Aquaporin Water Channels in Mammals

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Ten aquaporins have been cloned from various mammalian tissues. They are grouped according to their structure and function. The first group consists of 7 aquaporins; AQP0, 1, 2, 4, 5, 6, and 8. These channel molecules selectively transport water and do not transport glycerol and urea. The second group consists of 3 aquaporins; AQP3, 7, and 9. They transport not only water, but also small nonionic molecules such as glycerol and urea. The extensive tissue distribution and physiologic regulation by dehydration and hormones of these aquaporins suggest that aquaporins have important functions in water and solute transport in the body. However, the recent studies of knockout animals and humans with defective mutations of aquaporins showed unexpectedly small phenotypic effects. It is possible that other, unidentified aquaporins may compensate for these deficiencies. The future challenge of research in aquaporins should be the identification of their physiologic significance, and the discovery of new members, which will expand the research area of water metabolism and deepen our understanding of the physiology and pathophysiology of water transport in our body.

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The recent identification of a group of proteins, named aquaporins (AQPs), as highly water-permeating proteins in plasma membranes has opened a new field of research. This review focuses on recent progress in this field, especially concerning the aquaporins expressed in the kidney, and on 3 new members, AQP7, 8, and 9, recently cloned by us. Most reviews,[1,2] and even the latest review,[3] in this field discussed only the aquaporins from AQP0 to AQP5. Therefore, this review has the widest coverage of mammalian aquaporins. Information on the new aquaporins has made the reasons for their division into 2 groups much clearer,[4,5] and has suggested further analysis of some poorly studied areas of water-transport physiology; reproduction biology, and immunology.

Table 1 summarizes the properties of the mammalian water-channel family members cloned to date and deposited in the Gene Bank data base (http://www.ncbi.nlm.nih.gov/). They all belong to a channel family called the MIP family. Biochemical and structural studies indicate that each molecule consists of 6 transmembrane domains, with the amino- and carboxy-terminal ends located in the cell interior. Aquaporins consist of 2 repeats that are tandemly connected; each of which contains the conserved sequence motif of asparagine-proline-alanine (NPA box). This motif has been exploited for cloning new members of this family by using polymerase chain reaction-based homology cloning. Most of the aquaporins are reversibly inhibited by mercurials, with the exception of AQP4 and AQP7. The tissue distributions of the 10 aquaporins are summarized in Table 2. These distributions are based on the results of Northern blot analysis, and are not necessarily confirmed by immunohistochemical studies (results of rat and human studies have been combined, when available). The references cited in this review are limited only to the recent studies. The original and older references are easily found in the more extensive reviews of this field.[1–3]

CHARACTERISTICS AND TISSUE DISTRIBUTION

AQP0

AQP0 (Acc no: P30301) is the first member of the MIP family to be cloned. AQP0 is exclusively expressed in the fiber cell membranes of the eye lens, which constitute approximately 60% of the integral mem-

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Table 1. Characteristics of mammalian aquaporins.

Characteristic	Aquaporin									
	0	1	2	3	4	5	6	7	8	9
Protein size (amino acid)	263	269	271	292	301/323	265	282	269	263	295
mRNA size (kb)	1.4	2.8	1.9	1.8	5.5	1.6	2.2	1.5	1.5	3.5
N-linked glycosylation	–	+	+	+	–	+	–	–	+	+
Hg inhibition of water transport	–	+	+	+	–	+	+	–	+	+
Glycerol transport	±	–	–	+	–	–	?	+	–	?
PKA site	+	–	+	–	+	+	+	–	–	–
PKC site	+	+	+	+	+	+	+	+	+	+
Induction by dehydration	?	–	++	+	–	?	+	–	–	?
Exon numbers	4	4	4	6	4	4	4	6	?	6?
Gene locus	12q13	7p14	12q13	9p13	18q22	12q13	12q13	?	?	?

Hg, mercury; PKA, protein kinase A phosphorylation; PKC, protein kinase C phosphorylation; –, not present; ±, controversial; +, present; ++, strong induction; ?, unknown.

