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Aquaporins: water channel proteins of the cell membrane

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Abstract

Aquaporins (AQP) are integral membrane proteins that serve as channels in the transfer of water, and in some cases, small solutes across the membrane. They are conserved in bacteria, plants, and animals. Structural analyses of the molecules have revealed the presence of a pore in the center of each aquaporin molecule. In mammalian cells, more than 10 isoforms (AQP0–AQP10) have been identified so far. They are differentially expressed in many types of cells and tissues in the body. AQP0 is abundant in the lens. AQP1 is found in the blood vessels, kidney proximal tubules, eye, and ear. AQP2 is expressed in the kidney collecting ducts, where it shuttles between the intracellular storage sites and the plasma membrane under the control of antidiuretic hormone (ADH). Mutations of AQP2 result in diabetes insipidus. AQP3 is present in the kidney collecting ducts, epidermis, urinary, respiratory, and digestive tracts. AQP3 in organs other than the kidney may be involved in the supply of water to them. AQP4 is present in the brain astrocytes, eye, ear, skeletal muscle, stomach parietal cells, and kidney collecting ducts. AQP5 is in the secretory cells such as salivary, lacrimal, and sweat glands. AQP5 is also expressed in the ear and eye. AQP6 is localized intracellular vesicles in the kidney collecting duct cells. AQP7 is expressed in the adipocytes, testis, and kidney. AQP8 is expressed in the kidney, testis, and liver. AQP9 is present in the liver and leukocytes. AQP10 is expressed in the intestine. The diverse and characteristic distribution of aquaporins in the body suggests their important and specific roles in each organ.

Keywords: Antidiuretic hormone; AQP; AQP1; AQP2; AQP3; Aquaporin; Cell membrane; Epithelium; Kidney; Knockout experiment; Secretion; Water channel; Water transfer

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1. Contents

2. Introduction

Water is the most abundant and basic molecule in a cell, a unit of life. The cell separates its intracellular milieu from the external environment by the plasma membrane, whose basic structure is the phospholipid bilayer. By separating the interior of the cell from the harsh outside, the optimal environment of the cell interior, i.e., pH, ionic strength, concentration of inorganic ions, etc. is thus maintained. The phospholipid bilayer is basically permeable to small polar molecules, and water can slowly permeate the bilayer by simple diffusion. In addition, specialized integral membrane protein channels that facilitate the efficient permeation of water across the biomembranes have evolved. Such water channel proteins have been found in organisms ranging from bacteria to plants and animals, and named aquaporins (AQPs) (Agre et al., 1993b). In addition to water, aquaporins serve in the permeation of small molecules such as glycerol, urea, and even ions in special cases. Since multiple isoforms of aquaporins are differentially expressed in cells and tissues, their localization to specific cells and membrane domains is critical in understanding the role of each AQP in the transfer of water and small solutes. Histochemical examination therefore has played a pivotal role in the advancement of the AQP studies. This review describes the specific character, localization, and possible roles of each AQP isoform in mammalian cells. In addition, pathological aspects of AQPs are overviewed. In non-mammalian animal cells, plant cells, and bacteria, many aquaporins have been identified and studied, and they were reviewed and discussed elsewhere (Chrispeels et al., 1994; Park and Saier, 1996; Ishibashi et al.,

1998a; Hohmann et al., 2000; Johansson et al., 2000; Chaumont et al., 2001; Maurel et al., 2002).

3. Aquaporins

Identification and characterization of the water channel proteins and their homologues revealed that they form a large family of integral membrane proteins structurally related to a major intrinsic protein of mammalian lens MIP26 (Agre et al., 1993a, 1995, 1998, 2002; Verkman et al., 1996, 2000b; Sasaki et al., 1998; Ishibashi and Sasaki, 1998; Borgnia et al., 1999; Engel et al., 2000; Ishibashi and Sasaki, 2000b; Matsuzaki et al., 2002) (Table 1). Since water channel activity was found to be the most prominent physiological function, the name aquaporins (AQP) was proposed by Agre, et al. (1993b). They noted that not all proteins structurally related to MIP26 share water channel activity, and the term aquaporins should not be used to describe proteins that facilitate the movement of ions or other molecules such as glycerol. At that time, the channel-forming integral protein of 28 kDa (CHIP28), water channel of collecting duct (WCH-CD), and γ-tonoplast intrinsic protein (γ-TIP) were the major members of the aquaporin family. MIP26 and a glycerol facilitator of *Escherichia coli* GlpF were not included in the family. The term aquaporins is currently used to describe all these structurally related molecules, and now they are regarded as members of aquaporins. The term aquaglyceroporin is often used to identify those molecules with glycerol facilitator activity. Therefore, the term aquaporin is used in two ways. One is in a narrow sense as originally envisaged; aquaporins are restricted to the channel proteins permeable only to water. The other is in a broad sense; the term aquaporins includes all the structurally related proteins and hence, includes not only aquaporins in a narrow sense plus aquaglyceroporins but also all structurally related proteins of unknown functions.

4. Structure of aquaporin molecule

4.1. Hourglass model and tetrameric structure

Aquaporin is composed of a single peptide chain of approximately 270 amino acids. The deduced amino acid sequence of AQP1 predicted six membrane spanning domains with intracellular N and C termini (Preston et al., 1991) (Fig. 1). Other aquaporin molecules have a similar molecular configuration. The transmembrane orientation of the molecule was further confirmed using antibodies to each terminus and loop domains (Stamer et al., 1996). Two halves of AQP1 molecules are sequence-related and probably evolved by gene duplication. A single N-glycosylation site is present in the second extracellular loop (loop C). Non-glycosylated form as well as glycosylated form of apparently high molecular weight are usually detected

Table 1. Distribution of aquaporins in the mammalian body

AQP	Organ	Tissue/cell	References
AQP0	Eye	Lens fiber cell	Fitzgerald et al., 1983
AQP1	Kidney	Proximal tubule	
		Descending thin limb of Henle's loop	Denker et al. (1988), Maunsbach et al. (1997), Sabolic et al. (1992), Nielsen et al. (1993c, 1995c), Hasegawa et al. (1994b)
		Vasa recta(endothelial cell)	Sabolic et al. (1992)
	Blood vessel	Endothelial cell	Sabolic et al. (1992), Nielsen et al. (1993b), Hasegawa et al. (1994a), Nielsen et al. (1997a)
	Lymphatic vessel	Endothelial cell	Nielsen et al. (1993b), Koyama et al. (1999)
	Gall bladder	Epithelial cell	Nielsen et al. (1993b); Hasegawa et al. (1994a)
	Pancreas	Interlobular duct	Ko et al. (2002), Furuya et al. (2002)
		Acinar cells	Cho et al. (2002)
	Airway	Endothelial cell	Nielsen et al. (1993b), Nielsen et al. (1997a)
	Lung	Endothelial cell	Nielsen et al. (1993b), Nielsen et al. (1997a)
	Peritoneum	Capillary (endothelial cell)	Carlsson et al. (1996); Devuyst et al. (1998)
		Mesothelium	Lai et al. (2001)
	Efferent duct	Non-ciliated cell	Brown et al. (1993)
	Vas deferens, seminal vesicles, proximal parts of the vas deferens		Brown et al. (1993)
	Brain	Choroid plexus (epithelial cell)	Hasegawa et al. (1993), Nielsen et al. (1993b), Hasegawa et al. (1994a)
	Eye	Ciliary body (non-pigmented epithelial cell)	Nielsen et al. (1993b), Hasegawa et al. (1994a), Hamann et al. (1998)
		Iris (posterior and anterior epithelia)	Nielsen et al. (1993b), Hasegawa et al. (1994a), Hamann et al. (1998)
		Trabecular meshwork	Hamann et al. (1998)
		Lens (epithelium)	Nielsen et al. (1993b), Hasegawa et al. (1994a)
		Cornea (endothelium)	Nielsen et al. (1993b)

Table 1 (*continued*)

AQP	Organ	Tissue/cell	References
	Ear	Organ of Corti	Stankovic et al. (1995), Sawada et al. (2003)
		Vestibule	Stankovic et al. (1995), Sawada et al. (2003)
AQP2	Kidney	Collecting duct (principal cell)	Fushimi et al. (1993), Nielsen et al. (1993a), Nielsen et al. (1995a), Sasaki et al. (1994)
	Vas deferens	Epithelium (principal cell)	Stevens et al. (2000)
	Ear	Organ of Corti	Mhatre et al. (2002a)
AQP3	Kidney	Collecting duct (principal cell)	Ishibashi et al. (1994), Ma et al. (1994), Echevarria et al. (1994), Ecelbarger et al. (1995)
		Renal pelvis	Matsuzaki et al. (1999a)
	Ureter	Epithelium (basal and intermediate cells)	Matsuzaki et al. (1999a)
	Urinary bladder	Epithelium (basal and intermediate cells)	Matsuzaki et al. (1999a)
	Urethra	Epithelium (basal and intermediate cells)	Matsuzaki et al. (1999a)
	Oral cavity	Epithelium (basal and intermediate cells)	Matsuzaki et al. (1999a)
	Esophagus	Epithelium (basal and intermediate cells)	Matsuzaki et al. (1999a)
	Stomach		Matsuzaki et al. (1999a)
	Ileum		Matsuzaki et al. (1999a)
	Colon		Matsuzaki et al. (1999a)
	Nasal cavity	Epithelium (basal and intermediate cells)	Matsuzaki et al. (1999a)
	Trachea	Epithelium (basal cell)	King et al. (1997), Nielsen et al. (1997a), Matsuzaki et al. (1999a)
	Lung	Bronchus	King et al. (1997), Nielsen et al. (1997a), Matsuzaki et al. (1999a)
	Skin	Epidermis (basal and intermediate cells)	Matsuzaki et al. (1999a)
		Hair follicle	Matsuzaki et al. (1999a)
	Brain	Ependyma	Ma et al. (1994)
	Eye	Conjunctiva	Hamann et al. (1998)

Table 1 (*continued*)

