protein are approximately 20% identical, and certain domains have been retained among all known homologs; most distinctive is the three residue motif asparagine–proline–alanine (NPA; single-letter code for amino acids) within connecting loops B and E (Fig. 1a). Circular dichroism measurements of lens protein MIP revealed ~50% α-helix and ~20% β-structure [27], and studies of AQP1 from red cells confirmed this composition [28].

Several topological landmarks have been established unambiguously. Biochemical studies and immunoelectron microscopy demonstrated the cytoplasmic location of the amino and carboxyl termini of AQP1 [21,29,30•]. Recent discovery of the Colton blood group polymorphism at residue 45 in loop A of AQP1 (Ala45/Val45; Fig. 1a) demonstrated the extracellular location of this loop [31,32••]. Moreover, the *N*-glycosylation consensus site is in loop A of AQP1 [6]; however, the only *N*-glycosylation sites in AQP2, AQP3, AQP4, and AQP5 reside in loop C, thereby establishing the extracellular orientation of this domain [12,13•–15•,16,17•,18•,19,33•].

Structural controversies

Although the cDNAs encoding six mammalian aquaporins have been isolated, multiple variations in the coding sequences suggest that numerous errors exist in the reported sequences and warrant correction. Given that natural polymorphisms and naturally occurring mutations are now being discovered, it is essential that investigators pursue their homology cloning with standard methods, including isolation of intact