Table 2. Tissue distribution of mammalian aquaporins.

Characteristic	Aquaporin									
	0	1	2	3	4	5	6	7	8	9
Brain		+	–	+	++	–	–	–	–	–
Eye	+	+		+	+	+		±	–	+
Salivary gland		+		+	+	+		+	+	
Trachea		+		+	+	+		+	+	
Lung		+	–	±	+	++	–	–	–	+
Heart		+	–	–	+	–	–	+	–	–
Liver		+	–	+	–	–	–	–	+	+
Pancreas		–	–	+	+	–	–	–	+	–
Spleen		+	–	+	±	–	–	–	–	+
Small intestine		+	–	+	–	–		+	+	–
Colon		+		++	+	–		–	+	–
Kidney		+	++	++	+	–	+	+	–	–
Testis		–	±	–	–			++	++	–
Ovary		+	–	–	+	+		±	–	–
Placenta		+		–	–		–		+	–
Muscle		–		–	+		–			–
Leukocyte		–		±	–					++
Mammary gland						+		+		

–, absent; ±, very little; +, present; ++, abundant; blank, not known.

brane protein of the lens. Thus, it was originally named MIP26 (major intrinsic protein of 26 kD). In comparison to other aquaporins, AQP0 transports very little water when expressed in *Xenopus* oocytes.[6] Because a mutant AQP0 truncated at the carboxy-terminus resulted in no increase in water permeability, AQP0 itself may transport water. The reconstitution of AQP0 in planar lipid bilayers resulted in the formation of large, slightly anion-selective and voltage-dependent channels. However, the water permeability of these bilayers has not been critically examined. Interestingly, ion channel activity was not observed when AQP0 was expressed in oocytes. The water transport of AQP0 in oocytes is less effective, by approximately 42-fold, compared to that of AQP1, and its activation energy is approximately 7 kcal/mol. The mercurials had no effect on APQ0 water transport. Currently, the water channel activity of AQP0 is still controversial, and may not be its sole function.

The physiologic function of maintaining lens transparency can be explained by the molecular function of AQP0, because a mutant AQP0 caused cataracts in a mouse model.[7] Although AQP0 was identified more than 10 years ago, and functionally

examined extensively at that time, it was not identified as a member of the water channel family until 5 years ago. Therefore, previous studies should be carefully reexamined from the viewpoint that AQP0 is an aquaporin.

AQP1

AQP1 (P29975) is the first characterized water channel. It stimulates water transport when expressed in *Xenopus* oocytes, yeast vesicles, and mammalian cells, or when reconstituted into proteoliposomes. AQP1 is expressed widely in various epithelia and vascular endothelia. Because it is expressed in capillary endothelia, almost all organs express AQP1. However, the cellular localization of AQP1 may not be limited to vascular tissues. In the kidney, it is expressed at apical and basolateral membranes of proximal tubules and at the thin descending limb of the loop of Henle. It is also expressed in the endothelia of the vasa recta. Whether it is expressed at the glomerular capillary is controversial.

AQP1 is regarded as a constitutive water channel, and is not regulated by dehydration or by the antidiuretic hormone in the kidney. However, a recent report of AQP1 in cholangiocytes showed that expression was regulated by secretin.[8] Secretin induced an increase in osmotic water permeability of isolated rat cholangiocytes that was shown to be inhibited by mercuric chloride. Secretin increased AQP1 content in the plasma membrane, with a proportional decrease of AQP1 in intracellular membranes. Both the redistribution of AQP1 and the increase of membrane water permeability induced by secretin were inhibited by the microtubule blocker, colchicine, and by exposure to low temperatures. These observations suggest that secretin induces the microtubule-dependent targeting of AQP1-containing vesicles to the plasma membrane, which is similar to the regulation of AQP2 by antidiuretic hormone.