AQP	Organ	Tissue/cell	References
	Ear	Organ of Corti (spiral ligament, inner spiral tunnel)	Huang et al. (2002)
		Vestibules	Huang et al. (2002)
AQP4	Kidney	Collecting duct (principal cell)	Frigeri et al. (1995a, b)
	Skeletal muscle	Fast-twitch muscle	Frigeri et al. (1998)
	Stomach	Parietal cell	Misaka et al. (1996), Koyama et al. (1999)
	Nasal cavity	Ciliated cell	Nielsen et al. (1997a)
	Trachea		Nielsen et al. (1997a)
	Lung	Bronchus	Nielsen et al. (1997a), Kreda et al. (2001)
	Brain	Astrocyte	Nielsen et al. (1997b)
	Eye	Retina (Müller cell)	Nagelhus et al. (1998), Hamann et al. (1998)
		Ciliary body (non-Pigmented epithelial cell)	Hamann et al. (1998)
	Ear	Organ of Corti (supporting cell)	Minami et al. (1998), Takumi et al. (1998), Li et al. (2001)
		Vestibules (ciliated cell)	Mhatre et al. (2002b) Minami et al. (2001)
AQP5	Salivary gland	Secretory cell	Nielsen et al. (1997a), He et al. (1997), Funaki et al. (1998), Matsuzaki et al. (1999b)
	Stomach	Pyrolic gland	Matsuzaki et al. (2003)
	Duodenum	Duodenal gland	Matsuzaki et al. (2003)
	Pancreas	Intercalated duct cell	Burghardt et al. (2003)
	Airway	Glandular cell	
	Lung	Type I pneumocyte	Nielsen et al. (1997a)
	Sweat gland		Nejsum et al. (2002)
	Eye	Cornea	Raina et al. (1995), Hamann et al. (1998)
	Lacrimal gland	Glandular cell	Ishida et al. (1997), Matsuzaki et al. (1999b)
	Ear	Organ of Corti	Mhatre et al. (1999)
AQP6	Kidney	Collecting duct (intercalated cell)	Yasui et al. (1999a), Yasui et al. (1999b)

Table 1 (*continued*)

AQP	Organ	Tissue/cell	References
AQP7	Kidney	Proximal tubule (S3)	Ishibashi et al. (2000a), Nejsum et al. (2000)
	Adipose tissue	Adipocyte	Kishida et al. (2000)
	Immune system	Dendritic cell	
	Testis	Seminiferous tubules (spermatid)	Ishibashi et al. (1997b), Suzuki-Toyota et al. (1999)
	Ear	Organ of Corti (supporting cells)	Huang et al. (2002)
		Vestibule	Huang et al. (2002)
AQP8	Kidney	Proximal tubule	Elkjaer et al. (2001)
		Collecting duct	
	Salivary gland	Glandular cell	Koyama et al. (1997), Wellner et al. (2000)
		Myoepithelial cell	Elkjaer et al. (2001)
		Duct cell	Elkjaer et al. (2001)
	Liver	Hepatocyte	Ishibasi et al. (1997a), Koyama et al. (1997), Calamita et al. (2001a), Tani et al. (2001)
	Pancreas	Glandular cell	Hurley et al. (2001), Tani et al. (2001)
	Intestine	Epithelial cell	Elkjaer et al. (2001), Tani et al. (2001)
	Airway	Myoepithelial cell	Elkjaer et al. (2001)
	Testis	Seminiferous tubules (spermatogenic cells, Sertoli cell)	Ishibasi et al. (1997a), Elkjaer et al. (2001), Calamita et al. (2001b)
AQP9	Liver	Hepatocyte	Tsukaguchi et al. (1998)
	Testis	Seminiferous tubule	
		Leydig cells	Tsukaguchi et al. (1998), Nicchia et al. (2001)
	Epididymis		Pastor-Soler et al. (2001)
	Vas deferens		Pastor-Soler et al. (2001)
	Brain	Astrocytes	Tsukaguchi et al. (1998)
		Circumventricular organs	Nicchia et al. (2001)
	Leukocyte		Tsukaguchi et al. (1998)
	Ovary	Oocyte	Ford et al. (2000)
	Digestive tract	Goblet cell	Okada et al. (2003)
	Ear	Organ of Corti	Huang et al. (2002)
		Vestibule	Huang et al. (2002)
	Placenta	Syncytiotrophoblast	Damiano et al. (2001)
AQP10	Intestine	Epithelial cell	Hatakeyama et al. (2001), Morinaga et al. (2002)

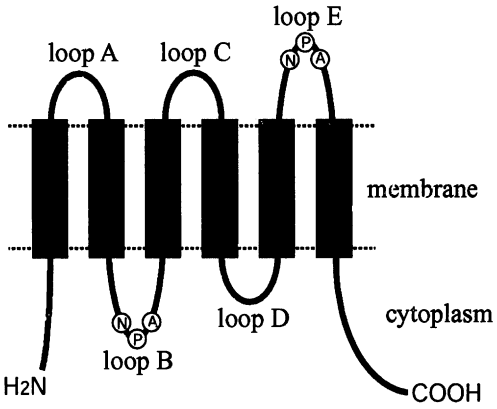


Fig. 1. Schematic representation of the structure of aquaporins. Each aquaporin isoform is composed of a single polypeptide chain of approximately 270 amino acids, and spans the membrane six times. The amino- and carboxyl-terminal ends are both in the cytoplasm and there are three extracellular loops (loops A, C, and E) and two intracellular loops (loops B and D). Highly conserved regions are present within the loops B and E, each of which contains the consensus motif, asparagine–proline–alanine (NPA; single letter code for amino acid). Reproduced from Matsuzaki et al. (2002) with permission from Blackwell Publishing.

by immunoblotting (Fig. 2). Water transport activity of AQP1 is inhibited by mercurial ion, and the site-directed mutagenesis revealed that cysteine 189 in the fifth hydrophilic loop (E-loop) is responsible for this mercurial sensitivity (Preston et al., 1993). The first intracellular loop (loop B) and the third extracellular loop (loop E) have highly conserved NPA (asparagine–proline–alanine) motifs (NPA boxes). When these two loops are folded into the lipid bilayer, connected in the center of the bilayer, and surrounded by transmembrane domains, they may form a hydrophilic path for water transfer through the lipid bilayer (Jung et al., 1994). This model is called an ''hourglass model'', and crystallographic studies later proved this basic design of the molecule.

Electron microscopic examination of the freeze fracture replica of the proteoliposomes reconstructed with purified AQP1 formed tetramers, indicating that AQP1 is present in the plasma membrane as tetramers (Verbavatz et al., 1993).

4.2. Crystallographic examination of AQP1 molecule and the comparison with GlpF

Electron microscopic examination of the frozen two-dimensional crystals of human AQP1 molecules revealed its molecular structure at 6–7 Å resolution (Cheng et al., 1997; Walz et al., 1997). As expected from the hourglass model, AQP1 has six tilted bilayer-spanning α-helices and a central pore-like region. Improvement of the resolution up to 3.8 Å (Murata et al., 2000) or 3.7–4 Å (Ren et al., 2000, 2001) revealed the detail of the pore region of the molecule that water molecules pass. Each

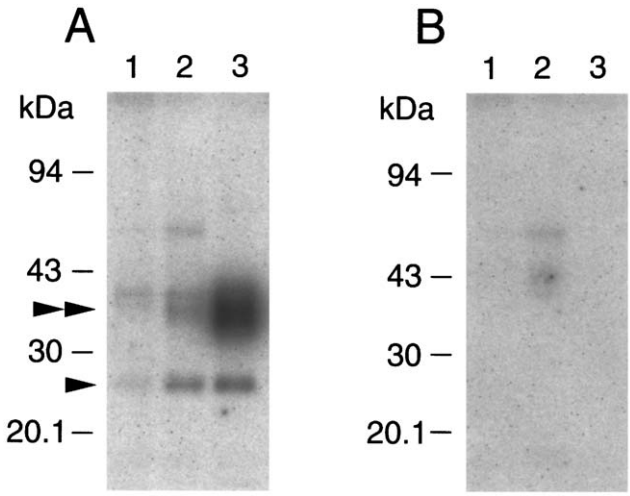


Fig. 2. Immunoblotting of rat tissues with antibody against AQP3. AQP3 is detected in the forestomach (lane 1), palmar skin (lane 2), and kidney inner medulla (lane 3). Non-glycosylated form of AQP3 (26 kDa; arrowhead) and glycosylated form (32–40 kDa; double-arrowhead) are detected (A). Both bands disappear when anti-AQP3 antibody was preincubated with COOH-terminus peptide of AQP3 protein used as immunogen (B). Reproduced from Matsuzaki et al. (1999a) with permission from the Histochemical Society.

aquaporin monomer has a central funnel-shaped opening at the extracellular and cytoplasmic faces. At each end of the funnels stretches a narrow tunnel for the selective transfer of water across the center of the molecule. A refined model was further presented by comparing the model from the high-resolution X-ray structures of the homologous bacterial glycerol transporter GlpF from *E. coli* (de Groot et al., 2001). The structure of AQP1 was clearly shown by the X-ray crystallographic analysis of a bovine AQP1 molecule at 2.2 Å resolution (Sui et al., 2001). The pore or filter region of AQP1 is a narrow tunnel. Four bound water molecules are localized in the pore along three hydrophilic nodes, which punctuate an otherwise extremely hydrophobic pore, and a minimal number of solute binding sites along the hydrophobic channel pore facilitate rapid water transport (Sui et al., 2001). The structure of the pore indicates that the transport of protons through AQP1 is energetically unfavorable.