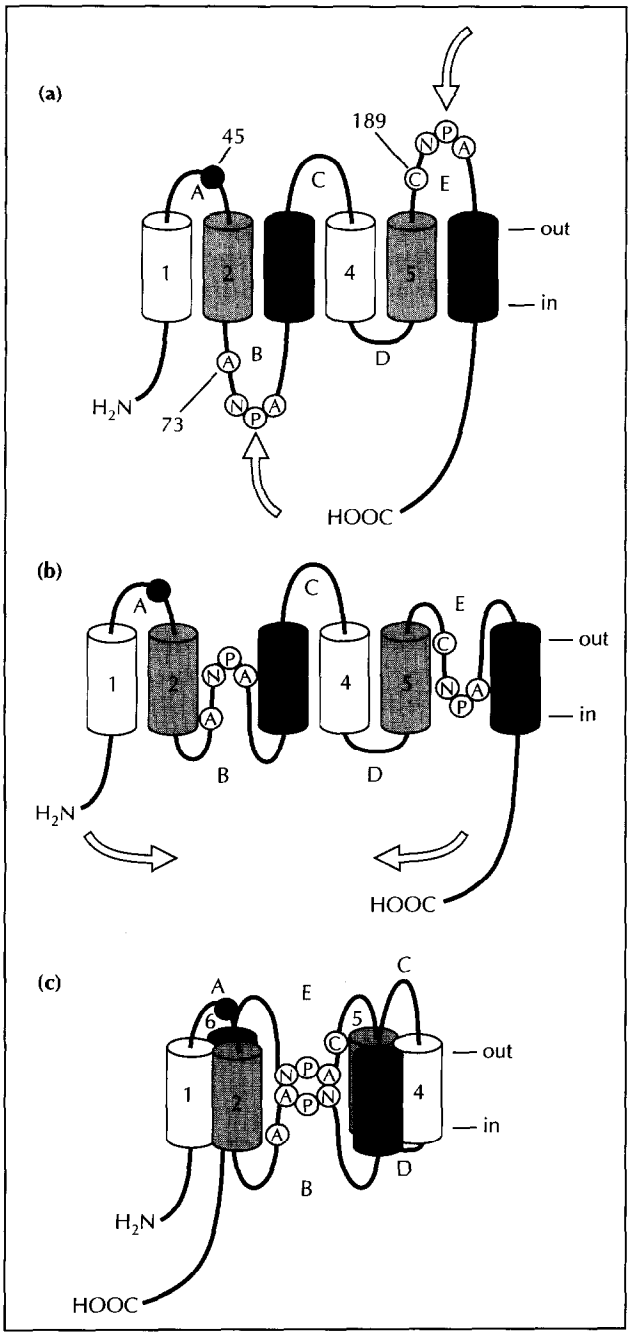

Fig. 1. Hourglass model of a single AQP1 subunit. **(a)** Six bilayer-spanning domains configured into two repeats each comprising three bilayer spans arranged in obverse symmetry. Represented are the synthetic (A73) and native (C189) mercury-sensitive sites, the NPA motifs in loops B and E, and the Colton blood group polymorphism (A45/V45). Arrows indicate the predicted folding of loops B and E into the bilayer. **(b)** Arrows indicate the predicted folding of the two repeats back upon each other. **(c)** Loops B and E juxtaposed to form a single aqueous pore (the 'hourglass'). AQP1 subunits assemble into a tetramer with the four sets of B and E loops constituting four central water pores. Reprinted with permission from Jung *et al.* [43••].

recombinants from cDNA libraries (not just by PCR amplification), sequencing of both strands of the cDNAs, and resolution of sequencing artifacts before publication. For example, the amino-terminal sequence reported for the rat homolog CHIP28k [9] surprisingly contains the nucleotide and deduced amino amino acid sequences corresponding to the human homolog, apparently because of the inclusion of sequence derived from the PCR primers designed on the basis of the human cDNA sequence [6]. The sequence of the kidney collecting duct homolog, AQP2, cloned from a Japanese individual [34] contained several differences from that obtained from a European [35••], suggesting a possible racial difference in the cDNAs; however, the discrepancies were subsequently found to be artifactual [34]. Two groups reported nearly identical sequences: MIWC cloned from rat lung [18•] corresponds to AQP4 cloned from rat brain [17•]. MIWC contains a proline at position 201 which probably represents a sequencing error, as this residue is a histidine in AQP4 and most other aquaporins; site-directed mutagenesis of AQP4 His201→Pro (MIWC) virtually abolished the osmotic water permeability assayed in *Xenopus* oocytes [17•]. As investigators have not agreed about the primary sequences, it is not surprising that three different laboratories have generated three different membrane topology models, outlined below.

Algorithm-generated β-barrels: the restricted pore model

Fischbarg *et al.* [36•] analyzed the deduced primary amino acid sequences of multiple aquaporins using several different computer-based algorithms. These investigators believed that the hydrophobic stretches may be too short to conform to known α-helical transmembrane spans, and they observed sequences with turn propensities at frequent intervals. They concluded that the subunit may be comprised mostly of β-structure [36•]. Although no biological experiments were performed, the investigators proposed a structure likened to a 'restricted pore' similar to bacterial porins and comprising a 16-stranded antiparallel β-barrel, a structure which they believe may be relevant to several other transport proteins. Although very interesting, their model is inconsistent with experimental evidence of others, as it predicts an absence of α-helical structure and projects a known *N*-glycosylation site to an intracellular location.

Site-directed mutilation: the four bilayer span model

Lingappa and colleagues [37] developed a method for establishing protein topology by truncating cDNAs encoding membrane proteins with multiple bilayer-spanning domains at potential extracellular and intracellular locations; DNA sequence encoding a 15 kDa epitope from bovine prolactin protein is spliced at the truncation site and the recombinants are expressed in microsomal membranes using rabbit reticulocyte lysate. Antibodies directed against the prolactin epitope are then used to map its disposition in the chimeric protein. This approach was used to map the topology of the MDR (multidrug resistance) protein but resulted in the

generation of an 'alternative model' in which several expected transmembrane domains failed to cross the bilayer [37]. As the truncation–prolactin recombinants contain only part of the polytopic integral membrane protein, no functional assessment is possible, and the relevance to the topology of the native protein is entirely uncertain. In contrast, other investigators demonstrated using functionally active recombinants that MDR exhibited the expected membrane topologies [38].

Using the truncation–prolactin method, Skach, Verkman, and colleagues [39••] attempted to map the topology of AQP1 and concluded that the protein has only four bilayer spans with the second hydrophobic domain residing entirely in the extracellular space. Also in contrast to the expected topology, their model predicts that the fourth hydrophobic domain and loop C both reside entirely in the cytoplasm, and the model lacks symmetry. It is notable that the mass of the prolactin epitope (15 kDa) dwarfed the mass of the AQP1 polypeptide in most of their constructs, so no functional assessment of water permeability was possible. Although thought-provoking, the authors have subsequently backed off this model and now argue that it may exist only in the endoplasmic reticulum (W Skach, personal communication), although other investigators feel this model represents the protein in a twisted, non-functional conformation (hence 'site-directed mutilation').