The water permeability by AQP1 has been shown to be stimulated by cyclic adenosine monophosphate-induced phosphorylation, although AQP1 itself does not have a consensus phosphorylation site by protein kinase A.[9] The same authors also reported that AQP1 acquires ion channel activity when stimulated by protein kinase A; however, the results are still controversial. Recent reports show that antidiuretic hormone stimulates, and atrial natriuretic peptide inhibits, AQP1-induced water permeability in oocytes. These observations seem to favor a cyclic adenosine monophosphate-dependent membrane shuttle mechanism for AQP1 regulation. However, these results should be confirmed by other studies examining its physiologic relevance.

AQP1 was shown to be transiently induced by platelet-derived growth factor in 3T3 fibroblastic cells as one of the delayed early-response genes.[10] The necessity of a water channel during cell division is not apparent. The process of the cell swelling by accumulation of water entering through a water channel may facilitate cell division, and merits further study of such a biologic role of aquaporins, especially AQP1. The previously identified aquaporin in bovine tissues that is highly related to AQP1, named CHIP29, seems to be the bovine orthologue of AQP1.

AQP2

AQP2 (L28112) is the first antidiuretic hormone-regulated water channel. AQP2 is localized at the apical membrane of the principal cells of collecting ducts. Water deprivation results in a drastic increase in messenger RNA and in the protein of AQP2. Antidiuretic hormone modulates AQP2 in 3 ways. First, the antidiuretic hormone stimulates *AQP2* gene transcription through a cyclic adenosine monophosphate-responsive element (CRE), which has been identified in the 5′ flanking region of the *AQP2* gene.[11] Second, the antidiuretic hormone induces redistribution of AQP2 from intracellular membranes to the apical plasma membrane in a microtubule-dependent manner by protein kinase A phosphorylation at the carboxy-terminus, specifically at the serine 256 site, whose mutation abolished this trafficking.[12] Third, the antidiuretic hormone stimulates water permeability in another protein kinase A-mediated manner, other than the exocytosis of AQP2, when expressed in oocytes.[13] However, the third observation is controversial, because in isolated kidney collecting duct vesicles, the direct phosphorylation of AQP2 by protein kinase A did not affect its water permeability.[14]

Recent efforts towards identifying the cellular machinery involved with regulation of membrane-vesicle trafficking are noteworthy. The different distributions of artificially expressed AQP2 between LLC-PK cells (porcine kidney) and MDCK cells (canine kidney) may be explained by different cellular components of this machinery. Another effort towards identifying the transcription factors that bind to the dehydration-responsive elements of AQP2 gene is still underway.

Until recently, the expression of AQP2 has only been shown in the kidney. However, AQP2 has now been shown to be present in the testis. Results of the initial Northern blot analysis of rat tissues had failed to show its presence in the testis, because expression of AQP2 in the testis is far less than that in the kidney. AQP2 was localized to the tails of late spermatids.[15] The physiologic significance of AQP2 expression in the testis remains to be clarified.

AQP3

AQP3 (P47862) is the first functionally unique water channel; it transports glycerol as well as water.

Although AQP0, 1, and 2 have been shown to transport glycerol by some investigators,[16] such transport was not observed consistently. Glycerol permeability has been confirmed by 3 independent discoverers of AQP3. The issue of water permeability of AQP3 was resolved when Yang and Verkman reported the comparison of 5 aquaporins water permeabilities in the oocyte system.[17] Although they initially reported no water permeability for AQP3, they finally confirmed its water permeability.

AQP3 is expressed at the basolateral membrane of the principal cells of the renal collecting ducts. The expression is mainly at the outer medulla to the cortex in the rat kidney. AQP3 expression at the inner medulla gradually decreases, where AQP4 is also expressed. In the colon, AQP3 is expressed at the basolateral membrane of the columnar epithelial cells at the surface. AQP3 is mainly expressed in the distal colon. Results of Northern blot analysis show that AQP3 is expressed widely in the gastrointestinal tract. However, immunohistochemical studies showed its presence only at the distal colon. Our initial report of AQP3 showed the presence of AQP3 messenger RNA in the spleen. A recent preliminary report showed AQP3 is present in red blood cells.[18] If this is confirmed, red blood cells should have at least 2 aquaporins, AQP1 and AQP3.