The analyses of the molecular structure of glycerol facilitator GlpF were carried out in parallel with those of AQP1. Cryoelectron microscopic (Braun et al., 2000) and X-ray diffraction studies (Fu et al., 2000) revealed its molecular structure. The structure of the pore of the GlpF shows its selective permeability for linear carbohydrates and indicates how ions and water are excluded (Fu et al., 2000). The elucidation of the molecular architecture of aquaporins has shed light on the mechanism of substrate specificity in AQP1 and GlpF at the molecular level (de Groot and Grubmuller, 2001). Further simulation studies of the permeation of water

through AQP1 molecule revealed that the specificity to water is primarily due to the size-exclusion effect of the pore (Kong and Ma, 2001). In addition, the locations of asparagine 76 and asparagine 192 in the conserved NPA motifs seem to provide necessary hydrogen-bonding interactions to maintain the water connectivity in the narrow constriction region of the pore. Similar results were obtained in GlpF molecules (Tajkhorshid et al., 2002).

5. AQP0

5.1. Cloning of AQP0

AQP0 was originally identified as a major intrinsic protein of 26 kDa (MIP, also called MIP26) in the lens. cDNA of bovine major intrinsic protein (MIP) was cloned and sequence data suggest that it passes the lipid bilayer six times with both C- and N-termini on the cytoplasmic side (Gorin et al., 1984). Identification of other homologous molecules of MIP revealed a large family of aquaporins (Ishibashi et al., 1998a).

5.2. Localization of AQP0

Expression of AQP0 is restricted to the lens. AQP0 is abundant in the lens fibers that are composed of compactly packed fiber-shaped cells of the epithelial origin. Immunoelectron microscopic examination on ultrathin frozen sections of the rat lens showed that AQP0 is distributed throughout the plasma membrane of the lens fiber cells (Fitzgerald et al., 1983). AQP0 is not present in the basal or lateral plasma membrane of the lens epithelial cells. No apparent difference of the distribution of AQP0 was observed between the junctional and non-junctional membranes. Detailed immunoelectron microscopic analysis using freeze-fracture-labeling technique for the identification of individual intramembranous particles revealed that AQP0 does not distribute at the plasma membrane uniformly. Rather, AQP0 constitutes microdomain structures at the plasma membrane (Zampighi et al., 2002). One type of microdomain is the mixed presence of AQP0 with other membrane proteins, although the density of AQP0 varies depending on the region of the cell. The second type is the localization of AQP0 within the gap junctions, i.e., the formation of mixed junctions. The third type is the "AQP0 junction" where clusters of AQP0 abut against an opposing particle-free membrane. The formation of microdomains in the lens fiber plasma membrane suggests that AQP0 may play roles other than water transport.

5.3. Function of AQP0

Because of its structural similarity to water-permeable aquaporins, channel activity for water and small solutes was expected. In a *Xenopus* oocyte expression

system, AQP0 exhibits osmotic water permeability in a mercurial-insensitive manner (Mulders et al., 1995; Chandy et al., 1997; Varadaraj et al., 1999). The water permeability of AQP0 is regulated by pH and Ca^{2+} (Nemeth-Cahalan and Hall, 2000). Because of its abundance and specialized distribution at the plasma membrane, a possible role of AQP0 in cell adhesion was suggested in addition to channel activity (Zampighi et al., 2002). Membrane sheets reconstituted from purified ovine lens AQP0 mostly consist of two layers. Examination by atomic force microscopy and cryoelectron microscopy revealed that AQP0 is arranged in precise register and apposing AQP0 molecules fit in a tight tongue-and-groove manner, suggesting its possible role in cell-to-cell adhesion in addition to water transport (Fotiadis et al., 2000). Although several functions such as gap junction, membrane ion channel, and membrane water channel have been proposed for the possible physiological role of AQP0, measurement of these activities in lens fibers in wild mice and cataract Frasner mice with mutated AQP0 revealed that water channel activity is reduced in the mutated AQP0 (Varadaraj et al., 1999). This result suggests that water channel is the only activity in vivo.

5.4. Mutation of AQP0 and cataract

Mutation of AQP0 results in congenital cataract (Shiels and Bassnett, 1996; Berry et al., 2000; Francis et al., 2000a; Okamura et al., 2003). In mice, cataract Frasner mutation is a transposon-induced splicing error that substitutes a long terminal repeat sequence for the C-terminus of AQP0 (Shiels and Bassnett, 1996). The lens opacity mutation is a single amino-acid substitution that inhibits targeting of AQP0 to the plasma membrane. These AQP0 mutants suggest that AQP0 plays a crucial role in the development of a transparent lens (Shiels and Bassnett, 1996). Cataract Tohoku is also a dominant cataract mutation with mutated AQP0, and targeted expression of mutated AQP0 revealed that it is localized intracellularly and causes opacity in transgenic mice (Okamura et al., 2003).

In humans, a point mutation of E134G or T138R results in a dominantly inherited cataracts (Berry et al., 2000). When the mutated AQP0 was expressed in *Xenopus* oocytes, loss of water channel activity due to impaired trafficking of mutated proteins was observed (Francis et al., 2000b). Coexpression of the mutated AQP0 with wild-type AQP0 exhibited dominant-negative behavior (Francis et al., 2000b). These observations indicate that plasma membrane localization of AQP0 and its water channel activity is important in maintaining the lens fiber characteristics, whose defect leads to cataract.

In the cataract Frasner mouse lens, mutated AQP0 is retained intracellularly. Scanning electron microscopy of a heterozygous cataract Frasner lens revealed the disorganization of the lens fibers resulting in disorganization of the optical axis (Shiels et al., 2000). The lens showed loss of uniform fiber cell stratification and aberrant end-curvature with irregularly swollen fibers, suggesting that AQP0 regulates the unique cellular architecture of the lens.

In AQP0-null mice, the formation of polymorphic opacities was observed (Shiels et al., 2001). Heterozygotes eyes were also affected. Reduction of osmotic water

permeability in the lens cells was observed together with the reduction of the focusing power. Ultrastructural examination revealed that heterozygote and homozygote lens lack the ordered packing arrangement of lens fibers (Al-Ghoul et al., 2003). These observations show that AQP0 plays an important role in maintaining the lens fiber structure, the loss of which seems to be related to the formation of cataract.

6. AQP1

6.1. Identification, cloning, and characterization

A novel integral membrane protein of Mr. 28 kDa was identified from the human erythrocyte ghost during the isolation of the 32-kDa Rh polypeptides (Denker et al., 1988). The antibodies to this 28-kDa protein showed that both the 28-kDa form and N-glycosylated form of higher molecular weight are present in the erythrocyte membrane. By immunohistochemical staining, this antigen has been shown to be present in the kidney proximal tubules. cDNA cloning of this 28-kDa integral membrane protein revealed that it has six-membrane spanning domains and intracellular N and C termini (Preston and Agre, 1991). The human AQP1 gene was identified and is located at chromosome 7p14 (Moon et al., 1993). Comparison of the sequence data indicates that it exhibits strong homology with the major intrinsic protein of bovine lens (MIP; AQP0) (Gorin et al., 1984). Similarities to membrane proteins in plants and bacteria (Baker and Saier, 1990) suggest that it is a membrane channel protein, and is hence named a "channel-like integral protein of 28 kDa" (CHIP28), and later AQP1 (Preston and Agre, 1991). The rat homologue of human CHIP28 was also cloned (Deen et al., 1992; Zhang et al., 1993). When expressed in *Xenopus* oocytes, CHIP28 increased the mercurial-sensitive osmotic water permeability, showing that CHIP28 is a water channel protein (Preston et al., 1992). Proteoliposomes reconstituted with CHIP28 solubilized and purified from human erythrocytes showed high osmotic water permeability (van Hoek and Verkman, 1992). Expression of AQP1 in cultured cells such as CHO cells also showed a selective increase of plasma membrane osmotic water permeability (Ma et al., 1993b). These studies demonstrate that CHIP28 is the human erythrocyte water channel.

6.2. AQP1 in the kidney

6.2.1. Localization in the kidney

Erythrocyte and kidney exhibit very high osmotic water permeability and the presence of a water channel was suggested. When AQP1 protein (CHIP28) was identified, antibodies were raised and immunohistochemical staining was carried out in the human kidney specimens (Denker et al., 1988; Maunsbach et al., 1997). AQP1 is present in the apical and basolateral surfaces of the proximal convoluted tubules