Back to the future: the hourglass model

The original report of MIP [4] contained a membrane topological model with six bilayer-spanning domains (Fig. 1a). Preston and colleagues [40•] sought to determine the topology of AQP1 by inserting a 31 residue E1 epitope from avian coronavirus at separate points in the molecule corresponding to the amino and carboxyl termini, loops B, C, D, and E. Importantly, the capacity of each mutant to transport water was measured after expression in *Xenopus* oocytes. The sites of the epitope tags were established using antibody labeling or vectorial proteolysis [40•] and predicted a membrane topology consistent with the six bilayer-spanning model originally proposed by Gorin and colleagues [4], thus advancing the field back to where it was in 1984.

Several observations have suggested a modification to the six bilayer span topology. Although initially proposed as residing at intracellular and extracellular locations, loops B and E both exhibit significant hydrophobic character. Moreover loops B and E are highly related, each containing the signature motif NPA, and introduction of the E1 epitopes at these sites led to loss of biological function [40•]. The site of inhibition of AQP1 by mercury has been demonstrated to be Cys189, adjacent to the NPA motif in loop E [41,42]. A series of site-directed mutations at this site showed that residues of greater mass obstructed osmotic water flow, whereas smaller residues did not, indicating that this site may correspond to a narrowing of the aqueous pore which is critical to proper protein folding and transit through the Golgi [41]. When a cysteine was introduced at residue 73, the corresponding position in loop B, mercury-sensitivity was again noted, and substitution by residues of greater mass abrogated the water permeability [43••]. Although loops B and E are at opposite ends of the polypeptide, they both appear to reside at critical narrowings of the aqueous pathway. To explain these observations, the 'hourglass' model was proposed by Jung *et al.* [43••], in which the amino- and carboxy-terminal halves of the molecule exist in an obversely symmetric orientation with loops B and E dipping into the membrane from opposite sides of the bilayer (Fig. 1b). The overlap of loops B and E would comprise a single, narrow aqueous channel with adjacent mercury-sensitive sites at inner and outer locations (residues 73 and 189; Fig. 1c).

The analysis of site-directed mutant forms of AQP1 also led to the conclusion that individual subunits each contain their own aqueous pore. Creation of tandem dimeric molecules with and without mercury-sensitive residues showed that subunits behaved independently, even when two subunits are expressed as a single polypeptide [43••,44•]. The importance of oligomerization was revealed by functional complementation studies in *Xenopus* oocytes. High water permeability resulted when cRNAs encoding recombinants with mutations in or adjacent to the NPA motifs were co-injected with a cRNA encoding a truncated polypeptide lacking the carboxy-terminal membrane-targeting domain; expression of the individual subunits produced no increase in water permeability [43••].

Although the osmotic water permeabilities of AQP1, AQP2, and AQP5 are inhibited by mercury, the structures of some homologs do not fit this simple paradigm. AQP3 [13•,14•] and the plant homolog γ-TIP [5] are reversibly inhibited by mercury even though they lack cysteines at the mercury-senstitive site. Also, the water permeabilities of AQP4 and MIP are insensitive to mercury even when a cysteine is substituted into their structures at sites adjacent to the second NPA [17•,18•,45•]. The structural explanations for these variations in mercury-sensitivity warrant additional study.

Biophysical features

Studies from multiple laboratories have confirmed that AQP1 and several homologous proteins are freely permeated by water but not ions or other small uncharged molecules (reviewed in [1]). Nevertheless, several biophysical issues remain unresolved.

MIP is most abundant membrane protein of lens; although it was the first member of the aquaporin family to be identified [4], its biophysical specificity remains uncertain. Although often referred to as an 'ion channel,' membrane conductance has only been

measured when MIP was reconstituted into black lipid membranes [46], with no increase in conductance noted when MIP was expressed in oocytes ([45•,47]; J Hall, personal communication). This behavior was also shown for the root protein NOD26 [48] and may apply to other homologous proteins. It has been demonstrated recently that MIP expressed in oocytes confers osmotic water permeability which is thermodynamically similar to that of the other aquaporins although the capacity is much less ([45•,47]; J Hall personal communication).