The physiologic significance of AQP3 in the body remains to be clarified. Although AQP3 seems to be constitutively expressed at the basolateral membrane, its expression is induced by dehydration in the kidney[19] and by glucocorticoid stimulation in pulmonary cell lines.[20] Although the induction by dehydration is smaller for AQP3 than that for AQP2, it has some physiologic relevance, especially in urine concentration, in view of the fact that AQP1, 4, and 7 are not induced in the kidney in dehydrating animals.

AQP4

AQP4 (P47863) is the first mercurial-insensitive water channel. This property is not unique to AQP4, as AQP7 is also mercurial insensitive. AQP4 is predominantly expressed in the brain, and is strongly expressed in cells lining the ventricular surface. These cells are not neurons, but glial cells; some are possibly neural stem cells.

The primary structure of AQP4 is close to that of the big brain (*bib*) gene of *Drosophila*. The loss of function of *bib* produces a brain twice as big as that found in the normal, wild-type fly. It is thought that *bib* may be important for the suppression of neural differentiation, because without *bib* function, all neural epithelial cells differentiate to neurons. Because the knockout mouse model did not show any gross neurologic defects in morphologic structure or physiologic function,[21] either AQP4 may not be the *bib* homologue, or the role of AQP4 may have changed in mammals.

AQP4 expression in the the kidney is restricted to the basolateral membrane of the inner medullary collecting duct cells. AQP3 and AQP4 are colocalized in some epithelial membranes, such as the collecting duct cells, colonic epithelia, and tracheal epithelia. Such redundant expression of aquaporins in the same membrane domain is intriguing, but is found in several tissues. For example, AQP1 and AQP7 are colocalized in the brush border membrane of proximal tubules. AQP2 and AQP7 are colocalized in the tail of late spermatids. AQP1 and AQP3 are colocalized in red blood cells. AQP1 and AQP8 are colocalized in the ductules of the small intestine. If the function of aquaporins is critical, such coexpression may suggest a safeguard against the loss of one of the aquaporins, to keep water permeability high. The reason for the relatively minor phenotypic changes of knockout animals and humans with defective aquaporins may lie in such redundant expressions of aquaporins.

Alternatively, functional and regulatory differences of the overlapping aquaporins may explain such redundancy. AQP4 is also unique in that its gene yields 2 distinct mRNA species corresponding to 2 polypeptides that differ at their amino-termini.[22] Both are functional aquaporins expressed in the same tissues, suggesting the presence of heterotetramers. The spliced-out, shorter form of AQP4 reported in the original study was not a functional water channel, although this was not confirmed by other researchers. Other aquaporins may have an alternatively spliced form, but only AQP4 is currently shown to have such isoforms.

AQP5

AQP5 (P47864) is expressed at apical membranes in exocrine glands, and does not seem to be expressed in the kidney, according to results of Northern and recent Western blot analyses.[23] As AQP5 is more closely related to AQP2 and is shown to have a protein kinase A consensus phosphorylation site, it is expected that the AQP5 molecule is translocated from intracellular vesicles to the apical membrane by neurohumoral stimulation. However, this was not the case with AQP5 expressed in the lacrimal gland.[24] Although tear secretion is stimulated by pilocarpine, the expression level of AQP5 in the apical membrane of pilocarpine-stimulated lacrimal glands was not increased. However, the carboxy-terminus region seemed to be modified (masked or altered structure) by pilocarpine, as suggested by the change in the immunohistologic response to the carboxy-terminus–recognizing antibody. The mechanism underlying the increase of water transport through AQP5 by neurohumoral stimulation may be a novel regulatory mechanism, which may be used by many aquaporins.

AQP6

AQP6 (Q13520) stimulates water transport very weakly, as is the case with AQP0, when expressed in *Xenopus* oocytes. AQP6-stimulated water transport, unlike that of AQP0, was inhibited by mercuric chloride.[25] It is selectively expressed in the kidney, and similarly in the cortex and medulla. Its exact localization inside the kidney has not been reported. Dehydration induced its expression, and AQP6 may participate in the urine-concentrating mechanism. It is surprising that AQP6 has attracted such limited interest from researchers in this field. Because water transport through AQP6 is small, other permeating molecules should be identified that may be related to the osmotic regulation of the kidney tubules.