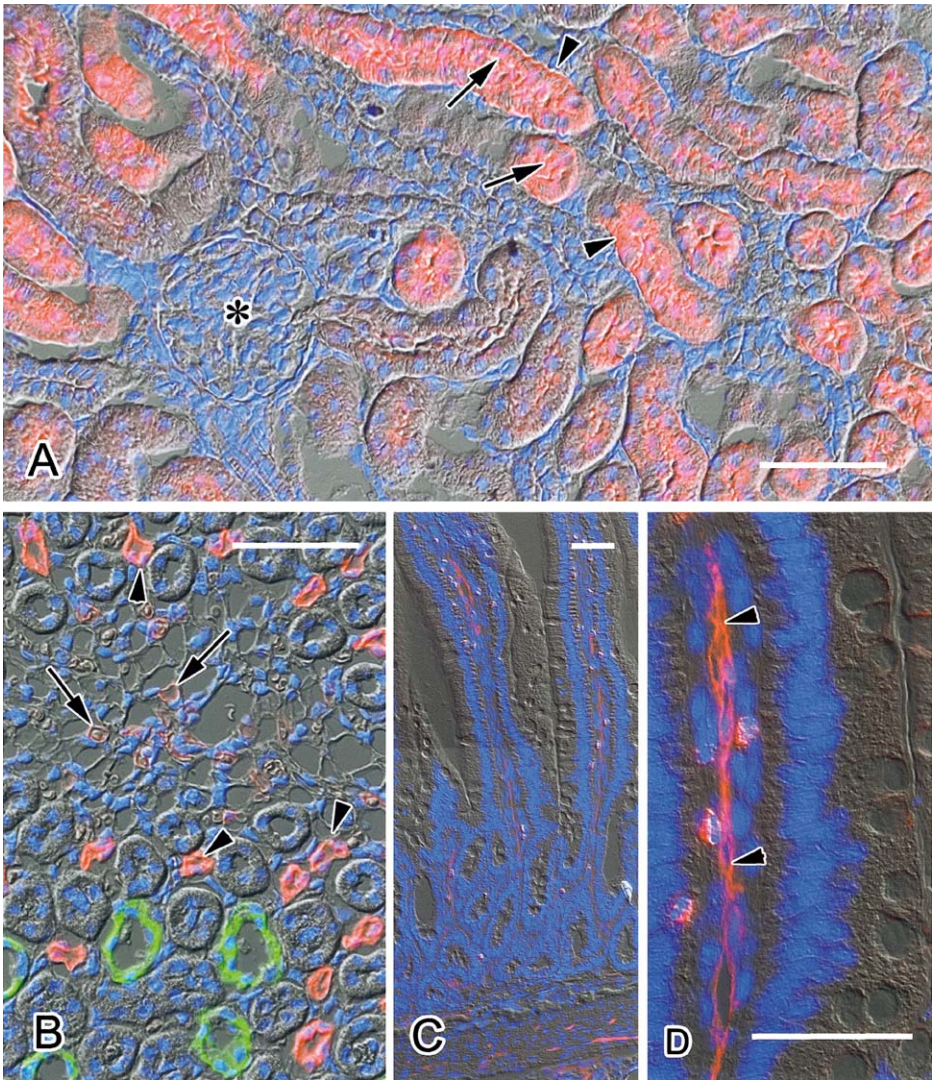


Fig. 3. AQP1 in the kidney and duodenum in the rat. Immunofluorescence images of AQP1 (red) are projected onto Nomarski images. Nuclei are counterstained with DAPI (blue). (A) Kidney cortex. AQP1 is expressed in the proximal tubules, where it is localized in the apical (arrows) and basolateral (arrowheads) membranes. *, glomerulus. (B) Kidney medulla. AQP1 is localized in the thin descending limb of Henle's loop (arrowheads) and blood vessels (arrows). Note that AQP3 (green) is expressed in the collecting ducts. (C) AQP1 in the duodenum. AQP1 is seen in the vessels in the lamina propria and submucosa. (D) AQP1 in the lymphatic vessels in the center of the lamina propria of the villus in the duodenum (arrowheads). Bars: 50 μm.

(Fig. 3A). AQP1 is also present in the apical and basolateral surfaces of the descending thin limb of Henle's loop, but is absent in other parts of the nephrons. In the rat kidney, similar results were obtained (Sabolic et al., 1992; Nielsen et al., 1993c, 1995c; Hasegawa et al., 1994b) (Fig. 3B).

Using microdissected tubules, an abundance of AQP1 was measured in various parts of the nephron by fluorescence-based ELISA (Maeda et al., 1995). Abundant AQP1 was detected in the proximal tubules and descending limb. On the other hand, AQP1 was undetectable in other parts of the nephron such as the ascending thin and thick limb, distal convoluted tubules, connecting tubules, and collecting ducts. In situ hybridization also confirmed the expression of AQP1 in these regions (Hasegawa et al., 1993). The localization of AQP1 is in accordance with the known water permeability characteristics of the kidney, supporting that AQP1 is mainly responsible for osmotic water permeation in these regions of the kidney.

AQP1 is an abundant protein in the proximal tubule cells in that it comprises 3.8% of the isolated proximal tubule brush border protein (Nielsen et al., 1993c). Since AQP1 is absent in the collecting duct where water absorption is regulated by antidiuretic hormone, AQP1 is responsible for the constitutive water reabsorption (Nielsen et al., 1993c).

In addition to uriniferous tubules, AQP1 is present in the vasa recta (Sabolic et al., 1992). Detailed immunoelectron microscopic examination in the rat kidney revealed that AQP1 is selectively localized in the non-fenestrated endothelium of descending vasa recta, whereas the fenestrated endothelium of ascending vasa recta and peritubular capillaries do not express AQP1 (Nielsen et al., 1995c). In the AQP1-null mice, NaCl-driven water permeability is markedly reduced in the outer medullary descending vasa recta in microperfusion measurement, suggesting that AQP1 in vasa recta is essential in concentrating urine (Pallone et al., 2000).

When AQP1 is expressed in polarized epithelial cells such as MDCK and LLC-PK_1 cells, AQP1 is localized to both the apical and basolateral domains and exhibits high transcellular osmotic water permeability sensitive to mercurial compounds (Deen et al., 1997).

6.2.2. Kidney in AQP1-null mouse and human

In order to analyze the role of AQP1 in the water absorption for the urine formation, AQP1-null mice were generated by targeted gene disruption (Ma et al., 1998). The osmotic water permeability in the proximal tubule membrane vesicles is reduced 8-fold compared with that from the wild mice. The AQP1-null mice lost body weight after water deprivation and became severely dehydrated due to the impaired urine concentrating ability. Microperfusion and in vivo micropuncture measurements in the proximal tubule and thin descending limb of Henle's loop in AQP-null mouse revealed decreased transepithelial water permeability and defective fluid absorption (Schnermann et al., 1998; Chou et al., 1999; Vallon et al., 2000). These observations show that AQP1 is a principal water channel in the proximal tubule and thin descending limb of Henle's loop and is indispensable for efficient urinary concentrating ability. This important role of AQP1 is further confirmed by the partial correction of the urinary concentrating defect in AQP1-null mice by

introducing AQP1 via adenovirus-mediated gene delivery (Yang et al., 2000a). These results also establish the cellular mechanism of water reabsorption; water passes through the epithelial layer not paracellularly but transcellularly via aquaporins.

Colton blood group antigen in human erythrocyte was identified to be AQP1 (Preston et al., 1994; Smith et al., 1994). Individuals lacking Colton antigen were identified whose AQP1 gene suffered from nonsense mutation or missense mutation resulting in non-functioning AQP1 molecules (Preston et al., 1994). Surprisingly they did not suffer any apparent clinical consequence. Functional analysis in erythrocyte in Colton-null phenotype revealed that AQP1 contributes more than 85% of the osmotic water permeability in erythrocyte membrane (Mathai et al., 1996). Recently, defective urinary concentrating ability was shown in an individual completely lacking AQP1 (King et al., 2001).

The remaining water transport capability in the proximal tubules of AQP1-null mice may be explained by the abundance of cotransporters such as Na^+-dependent glucose cotransporters (SGLT2 and SGLT1) in the brush border of the proximal tubule cells (Zeuthen et al., 2001). According to the molecular water pump hypothesis, a typical cotransporter SGLT1 transports 260 water molecules coupled to each sugar molecule transported and contributes to the transepithelial transfer of water (Loo et al., 1996).

6.3. AQP1 in the blood and lymphatic vessels

As is typically seen in the lung and kidney, AQP1 is present in the endothelial cells of the continuous non-fenestrated capillaries and venules (Sabolic et al., 1992; Nielsen et al., 1993b, 1997a; Hasegawa et al., 1994a). Electron microscopic immunocytochemistry revealed that AQP1 is localized in both the luminal and contraluminal plasma membrane of endothelial cells including caveolae and seems to serve in the transendothelial transfer of water (Nielsen et al., 1993b).

AQP1 is also present in the endothelial cells of lymphatic vessels in the submucosa and lamina propria in the rat gastrointestinal tract (Nielsen et al., 1993b; Koyama et al., 1999) (Figs. 3C and D). AQP1-null mice showed weight loss on a high-fat diet due to defective dietary fat processing suggesting a defect in the absorption of fat in the central lacteal endothelium in the intestinal villi (Ma et al., 2001).

In the rat lymph node, AQP1 is expressed in the endothelial cells of the lymphatic endothelium and high endothelial venules (Ohtani et al., 2003). In both endothelial cells, AQP1 is localized at the luminal and abluminal membranes. In combination, AQP1 may serve in the transendothelial transfer of water, thereby serving in the lymph-to-plasma flux of water.

6.4. AQP1 in the bile formation

AQP1 is expressed in the epithelial cells of the gall bladder where it is localized in both the apical and basolateral membrane (Nielsen et al., 1993b; Hasegawa et al., 1994a). AQP1 is also expressed in the cholangiocytes, the epithelial cells lining the

bile duct (Nielsen et al., 1993b) and seems to be responsible for mercurial-sensitive water transport in bile secretion (Roberts et al., 1994). AQP1 is not expressed in hepatocytes (Nielsen et al., 1993b; Hasegawa et al., 1994a; Roberts et al., 1994). Secretin induced the increase of mercurial-sensitive osmotic water permeability in isolated rat cholangiocytes (Marinelli et al., 1997). Cell fractionation analysis indicated that secretin induced the translocation of AQP1 from the microsomal fraction to the plasma membrane fraction (Marinelli et al., 1997). Light microscopic immunoperoxidase labeling showed that AQP1 is present diffusely in the cytoplasm and secretin treatment induces its concentration to the apical region, suggesting the cAMP-dependent exocytic insertion of AQP1-bearing vesicles into the apical plasma membrane (Marinelli et al., 1999). However, the immunolabeling of AQP1 is weak in cholangiocytes, and further studies are needed to verify the localization and dynamics of AQP1. The role of AQP1 in bile secretion was evaluated in AQP1-null mice (Mennone et al., 2002). AQP1 does not seem to be rate limiting for water movement.

6.5. AQP1 in the pancreas

Pancreas secretes a large volume of near-isotonic pancreatic juice containing bicarbonate and hydrolytic enzymes, suggesting the contribution of water channels in this process. In the rat, the measurement of osmotic water permeability of pancreatic duct cells in the isolated interlobular duct segments by time-lapse videomicroscopy indicated the presence of a mercurial-sensitive water channel (Ko et al., 2002). Immunohistochemical examination revealed that AQP1 is rich in both the luminal and basolateral sides of the interlobular duct cells (Ko et al., 2002; Furuya et al., 2002). AQP1 was not found in the acinar cells, centroacinar cells, or intercalated ducts (Furuya et al., 2002).