The selectivity of aquaporins for water is also an area of significant interest. Although pore size may explain the inability of AQP1 to transport urea, it does not explain its failure to conduct ions or protons [30•], as the latter exist in solution as H_3O^+. The low activation energy for aquaporin-mediated water transport indicates that water crosses the bilayer as a single-file column, so it is reasonable to expect that the orientation of charged residues within the aqueous pore may restrict permeability to ions. This specificity may be physiologically essential for normal renal concentration of water during the excretion of acid. Although creation of a site-directed mutant protein which is permeable to water and protons may be feasible, such a recombinant has not yet been reported.

The long-standing controversy over whether water and urea permeate the same pathway was resolved by identification of urea carriers which exhibit large capacity for urea but not water [49,50•]. As predicted by Macey and Youssef [51], separate water and urea transporters exist in both red cells and renal medulla. The inability of aquaporins to conduct urea may be explained by the restricted size of the pore; however recent studies have demonstrated a small degree of permeation of AQP3 by urea and glycerol [13•,14•]. A major exception to the selectivity rule has been demonstrated for the homologous bacterial protein, GlpF (glycerol facilitator) which transports glycerol but not water [5,52]. GLIP, a protein from rat kidney, was reported to be a stilbene-inhibitable glycerol transporter which is not permeated by water [15•]. Unfortunately a major controversy erupted when it was found that the sequence of GLIP is virtually identical to that of AQP3, whose water permeability had been established by two independent laboratories [13•,14•]. Moreover, the Northern hybridization analysis documenting the size of the transcript and tissue distribution of GLIP (5.5 kb, major site of expression in brain) is incompatible with the studies of AQP3 (1.8 kb, major site is kidney with no expression in brain). Thus much remedial work is now necessary to resolve these discrepancies.

Tissue distributions and physiological roles

Aquaporin-1

Although AQP1 is thought to be a simple, constitutively activated membrane water pore, its tissue distributions and developmental expression patterns are complex (Table 2a). Initial studies by Denker *et al.* [53] and detailed immunolocalization studies using affinity-purified antibodies to the amino and carboxyl termini of AQP1 [29] or immune serum [54] revealed expression in the proximal tubule and in the descending thin limb in the kidney, where it is believed to contribute to the countercurrent multiplier mechanism responsible for water conservation by the proximal nephron (Fig. 2). AQP1 is not expressed in other nephron segments or in the collecting duct [29], but studies with immune serum suggested its presence in descending vasa recta (part of the medullary blood supply) [54], a site where recent studies have documented partial inhibition of water flux with mercurials and defined the presence of AQP1 with affinity-purified antibodies [55]. AQP1 has recently been quantitated in nephron segments by ELISA [56•]. AQP1 is also abundantly expressed in multiple extrarenal sites [57], indicating a major role in transepithelial water transport within multiple organs and suggesting a role in secretion of spinal fluid, reproductive fluids, aqueous humor [58], and bile [59]. AQP1 expression is not restricted to secretory or absorptive epithelium and it is abundant in capillary endothelium where it may contribute to vascular permeability [57]. A strong *in situ* hybridization signal was noted in the mesenchyme surrounding maturing bone [60], and immunolabeling of fibrocytes of inner ear [61] and of smooth muscle cells surrounding unlabeled epididymis epithelium [62] have been reported. Labeling of smooth muscle cells appeared not to be a general phenomenon as it was absent from the thick layer of smooth muscle cells surrounding the vas deferens [62].