AQP7

AQP7 (AB000507) has highest homology with AQP3, and stimulates urea and glycerol permeability, as well as that of water.[26] Unlike that of AQP3, the water permeability of AQP7 is mercury insensitive. Although rat AQP7 has a very short carboxy-terminus, human AQP7 has a long carboxy-terminus (Acc no: AB006190).[27] Such divergence of aquaporin structure in different species is unusual. Our preliminary examination of its genomic structure showed that this divergence cannot be explained by alternative splicing. The possibility of 2 highly related genes should be considered.

AQP7 is predominantly expressed in the testis, where AQP7 is localized to the mid and tail portions of late spermatids. AQP7 is also expressed in mature sperm, and may correspond to the functional water channel observed in human sperm, which is mercury insensitive. As the osmolarity of seminiferous tubules is hypertonic (385 mOsm/kg), water channels of late spermatids may facilitate the process of slimming the cytoplasm, especially in the tail portion, which is otherwise bulky. Recently, the role of a glycerol facilitator of yeast in cell fusion during mating has been reported.[28] If the osmotic state of a cell can regulate cell fusion, the role of AQP7 may lie in the process of sperm-egg fusion. AQP7 is also expressed in the kidney, although in small amounts when compared to its expression in the testis. Immunohistochemical analysis showed its expression at the brush border membrane of proximal tubules. AQP7 may compensate for the defect in AQP1 in the proximal tubules in Colton-null people.[29] The presence of AQP7 in adipose tissue has been documented.[27] Because AQP7 transports glycerol, its role in lipid metabolism is worthy of further investigation.

AQP8

AQP8 (AB005547) is a water-selective aquaporin, and its primary structure is unique among other aquaporins, especially in amino-terminus half. Its carboxy-terminus half is similar to plant AQP-γTIP, which is localized to intracellular tonoplast membranes. Therefore, the possibility that AQP8 is present at the intracellular membrane should be entertained. It is most abundantly expressed in the testis.[30] A preliminary immunohistochemistry of the testis showed that it is localized to the heads of late spermatids. It seems that sperm have at least 3 aquaporins: AQP2, 7, and 8. This unexpected complexity of sperm aquaporins calls for further analysis of the role of aquaporins in reproduction biology.

AQP8 is also expressed in small amounts in the liver, pancreas, placenta, small intestine, and salivary gland. In-situ hybridization showed its expression in hepatocytes and in the pancreas.[31] The absence of a functional water channel has been reported in isolated hepatocytes.[32] It is possible that AQP8 is localized at the intracellular membranes, or is limited to the membrane area of the biliary intercellular canaliculi of hepatocytes.

AQP9

AQP9 (AB008775) is the latest water channel to be discovered that also permeates urea. It has been cloned from liver cells, and may function as a urea transporter. Because liver tissue is the main producer of urea, the presence of some urea-exit mechanism from hepatocytes has been suspected. The 2 previously identified urea-transporter genes are not expressed in the liver. AQP9 is a good candidate for this urea-exit pathway. However, AQP9 is abundantly expressed in peripheral leukocytes. The lung and spleen, but not the thymus, express AQP9 in small amounts. Therefore, AQP9 is most likely expressed at a monocyte-level system.

AQP9 may be localized in the Kupffer cells in the liver. The functional water channel of leukocytes was not identified in previous studies.[33] However, the glycerol permeability of leukocytes has been shown, and it may be important for the cryopreservation of blood with glycerol. AQP9 may be important for cell-volume regulation in leukocytes, similar to the role suggested for AQP1 in red blood cells. A possible immunologic function for AQP9 in leukocytes should be investigated.