In the human pancreas, AQP1 was originally reported to be expressed only in the acinar cells (Hasegawa et al., 1994a). A later report showed that AQP1 is not present in the acinar cells but its expression is restricted to the centroacinar cells and epithelial cells of intercalated and intralobular duct cells (Burghardt et al., 2003). AQP1 is absent in the large interlobular ducts. AQP1 is also expressed in the blood vessels in the pancreas (Nielsen et al., 1993b; Hurley et al., 2001; Ko et al., 2002). The differences in the distribution of AQP1 between rat and human pancreases may be species differences.

AQP1 in the duct epithelial cells suggests its contribution to the secretion of pancreatic juice in the ducts. Localization of AQP1 is similar to that of the cystic fibrosis transmembrane conductance regulator (CFTR), suggesting that CFTR and AQP1 may serve in the electrolyte secretion and water transfer, respectively (Hasegawa et al., 1994a; Burghardt et al., 2003).

To evaluate the role of AQP1 in the pancreas, AQP1-null mice were analyzed (Ma et al., 2001). AQP1 deletion did not affect the fluid secretion rate, volume, pH, or amylase activities. These results indicate that AQP1 in the pancreas is not indispensable for the secretion of pancreatic juice.

6.6. AQP1 in the secretory granules

The swelling of secretory granules occurs in the exocytosis. In the isolated zymogen granules of rat pancreas, its swelling is induced by GTP and inhibited by mercurial and anti-AQP1 antibodies (Cho et al., 2002). Immunofluorescence and immunogold electron microscopic examination showed the localization of AQP1 in the zymogen granules, although the labeling is weak (Cho et al., 2002). Further studies are needed to establish the presence and role of AQP1 in the exocytosis of granules.

6.7. AQP1 in the airway and lung

Since the lung is highly permeable to water, important roles of aquaporins in the water handling in the lung were suggested. AQP1 is expressed in the endothelial cells of capillaries and venules in the airway and lung (Nielsen et al., 1993b, 1997a). The role of AQP1 in the lung was evaluated in AQP1-null mice and humans (for a review see Verkman et al., 2000a). A measurement of osmotically driven water transport across microvessels in the AQP1-null mice revealed that water permeability is reduced by 10-fold in the AQP1-null mice (Bai et al., 1999). This observation suggests that water movement occurs transcellularly via AQP1. However, a comparison of neonatal lung fluid balance, adult lung fluid clearance, and formation of the lung edema after acute lung injury in AQP1-null and wild mice demonstrated that AQP1 is not required in these processes (Song et al., 2000a). In normal humans, an intravenous injection of saline induced an increase in the thickness of the airway wall, suggesting the peribronchiolar edema formation, whereas edema was not formed in AQP1-null humans (King et al., 2002). This observation suggests that AQP1 is responsible for an increase in extravasation of fluid across the endothelium and AQP1 is a determinant of vascular permeability in the lung. Since AQP1 seems to serve in the extravasation as well as clearance of fluid through the endothelial wall, and the edema formation seems to be the result of a change in the balance of these two routes, further studies are needed to determine the physiological and pathological role of AQP1 in blood vessels.

In the pleural cavity, AQP1 is present in the endothelial cells near the pleura and in the mesothelial cells covering the visceral pleura (Song et al., 2000b). Although AQP1 facilitates rapid osmotic equilibration across the pleural surface, results of AQP1-null mice suggest that AQP1 does not play a role in clinically relevant pleural fluid accumulation or clearance (Song et al., 2000b).

6.8. AQP1 in the peritoneum

Water transport is a critical event in the peritoneal dialysis. It was inhibited by mercurials and the presence of AQP1 in the capillary endothelium was shown immunohistochemically (Carlsson et al., 1996; Devuyst et al., 1998). In addition, AQP1 was also detected in the human mesothelial cells and was upregulated by osmotic agents (Lai et al., 2001). These observations suggest that AQP1 is

responsible for water movement in the peritoneal dialysis. In fact, knockout mice showed the reduction of osmotically driven water transport across the peritoneal barrier in peritoneal dialysis (Yang et al., 1999).

6.9. AQP1 in the male reproductive organ

AQP1 is present in the efferent duct in the rat male reproductive system (Brown et al., 1993). AQP1 is localized to both the apical and basolateral membrane of the non-ciliated cells. In addition, AQP1 was found in epithelial cells of the ampula of the vas deferens, seminal vesicles, and prostate (Brown et al., 1993). AQP1 in the male reproductive tract may mediate the water absorption of the luminal fluid.

6.10. AQP1 in the brain and choroid plexus

In situ hybridization showed that AQP1 is strongly expressed in the epithelium of the rat choroids plexus (Hasegawa et al., 1993). Immunohistochemical examination showed that AQP1 is restricted in the apical microvilli of the villous-like processes of the epithelial cells, whereas the basolateral membranes of the epithelial cells and endothelial cells of the underlying fenestrated capillaries are devoid of AQP1 (Nielsen et al., 1993b; Hasegawa et al., 1994a). Since choroids plexus is the site of cerebrospinal fluid production, the concentration of AQP1 in the apical membrane of its epithelial cells suggests that AQP1 may be involved in the secretion of cerebrospinal fluid. Reduced expression of AQP1 and its recovery after space flight was reported, suggesting the regulation of the AQP1 expression by gravity (Masseguin et al., 2000). No aquaporins have been assigned to the basolateral membrane yet, and the mechanism by which water crosses it remains elusive.

6.11. AQP1 in the eye

The eye is a specialized organ containing a large volume of extravascular fluid. Multiple types of aquaporins are differentially expressed in the eye (Hamann et al., 1998) and may be involved in maintaining the transparency of the lens and cornea, regulating intraocular pressure, signal transduction, and tear secretion (for a review, see Hamann, 2002; Verkman, 2003).

In situ hybridization showed that AQP1 is expressed in the ciliary body, iris, and lens epithelium (Hasegawa et al., 1993). Immunohistochemical examination showed that AQP1 is present in the corneal endothelium (Nielsen et al., 1993b) and keratocytes (Hamann et al., 1998) and may be involved in preventing the cornea from swelling. In fact, in the AQP1-null mice, the impaired recovery of corneal transparency and thickness after hypotonic swelling indicate that AQP1 in the cornea is involved in the active extrusion of fluid from the corneal stroma across the corneal endothelium (Thiagarajah and Verkman, 2002). This observation also suggests that upregulation of AQP1 expression may reduce corneal

swelling and opacification after injury (Nielsen et al., 1993b; Thiagarajah and Verkman, 2002).

In the ciliary body, AQP1 is present in the non-pigmented cells in the epithelium of the ciliary body but is absent in the apposing pigmented epithelial cells, whereas in the iris AQP1 is present in both the posterior and anterior epithelia (Nielsen et al., 1993b; Hasegawa et al., 1994a; Hamann et al., 1998). Since the ciliary body is the site of production of aqueous humor, AQP1 in the non-pigmented epithelial cells may play a role in the transfer of water. Direct measurement of aqueous humor production in mice lacking AQP1 and/or AQP4 revealed that AQP1 and AQP4 are involved in intraocular pressure regulation by facilitating the transfer of water in the ciliary body although the reduction in knockout mice is modest (Zhang et al., 2002).

AQP1 is expressed in the cells of trabecular meshwork, where aqueous humor is drained (Hamann et al., 1998). When the AQP1 protein level in the meshwork cells in culture was altered by the transfection of AQP1 or the transfer of antisense cDNA, the resting intracellular volume increased when the AQP1 level was high, and it decreased when the AQP1 level was low (Stamer et al., 2001). These observations suggest that AQP1 may regulate the paracellular permeability of the trabecular meshwork cells by changing the cell volume.

In the lens, the differential expression of aquaporins is prominent in that AQP1 is expressed in the epithelial cells (Nielsen et al., 1993b; Hasegawa et al., 1994a), whereas AQP0 is expressed in the lens fibers (Fitzgerald et al., 1983; Hamann et al., 1998).

6.12. AQP1 in the ear

The maintenance of homeostasis of ion and volume in the inner ear is crucial to maintain the function of sensation in the inner ear. Water transport in the perilymph and the endolymph is critically important. An abnormal increase of endolymph volume termed endolymphatic hydrops occurs in Meniere's disease and may be due to the disorder of fluid homeostasis, suggesting the involvement of aquaporins. Multiple types of aquaporins are expressed, among which AQP1 and AQP4 are most abundant (Huang et al., 2002). AQP1 is expressed in the fibrocytes lining the bony and membranous labyrinth of the inner ear of the guinea pig (Stankovic et al., 1995). In the rat, AQP1 is rich in the cells in the spiral ligament close to the bony wall, and the intermediate cells in the stria vascularis of the cochlea (Sawada et al., 2003). AQP1 is also present in the cells lining the perilymphatic surface of the scala vestibuli and the scala tympani. AQP1 may be involved in the precise volume control of the compartments in the inner ear to maintain the function of hearing. However, the deletion of AQP1 did not affect hearing in mice (Li and Verkman, 2001). AQP1 therefore may serve a secondary role in the ear.

In the middle ear, AQP1 is expressed in the capillary endothelial cells and fibroblasts (Minami et al., 2001; Huang et al., 2002).

7. AQP2

7.1. Cloning

AQP2 was identified and cloned as an antidiuretic hormone (ADH)-regulated water channel of the kidney collecting ducts (WCH-CD) in the rat (Fushimi et al., 1993) and in human (Sasaki et al., 1994). When expressed in *Xenopus* oocytes, AQP2 exhibited osmotic water permeability showing that AQP2 is a water channel.

7.2. Tissue distribution

Expression of AQP2 is basically confined to the kidney, where it is present in the collecting duct. Immunohistochemical examination revealed that AQP2 is expressed in the principal cells of the collecting duct (Fig. 4). In the basal state, AQP2 is stored in the intracellular vesicular compartment. Upon ADH stimulation, AQP2 rapidly moves from the intracellular storage sites to the apical plasma membrane, where it serves as a major water channel responsible for the concentration of urine (Fushimi et al., 1993; Nielsen et al., 1993a, 1995a) (Fig. 4). In addition to the kidney, AQP2 was reported to be expressed in the vas deferens (Stevens et al., 2000), and less abundantly in the inner ear (Mhatre et al., 2002a).