Several discrepancies in AQP1 distribution have been reported, including its location within lung and airways, gut, and exocrine glands (Table 2b). Use of affinity-purified antibodies revealed prominent labeling of AQP1 in a subset of capillaries surrounding bronchii and bronchioles, whereas less prominent labeling was confined to respiratory sections and no labeling was observed of bronchial epithelium [57]. Expression in lung was further studied by Folkesson *et al.* [63] who described inhibition of lung water permeability by mercurials; however, concern for the toxicity of this agent in lung is being raised. Hasegawa *et al.* [64] reported expression of AQP1 in tracheal and bronchial epithelium, colonic epithelial crypt cells, apical and basolateral membranes of pancreatic acinus cells, salivary gland epithelium, basolateral membranes of sweat glands and duct cells, but these findings conflict with previous and newer findings using thin cryosections and affinity purified antibodies. Also unexplained is the transient expression of AQP1 in some tissues such as fibroblasts where AQP1 was found among delayed early response genes [7]. Some of these discrepancies are probably due to methodological differences. Future studies should be pursued only with affinity-purified antibodies raised against highly purified antigen and with documentation of the presence of the protein in question both by immunoblotting and immunocyto-

Table 2. Tissue distribution of mammalian aquaporins.

Aquaporin	(a) Sites where expression is established
MIP (AQP0)	Lens fiber cells
AQP1	Red cells Kidney proximal tubule, descending thin tubule (I–III), descending vasa recta Eye corneal endothelium iris, ciliary and lens epithelia Choroid plexus (apical membrane only) Male reproductive tract Hepatobiliary duct and gall bladder Capillary and venule endothelia Lacteals and lymphatics
AQP2	Kidney collecting duct principal cells (intracellular vesicles and apical membranes)
AQP3	Kidney collecting duct principal cells (basolateral membrane)
AQP4	Brain hypothalamus (paraventricular and supraoptic nuclei) Purkinje and ependymal cells Kidney collecting duct principal cells (basolateral membrane) Stomach parietal cells
AQP5	Salivary and lachrimal glands (apical membrane) Corneal epithelium Lung
	(b) Sites where expression is disputed
AQP1	Sweat glands Large airway epithelium Colon epithelium Pancreas secretory epithelium Uterus (cellular localization unknown)

chemistry. This is highlighted by the fact that some immune sera raised against CHIP28/AQP1 reacted with MIP and other proteins [65]. Thin cryosections for immunocytochemistry allow a high degree of resolution and may provide detailed information about cellular and subcellular expression (see Fig. 3), which may be more difficult to obtain by procedures using thicker sections.

Although investigators readily attributed a large variety of tissue water movements to AQP1, the importance of this protein has been questioned after the surprising finding that rare patients who lack the Colton blood group antigens have 'knockout' mutations in AQP1, yet suffer no obvious clinical defect [32••]. Red cells from these individuals exhibit a marked delay in osmotic water permeability. It is not presently known why the patients fail to exhibit any apparent pathophysiological consequences in kidney, brain, eye, or other organ systems, and three hypothesis can be proposed: redundant expression of multiple aquaporins may confer complete compensation in many tissues; paracellular pathways of water transport or other non-aquaporin mechanisms may exist; or the real physiological roles of AQP1 are not known.

Major intrinsic protein (Aquaporin-0)

In contrast to AQP1, the distribution and physiological importance of the other known aquaporins are more easily explained. The first identified member of this family, MIP, is expressed exclusively in membranes of lens fiber cells [4]. The physiological importance of MIP in maintenance of lens transparency was demonstrated in the CAT mouse, a murine model for congenital cataracts resulting from mutations in the *Mip* gene ([66]; A Shiels, personal communication).

Aquaporin-2

AQP2 is expressed exclusively in kidney collecting duct principal cells. Most AQP2 is localized to apical plasma membranes and subapical vesicles [67]; although there are some discrepancies in the published membrane distribution of AQP2 in collecting ducts, these may reflect differences in rat strains and axial variations in the polarized distribution of AQP2 along the collecting duct. Nevertheless, multiple lines of investigation indicate that AQP2 is the predominant vasopressin-regulated water channel of kidney and is essential for regulation of body water balance [12,67]. Its physiological importance was dramatically demonstrated by the identification of patients suffering from a severe form of nephrogenic diabetes insipidus [35••,68] resulting from mutations in AQP2 that cause expression of misfolded proteins [69]. Brattleboro rats, a vasopressin-deficient strain, exhibit central diabetes insipidus and have a marked reduction of AQP2 [70•]. Reduced AQP2 levels were also identified in the important clinical syndrome of lithium-induced nephrogenic diabetes insipidus [71•].