CLASSIFICATION OF AQUAPORINS

The phylogenetic analysis and the functional studies of aquaporins suggests that aquaporins can be divided into 2 groups.[5] Figure 1 shows the schematic phylogenetic tree of 10 mammalian aquaporins. The first group consists of 7 aquaporins; AQP0, 1, 2, 4, 5, 6, and 8. They selectively transport water, and usually do not transport glycerol and urea. The second group consists of 3 aquaporins; AQP3, 7, and 9. They not only transport water, but also transport glycerol and urea.

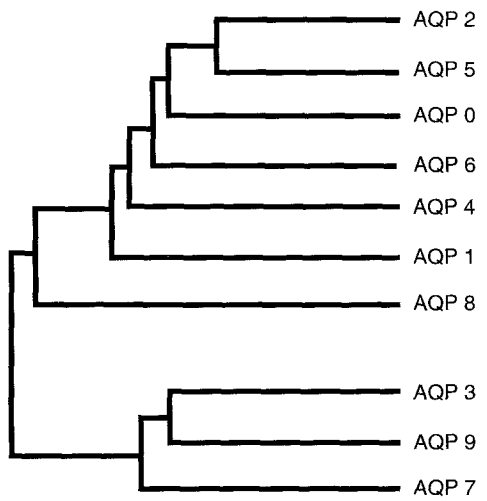

Fig. 1. Phylogenetic tree showing the two subgroups in mammalian aquaporins (Clustral method).

The molecular basis for this separation is currently not clear. Some speculate the presence of the separate pathways for water and small solutes.[34] However, our analysis with AQP3 showed that water and glycerol share a common pore, because both pathways were inhibited by mercuric chloride and by mutations introduced to the putative pore-forming region.[35] The definitive proof awaits the comparison of the 3-dimensional structure of AQP1 and AQP3. The 3-dimensional structure of AQP1 is currently known at approximately 6Å resolution from 3 laboratories.[36–38] The 3-dimensional structure of AQP3 is unknown.

The analysis of the human genome structures of aquaporins also supports this division of aquaporins. All members of each group share similar exon-intron boundaries within the group, with the exception of AQP8. This suggests that both groups originated from separate ancestors before the introduction of the intron. Furthermore, the chromosomal localization of human aquaporins showed that AQP0, AQP2, AQP5, and AQP6 are colocalized at chromosome 12q13, suggesting that they derived from gene duplication.[39] Although AQP8 is functionally similar to AQP1, its primary structure is deviated from that of AQP1, and our preliminary genome analysis of AQP8 showed a different structure of AQP8 compared to that of AQP1. Therefore, the first group can be further divided into 2 subgroups.

The reason for the dominant distribution of the first group is unclear. The primers used for polymerase chain reaction-based cloning may be favorable for the first group. However, all aquaporins of plants belong to the first group.[4] Therefore, there may be some selection bias for the first group. Such evolutionary constraints may be an important clue for the clarification of the physiologic significance of aquaporins.

SUMMARY AND PERSPECTIVE

Aquaporins are members of the MIP family, which is ubiquitously expressed in nature. *Arabidopsis thaliana* has more than 23 different MIP proteins, and at least 10 are aquaporins.[40] We may expect more members to be discovered in mammals. We assume that life processes need more aquaporins, because aquaporins play an important role as water transporters, and water is vital for living organisms. However, the recent knockout animals and individuals with defective mutations in aquaporins showed unexpectedly minor effects. People with defective AQP1 live a normal life, although their red blood cells have low water permeability. People with defective AQP2 have only polyuria and nephrogenic diabetes insipidus. Mice with a defect in AQP4 have only a minor defect in urine-concentra-ting ability.[21]

It is possible that the other unidentified aquaporins may compensate for these defects. Alternatively, the relatively high water permeability of plasma membranes due to the presence of solute transporters[41] and channels[42] may have minimized the need for specialized water channels. Therefore, the challenge for the future research in aquaporins should be not only to find unidentified new molecules that may be unrelated to the current aquaporins, but more importantly, to clarify the physiologic and pathologic significance of each aquaporin. The results from the studies with lower animals, plants, or even bacteria, may shed light on the role of this ancient, well-conserved protein family.

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