7.3. Translocation of AQP2 from the intracellular pool to the plasma membrane

Vasopressin stimulates the water reabsorption in the principal cells of the kidney collecting ducts. The increase of the osmotic water permeability at the apical plasma membrane was considered to be mediated by the exocytic insertion of the putative water channels stored in the cytoplasmic vesicles (Verkman, 1989). AQP2 is the water channel molecule responsible for this translocation from the intracellular pool to the plasma membrane. By using antibodies specific to AQP2, this process was clearly demonstrated histochemically (Fushimi et al., 1993; Nielsen et al., 1995a, 1993a; Yamamoto et al., 1995) (Fig. 4). Translocation of AQP2 from the intracellular pool to the plasma membrane is also shown in porcine renal epithelial cell line LLC-PK_1 cells stably transfected with AQP2 (Katsura et al., 1995). Translocation of AQP2 from the intracellular store to the plasma membrane by ADH stimulation and retrieval after washout occur even when de novo AQP2 synthesis is blocked by cycloheximide. Retrieval to the intracellular pool was also observed in water-loaded rat kidney (Saito et al., 1997). The second stimulation results in the reappearance of the retrieved AQP2 to the cell surface, indicating that AQP2 shuttles between the intracellular storage vesicles and the plasma membrane (Katsura et al., 1996). The details of the translocation process of AQP2 are also described and discussed in recent reviews (Verkman, 2000; Nielsen et al., 2002; Brown, 2003).

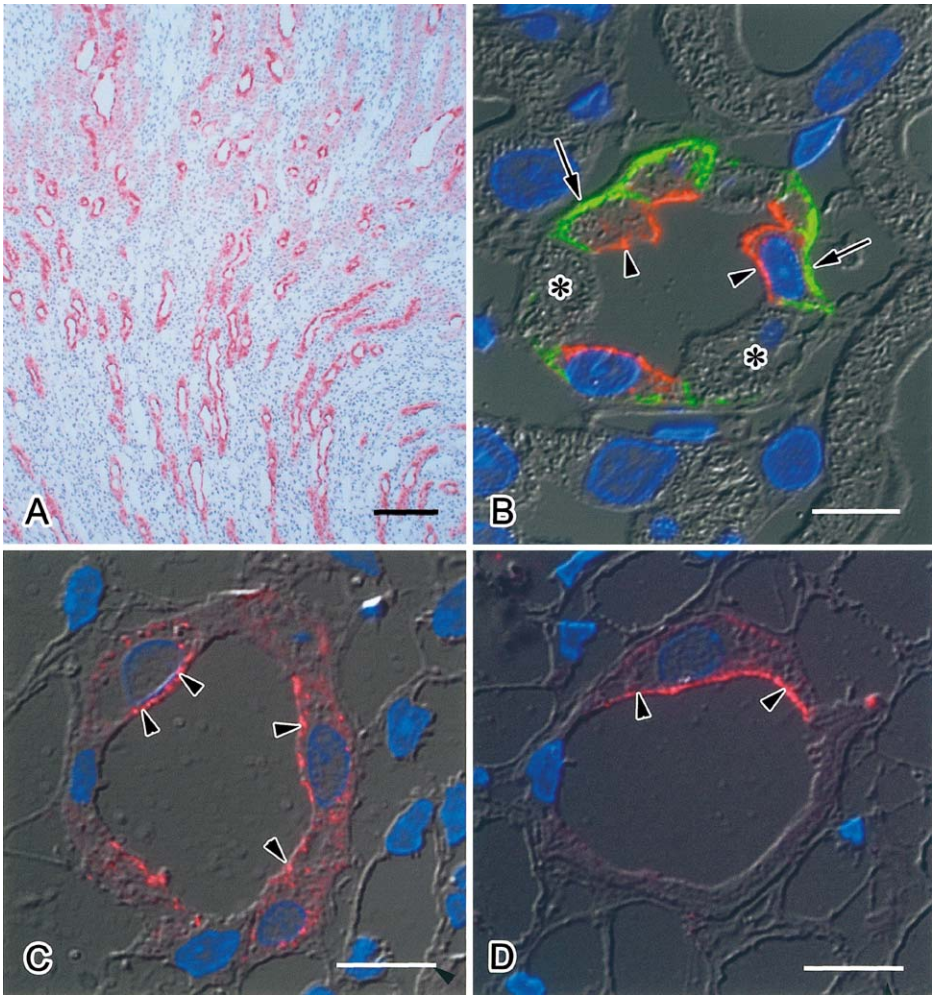


Fig. 4. AQP2 in the rat kidney. (A) A survey view by immunoperoxidase labeling. AQP2 is expressed in the collecting ducts. (B) AQP2 and AQP3 in the collecting duct. AQP2 (red) is localized in the apical side (arrowheads), whereas AQP3 (green) is in the basolateral side (arrows) in the principal cells. Note that neither AQP2 nor AQP3 is expressed in intercalated cells (*). (C, D) Effect of ADH on AQP2 localization. In the control water-loaded specimens (C), AQP2 is localized mainly in the subapical cytoplasmic vesicles (arrowheads in C). Translocation of AQP2 to the apical plasma membrane (arrowheads in D) occurs by the administration of ADH (D). Bars: 10 μm. C, D was reproduced from Tajika et al. (2002) with permission from Blackwell Publishing.

7.4. Subcellular localization of AQP2

AQP2 is unique in that most of cellular AQP2 is retained in an intracellular compartment in an unstimulated condition. Characterization of this intracellular

compartment and its dynamics will serve as a good model system in other plasma membrane molecules whose cell surface localization is regulated by translocation mechanism and will provide a clue to cure diabetes insipidus.

In the principal cells of the rat kidney collecting ducts, AQP2 is mainly localized in the vesicles in the supranuclear region. Double labeling with lysosomal markers cathepsin D and lgp-110, endoplasmic reticulum marker calnexin, trans-Golgi network marker TGN38, and Golgi marker Golgi 58k revealed that none of them colocalized with AQP2 vesicles in the basal and ADH-stimulated cells (Figs. 5 and 6) (Tajika et al., 2002). Only a small fraction of AQP2 vesicles colocalized with early endosome marker EEA1. These observations show that AQP2 is stored in a unique intracellular compartment distinct from lysosome, endoplasmic reticulum, trans-Golgi network, and the Golgi. Partial colocalization with EEA1 suggests that the endosomal system may be involved in the trafficking of AQP2. Lowering of pH in the endosomal and lysosomal compartments by inhibiting vacuolar type H^+-ATPase with bafilomycin A1 blocked the recycling pathway and AQP2 was accumulated in the perinuclear compartment adjacent to the Golgi apparatus with or without vasopressin stimulation (Gustafson et al., 2000).

AQP2 constitutively recycles between the cell surface and intracellular compartment (Gustafson et al., 2000) as is seen in insulin-regulatable glucose transporter GLUT4 (Watson and Pessin, 2001). The apparent increase of AQP2 on the cell surface may be due to the increase in exocytic insertion of AQP2 from the intracellular pool or the decrease in endocytic removal of AQP2 from the cell surface.

Immunogold electron microscopic examination of ultrathin sections of collecting duct principal cells revealed that AQP2 is localized in the clathrin-coated pits on the apical cell surface (Sun et al., 2002). A fracture-label in LLC-PK_1 cells expressing AQP2 also showed that intramembranous particles labeled for AQP2 distribute randomly on the plasma membrane by forskolin stimulation. After forskolin washout for 10 min, AQP2 labeling became concentrated over intramembranous particle clusters in the membrane invaginations along with a flat area suggesting clustering of AQP2 in the coated pits. When GTPase-deficient dominant-negative K44A dynamin 1 and dynamin 2 mutants were expressed, AQP2 accumulated on the cell surface due to inhibition of endocytosis. These results indicate that cell surface AQP2 is also controlled by the dynamin- and clathrin-dependent endocytosis of AQP2 (Sun et al., 2002).

7.5. Regulation of AQP2 trafficking by phosphorylation

Vasopressin binds to the vasopressin V2 receptor located at the basolateral membrane of the kidney principal cells, leading to an increase in the cAMP level. Elevation of the cAMP level is sufficient to trigger the translocation of AQP2 stored in the cytoplasmic compartments of the plasma membrane (Lorenz et al., 2003) (Fig. 6).

Vasopressin induces the phosphorylation of AQP2 at serine 256 on the cytoplasmic terminus by protein kinase A (Fushimi et al., 1997; Katsura et al.,