The molecular controls for regulation of AQP2 involve short-term (minutes) and long-term mechanisms (hours to days). Three mechanisms for vasopressin activation of membrane water permeability have been postulated: targetted exocytosis of intracellular AQP2 vesicles to the apical plasma membrane; direct activation of AQP2 in the plasma membrane by protein kinase A phosphorylation; or both mechanisms acting in parallel. The vesicle shuttle hypothesis originally proposed by Wade (reviewed in [72]) has been supported by multiple recent reports [73,74•,75] which together have shown translocation of the AQP2 protein to the apical membrane (Fig. 3) and induction of water permeability in isolated collecting ducts. Much current effort by multiple groups is now devoted to identifying

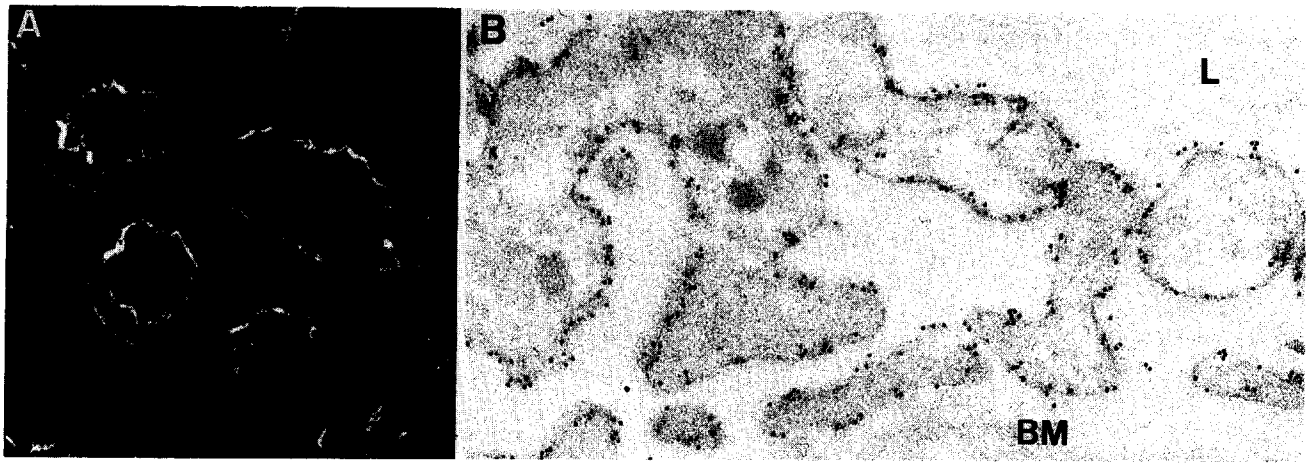

Fig. 2. Immunolabeling of AQP-1 in kidney. Both apical and basolateral plasma membranes exhibit extensive labeling. **(A)** Immunofluorescence microscopy of proximal tubules, adapted from Sabolic *et al.* [54]. **(B)** Immunogold electron microscopy of descending thin limb, adapted from Nielsen *et al.* [29]. Magnification (A) ×500; (B) ×48 000. BM, basement membrane; L, lumen.

the cellular machinery involved in membrane vesicle trafficking and examining the role of phosphorylation in AQP2 function. Important first steps have been the identification of synaptobrevin (VAMP2) associated with AQP2-containing vesicles (important for vesicle targeting) [76•,77••]; correlation of cAMP-stimulated AQP2 phosphorylation with increased water permeability of intact *Xenopus* oocytes [78•]; vasopressin-induced redistribution of AQP2–myc recombinant proteins expressed in LLC-PK epithelial cells [79•]; and the observation that direct phosphorylation of AQP2 in isolated collecting duct vesicles does not change the water permeability [80]. Detailed studies are warranted to document the roles of these cellular components and phosphorylations in the acute and chronic actions of vasopressin, and much new information is expected to emerge soon.

Aquaporins-3 and -4

Although AQP3 was cloned from renal collecting duct by three groups [13•–15•], this homolog is the subject of much controversy (described above). Nevertheless, immunocytochemistry has shown that, within kidney, AQP3 is almost exclusively present in the basolateral plasma membranes of collecting duct principal cells [81,82].