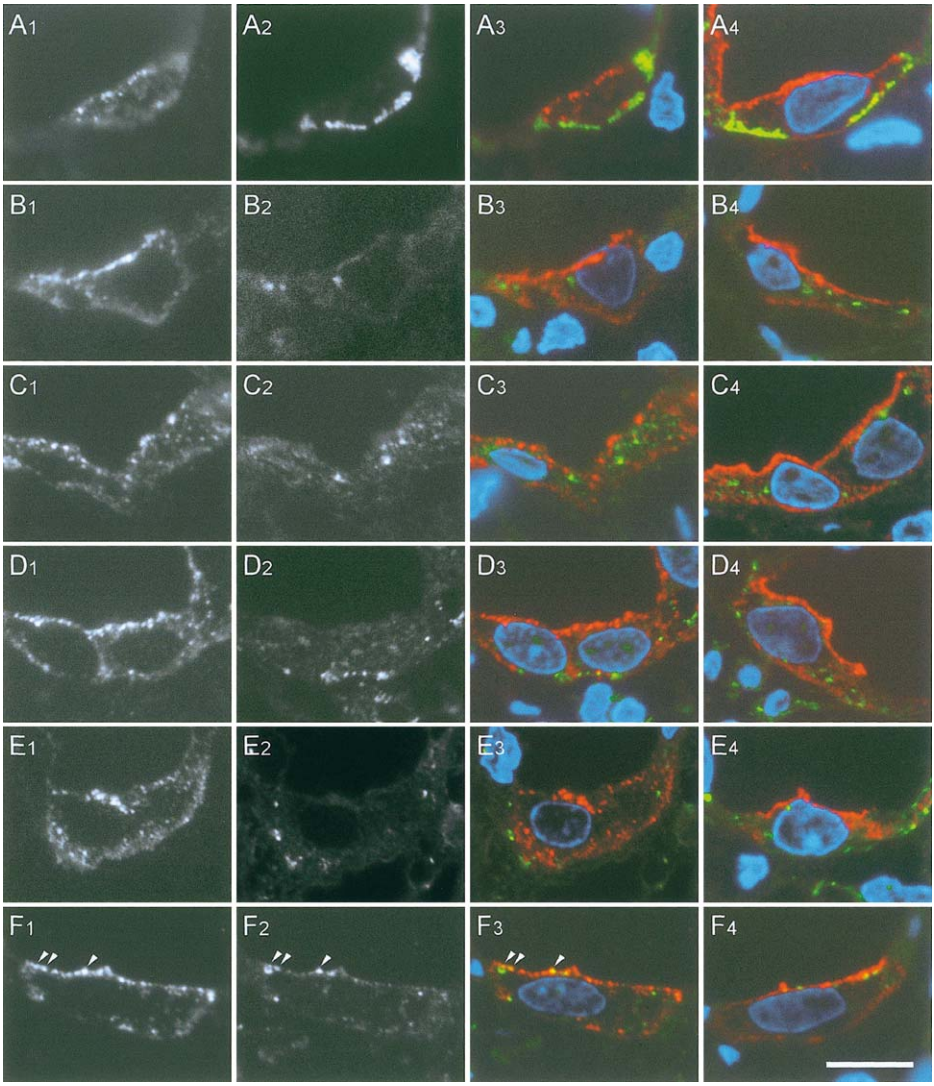


Fig. 5. Comparison of the localization of AQP2 with AQP3 (A), Golgi 58K (B), TGN38 (C), lgp-110 (D), cathepsin D (E), and EEA1 (F) in the principal cells of medullary collecting ducts in the control water-loaded (three panels labeled 1–3 in each row), and ADH-administered (right-most panels labeled 4) animals. The left columns (A1–F1) show AQP2 staining. The second column shows the labeling for AQP3 (A2), Golgi 58K (B2), TGN38 (C2), lgp-110 (D2), cathepsin D (E2), and EEA1 (F2). Merged images are shown in the third column (A3–F3). Merged images of the ADH-administered specimens are illustrated in the right most columns (A4–F4). In merged images, AQP2 is shown in red, whereas AQP3 and organelle marker proteins in green. Arrowheads in part F indicate colocalization of AQP2 with EEA1. Nuclear DNA is stained with DAPI and shown in blue. Semithin cryosections of 0.3–0.5 μm thickness. Bar: 10 μm. Reproduced from Tajika et al. (2002) with permission from Blackwell Publishing.

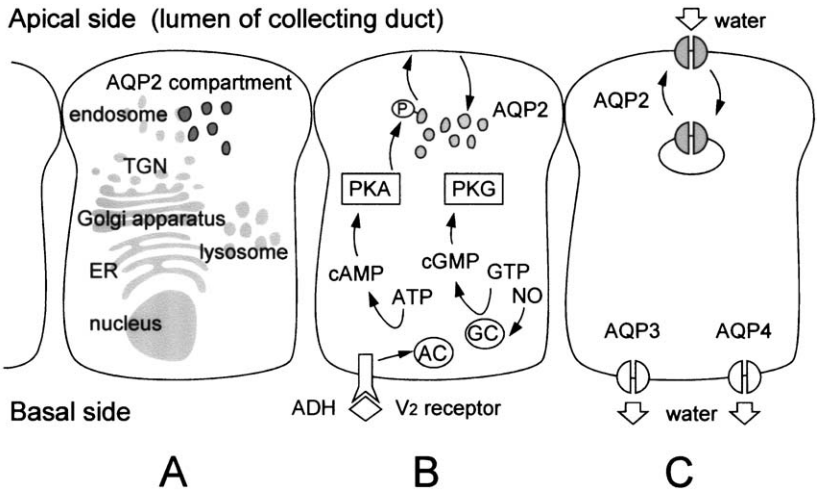


Fig. 6. Localization of AQP2 in kidney principal cells and its translocation to the plasma membrane. (A) AQP2 resides in the apical vesicular storage compartment distinct from the endoplasmic reticulum, Golgi apparatus, trans-Golgi network, lysosome, and endosome. (B) ADH binds to the vasopressin V2 receptor and subsequent activation of adenylate cyclase (AC), and protein kinase A (PKA) results in the phosphorylation of AQP2 in the apical storage vesicles and their translocation to the apical membrane. (C) Activation of guanylate cyclase (GC) by NO leads to the activation of protein kinase G (PKG) and also results in the phosphorylation of AQP2 and its translocation. Translocation of AQP2 to the apical plasma membrane allows the transcellular transfer of water as follows: AQP2 in the influx of water to the cell; AQP3 and AQP4 in the basolateral membrane in the efflux from the cell.

1997). Translocation of AQP2 to the plasma membrane is completely abolished by protein kinase A inhibitor H-89 (Katsura et al., 1997). When point-mutation of serine 256 to alanine (S256A) was introduced in AQP2 and expressed in LLC-PK_1 cells, translocation of AQP2 from the intracellular pool to the plasma membrane by cAMP was completely abolished. Phosphorylation of serine 256 and subsequent translocation of AQP2 were also shown to occur in response to vasporessin stimulation in principal cells in the kidney (Nishimoto et al., 1999). The effect of phosphorylation on the subcellular localization was further analyzed by the expression of phosphorylated and non-phsphorylated AQP2s in *Xenopus* oocytes. Phosphorylation of at least three monomers per AQP2 tetramer is suggested to be required for its localization at the plasma membrane (Kamsteeg et al., 2000). On the other hand, by mutating possible phosphorylation sites, it was proposed that phosphorylation of serine 256 is necessary and sufficient for expression of AQP2 in the apical membrane, but is not dependent on the endocytosis induced by protein kinase C (van Balkom et al., 2002).

Direct visualization of phosphorylation at serine 256 of AQP2 was carried out using antibodies that selectively recognize AQP2 phosphorylated at serine 256 (Christensen et al., 2000). By immunoperoxidase light and immunogold electron

microscopy, phosphorylated AQP2 is localized in both the apical plasma membrane and the intracellular vesicles of principal cells in the rat kidney. By treatment with ADH agonist, phosphorylated AQP2 disappeared from the plasma membrane with only marginal labeling in the cytoplasmic vesicles. In the Brattleboro rats lacking ADH secretion, phosphorylated AQP2 was present intracellularly, and ADH treatment resulted in a 10-fold increase of AQP2 at the plasma membrane although the overall amount of phosphorylated AQP2 remained unchanged. These results show that phosphorylated AQP2 is present at the plasma membrane and intracellular vesicles and suggest the important role of phosphorylation in the distribution of AQP2.

Signal transduction pathways other than vasopressin V2 receptor/protein kinase A have recently been uncovered leading to the translocation of AQP2 from the intracellular pool to the plasma membrane (Fig. 6). Using rat kidney slices and LLC-PK_1 cells stably expressing AQP2, nitric oxide donor sodium nitroprusside (SNP) induced the translocation of AQP2 from the intracellular pool to the plasma membrane (Bouley et al., 2000). The process is not dependent on the increase of the intracellular cAMP level. Rather, an increase in the cGMP level was observed. The addition of exogenous cGMP or an atrial natriuretic factor that increases the cGMP level also stimulated the membrane insertion of AQP2. In the cGMP-dependent translocation, phosphorylation of serine 256 occurs as well. These observations suggest that cGMP mediate the translocation of AQP2 to the plasma membrane via phosphorylation of AQP2 in the same way as in the cAMP-dependent manner. The relationship between protein kinase A, protein kinase G and phosphorylation of AQP2 remains to be clarified.

7.6. Fusion machinery of AQP2 vesicles to the plasma membrane

The docking and fusion of cytoplasmic synaptic vesicles to the plasma membrane in the neuronal cells are mediated by SNARE proteins (Sollner and Rothman, 1996). A similar mechanism works in the exocytosis of secretory granules in pancreatic islet cells (Gerber and Sudhof, 2002) and the exocytic insertion of insulin-regulatable glucose transporter GLUT4 in adipocytes and muscle cells (Elmendorf, 2002; Watson and Pessin, 2001). Immunoblotting showed that vesicle-associated membrane protein 2 (VAMP-2) but not VAMP-1 is expressed in the kidney. Immunoelectron microscopy revealed that VAMP-2 is colocalized with AQP2 vesicles in the principal cell (Nielsen et al., 1995b). Cleavage of VAMP-2 by tetanus toxin abolished the cAMP-dependent translocation of AQP2 from the intracellular pool to the plasma membrane in CD8 rabbit cortical collecting duct cells transfected with AQP2, indicating that VAMP-2 plays a critical role in the fusion step in the translocation of AQP2 (Gouraud et al., 2002). In addition, SNAP-25-associated Hrs-2 protein colocalizes with AQP2 vesicles in the rat kidney principal cells (Shukla et al., 2001). The putative targeting protein syntaxin-4 is expressed in the rat kidney collecting duct cells. Immunoperoxidase and immunogold electron microscopy revealed that it is localized at the apical plasma membrane of the principal cells

(Mandon et al., 1996). These observations suggest that VAMP-2 and syntaxin-4 constitute a fusion machinery of AQP2 vesicles to the apical plasma membrane.