RNase protection studies with AQP4 probes revealed that brain was the predominant site of expression; *in situ* hybridization identified a strong signal for AQP4 over several tissues [17•] including the paraventricular and supraoptic nuclei in the hypothalamus, which project axons to the neurohypophysis and also contain osmoreceptors responsive to the release of vasopressin [83]. Therefore, AQP4 is very likely to be the osmoreceptor through which the central nervous system senses the need for antidiuresis. In addition, AQP4 has been detected in ependymal cells lining the ventricles [17•,18•], in basolateral plasma membranes of kidney collecting duct principal cells and in gastric parietal cells [82,84]. Although Northern and *in situ* hybridization indicated the presence of AQP4 in multiple tissues, including lung, salivary glands and in thin structures in kidney inner medulla [18•], the cellular localizations await documentation by immunocytochemistry. No immunocytochemical labeling was found in thin structures in kidney inner medulla where only collecting duct principal cells were labeled [82,84]. The marked difference in cellular localization between certain studies of AQP4 mRNA [18•] and immunocytochemical analysis of AQP4 protein warrant further investigation.

As AQP3 and AQP4 are both expressed in the basolateral plasma membrane of collecting duct principal cells, it appears that multiple aquaporins may be co-localized in the same membrane domain, an apparent redundancy which remains unexplained. Axial heterogeneity in the expression of AQP3 and AQP4 along the collecting duct may be one explanation [81,84]. Thus AQP3 and AQP4 may function separately in different parts of the collecting duct, however, there is substantial overlap in the sites of expression. Although AQP3 and AQP4 are water-selective channels, they also transport other compounds to a limited degree (see above). Thus, another explanation may be ascribed to potential differences in function. No mutant phenotypes are yet known for AQP3 or AQP4, so their respective physiological functions remain speculative.

Aquaporin-5

The cDNA encoding this homolog was recently isolated from a rat submandibular gland library, and the mRNA was identified in salivary and lacrimal glands, corneal epithelium, and lung tissues [33•]. Preliminary studies indicate that this protein is abundant in the apical membranes of these tissues (S Nielsen, unpublished data). The presence of a protein kinase A consensus phosphorylation site in AQP5 suggests that it also may be under neurohormonal regulation, consistent with a

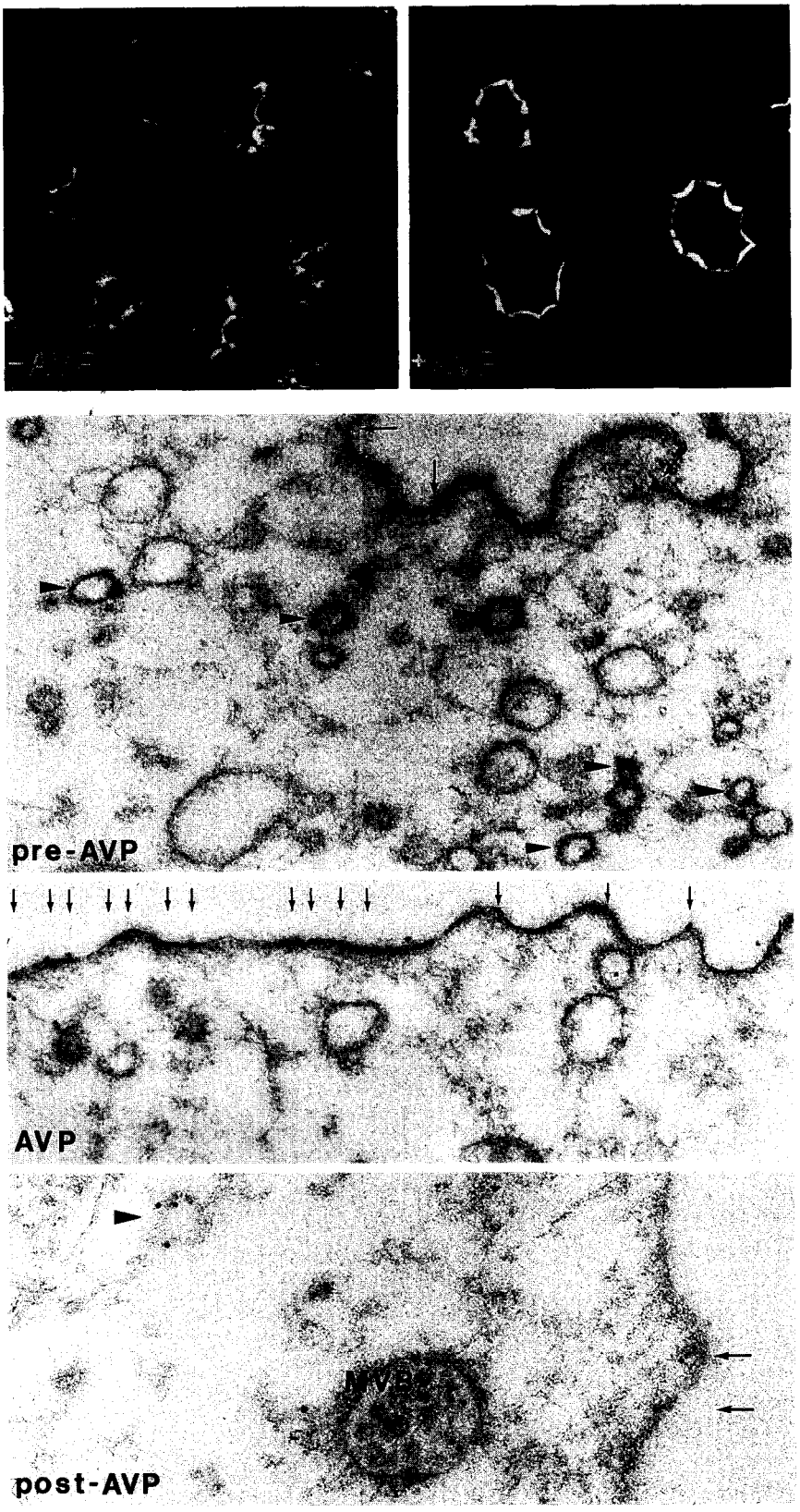

Fig. 3. Immunolabeling of AQP2 in kidney collecting ducts. Upper panels: immunofluorescence microscopy of inner medullary collecting ducts from Brattleboro rats before (–AVP) or after (+AVP) vasopressin-induced change in AQP2 localization to the subapical regions, adapted from Sabolic *et al.* [73]. Magnification ×450. Lower panels: immunogold electron microscopy of AQP2 in ultrathin sections (40 nm) from isolated, perfused inner medullary collecting ducts fixed in absence of vasopressin (pre-AVP), after 40 min exposure to AVP (AVP), and 40 min after washout of AVP from peritubular bath (post-AVP); adapted from Nielsen *et al.* [74•]. Magnification ×55 000. Arrows point to labeled apical plasma membrane and arrowheads to labeled vesicles. Vasopressin treatment results in a reversible increase in apical plasma membrane labeling of AQP2 in parallel with an increase in osmotic water permeability. MVB, multivesicular body.

major role in the secretion of tears, saliva, and sputum. Although no mutant phenotypes are yet known, it was hypothesized that the presence of an extracellular antigenic domain may be involved in some forms of

Sjögren's syndrome, an autoimmune disease affecting these tissues and causing lack of tear and saliva formation [33•].

Conclusions

Taken together, the studies reviewed here provide strong support to the hypothesis that members of the aquaporin family of membrane proteins play key roles in transmembrane water permeability in many mammalian tissues. Nevertheless, it should not be assumed that the importance of the recognized members of the aquaporin family is fully understood, and both critical thinking and impeccable experimental technique will be essential for further understanding of these fascinating proteins. Although efforts are needed to redress several published incompatibilities, it is likely that much future effort will be required to identify and characterize additional members of the aquaporin family and search for their involvement in clinical disorders. It is highly likely that the existing list of mammalian aquaporins is far from complete, and investigators are regularly finding numerous cDNAs within mammalian tissues. Thus, the molecular, cellular, and clinical characterization of the aquaporins may be in its infancy.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
- • of special interest
- •• of outstanding interest

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