7.7. Cytoskeleton and AQP2 translocation

Actin depolymerization by ADH was observed in the amphibian bladder and mammalian kidney (Hays et al., 1993). Recently, inhibition of Rho GTPase with *Clostridium difficile* toxin B or *Clostridium limosum* C3 fusion toxin induced actin depolymerization and translocation of AQP2 in the absence of hormonal stimulation in AQP2-transfected CD8 cells (Tamma et al., 2001). A similar effect was observed in Rho kinase inhibitor Y-27632. Constitutively active RhoA induced the formation of stress fibers, and inhibited the translocation of AQP2 to the plasma membrane. These observations indicate that Rho inhibits cAMP-dependent translocation of AQP2 into the plasma membrane by controlling the organization of actin cytoskeleton.

Disruption of actin cytoskeleton by cytochalasin D induced translocation of AQP2 to the plasma membrane, suggesting that depolymerization of F-actin is sufficient for the translocation of AQP2 (Klussmann et al., 2001). Detailed analysis indicates that the regulation of actin cytoskeleton upon elevation of the cAMP level and concomitant AQP2 translocation is carried out by RhoA inhibition through Rho phosphorylation to stabilize it in the inactive form as well as its interaction with Rho GDP dissociation inhibitor (Tamma et al., 2003). These observations suggest that Rho plays a critical role in the signal transduction of ADH for the translocation of AQP2 to the plasma membrane.

7.8. Basolateral localization

In addition to apical localization of AQP2 upon stimulation, basolateral membrane localization is seen in the principal cells of the inner medullary collecting ducts and connecting tubules (Nielsen et al., 1993a; Christensen et al., 2003; Jeon et al., 2003). Expression of AQP2 in MDCK cells showed that basolateral localization is not due to possible heterotetramerization of AQP2 with other basolateral aquaporins such as AQP3 and AQP4 or the hyper-expression level of AQP2 (van Balkom et al., 2003). Rather, hypertonic treatment resulted in the basolateral targeting of AQP2 upon stimulation. Since the interstitium of the kidney medulla is hypertonic, osmolarity may regulate the targeting of AQP2 in the kidney cells (van Balkom et al., 2003).

Basolateral localization of mutated AQP2 is also found in autosomal recessive nephrogenic diabetes insipidus when expressed in MDCK cells (Marr et al., 2002b; Asai et al., 2003).

7.9. Urinary excretion of AQP2

AQP2 is excreted in the human urine (Kanno et al., 1995). The amount of urinary excretion of AQP2 is decreased by dehydration, and increased upon ADH

stimulation. In patients with central diabetes insipidus, an increase of urinary excretion of AQP2 was observed as well, whereas no increase of AQP2 excretion was seen in patients with nephrogenic diabetes insipidus. These results show that urinary AQP2 serves as a good index of the action of ADH in the kidney (Kanno et al., 1995). The amount of AQP2 excretion is the same in men and women, and is not affected by age. It showed positive correlation with urine osmolarity (Rai et al., 1997). In the rat, approximately 3–4% of total AQP2 in the kidney is excreted daily (Rai et al., 1997; Wen et al., 1999). This fraction does not change by dehydration for 3 days (Rai et al., 1997). Immunoelectron microscopic examination of the pellet of urine samples revealed that AQP2 was present in small vesicles of various sizes and membrane fragments, some of which were multivesicular bodies (Wen et al., 1999). AQP2 and AQP1, both of which were found at the apical membrane of urinary epithelial cells, are detected in the urinary excretion, whereas basolateral aquaporin AQP3 is not detected, suggesting that the urinary secretion of AQP2 does not occur by whole cell shedding. By comparing the urinary excretion of AQP2, the expression level of AQP2, and the apical localization of AQP2 in the ADH stimulation, urinary excretion was found to be closely related to the apical localization of AQP2, suggesting that urinary excretion is mediated by an ADH-dependent apical pathway (Wen et al., 1999). The detailed cellular process of AQP2 excretion in the apical membrane of collecting duct cells is not clear. Apical plasma membrane containing AQP2 may be susceptible to shearing force, be shed off, and form vesicles.

7.10. GFP-labeled AQP2

To see the dynamics of AQP2 in living cells, fusion protein of AQP2 with green fluorescent protein (GFP) was expressed in cells and animals. Diffusion of AQP2 and AQP1 was measured in LLC-PK_1 cells expressing GFP-tagged AQPs by the fluorescence-recovery-after photobleaching (FRAP) method (Umenishi et al., 2000). Elevation of the cAMP level by forskolin induced a decrease in the mobility of AQP2 whereas it did not affect that of AQP1. Disruption of the actin cytoskeleton by cytochalsin D blocked the action of forskolin. The effect of forskolin was dependent on the phosphorylation of AQP2, since the mutation of the protein kinase A phosphorylation consensus site (S256A) at the AQP2 C-terminus diminished the effect of forskolin. These examinations suggest that cAMP induces the immobilization of phosphorylated AQP2 at the plasma membrane by actin cytoskeleton, which may form specialized membrane domains for efficient membrane recycling (Umenishi et al., 2000). When two fusion proteins of AQP2 with GFP were examined, one with GFP attached to the C-terminus of AQP2, and the other with GFP attached to the N-terminus, the addition of GFP to the C-terminus resulted in both the apical and basolateral localization in LLC-PK_1 cells (Gustafson et al., 1998). Moreover, AQP2-GFP failed to respond to ADH stimulation. On the other hand, the addition of GFP to N-terminus of AQP2 retained the polarized localization at the plasma membrane and remained responsive to ADH. These results indicate that trafficking signals may exist in the C-terminus regions of AQP2 and that care should be taken in using GFP-tagged AQP2 to study the trafficking of

AQP2. The importance of the C-terminus in the targeting of AQP2 is also evident in the dominant-negative type of nephrogenic diabetes insipidus (Marr et al., 2002b; Asai et al., 2003).

A transgenic mouse was created expressing GFP under the control of the promoter region of 9.5 kb of mouse AQP2 (Zharkikh et al., 2002). In this transgenic mouse, GFP was selectively expressed in the principal cells of the kidney collecting ducts. Expression of GFP was regulated in a manner similar to that of endogenous AQP2 such as increase by dehydration. Expression of AQP2 and GFP is maintained in the primary culture of the cells obtained from the kidney medulla, and increased in response to cAMP or ADH. This model system may be useful in the study of the differentiation of principal cells.

7.11. Pathogenesis and AQP2

7.11.1. Congenital nephrogenic diabetes insipidus

Nephrogenic diabetes insipidus is a disorder characterized by the inability to concentrate urine and resultant polyuria caused by the insensitivity to ADH in renal tubule cells (Morello and Bichet, 2001). Most cases are acquired forms caused by the drug administration, etc. Through lithium therapy, nephrogenic diabetes insipidus is induced where a marked reduction of AQP2 expression occurs (Marples et al., 1995).

Congenital diabetes insipidus is a rare inherited disorder. About 90% of the cases are the X-linked recessive disorder which is caused by mutation in arginine vasopressin receptor 2 gene (AVPR2) coding the vasopressin V2 receptor. The rest of the cases are autosomal recessive or dominant mutations of the AQP2 gene located in chromosome 12q13 (Sasaki et al., 1994; Vargas-Poussou et al., 1997; Morello and Bichat, 2001).

7.11.2. Autosomal recessive nephrogenic diabetes insipidus

In autosomal recessive nephrogenic diabetes insipidus, the mutations are mostly found between the first and sixth transmembrane domains of the molecule. Two types of mutations in AQP2 have been identified (van Lieburg et al., 1994; Kuwahara, 1998; Goji et al., 1998).

One type such as T125 M or G175R is a loss of the water channel function. Immunohistochemical labeling of *Xenopus* oocytes expressing mutant AQP2 genes revealed that these two mutant AQP2 proteins are localized at the plasma membrane but exhibit no significant water channel activity, suggesting that trafficking to the plasma membrane is normal but the channel function is disabled (Mulders et al., 1997).

Another type is the misrouting of mutated AQP2 molecules to the endoplasmic reticulum and failure to reach the plasma membrane. This type is most frequently found in patients of autosomal recessive diabetes insipidus. When expressed in *Xenopus* oocytes or cultured mammalian cells, water channel activity does not increase in the cells. Immunohistochemical examination revealed that they are retained inside the cell. Immunoblotting showed that these mutated AQP2 molecules are of endoplasmic reticulum form of approximately 32 kDa with high content of

mannose in the sugar moiety (Deen et al., 1995). These mutant AQP2 proteins are expressed as monomers and do not form oligomers with the wild-type AQP2 (Kamsteeg et al., 1999). Therefore, when wild and mutated AQP2 molecules are expressed simultaneously in heterozygous cells, they do not interact with one another and hence wild-type AQP2 serves as an active ADH-regulatable water channel. Cell biological analysis revealed that AQP2 molecules are misfolded in the endoplasmic reticulum and retained there (Tamarappoo and Verkman, 1998; Marr et al., 2002a). The misfolding of mutant AQP2 molecules can be corrected by chemical chaperones (Tamarappoo and Verkman, 1998). In fact, when CHO cells expressing such AQP2 mutant proteins are treated with chemical chaperon glycerol, AQP2 retained in the endoplasmic reticulum is rerouted to the plasma membrane and endosomes (Tamarappoo and Verkman, 1998). In such a case, facilitation of proper protein folding in the endoplasmic reticulum and subsequent rerouting may have therapeutic efficacy in nephrogenic diabetes insipidus (Tamarappoo et al., 1999).

7.11.3. Dominant nephrogenic diabetes insipidus

The mutations of autosomal dominant nephrogenic diabetes insipidus were found in the C-terminus of AQP2 with the extension of the tail (Mulders et al., 1998; Kuwahara et al., 2001; Kamsteeg et al., 1999). The mutated AQP2s are localized in aberrant intracellular compartments such as the Golgi apparatus (Mulders et al., 1998), late endosomes/lysosomes (Marr et al., 2002b), or basolateral membrane (Marr et al., 2002b; Asai et al., 2003). When wild-type AQP2 is coexpressed with the dominant-type AQP2 mutant, aberrant localization of not only the mutant AQP but also wild-type AQP2 was seen (Mulders et al., 1998). Such a dominant-negative effect is not seen when wild-type AQP2 is coexpressed with the recessive-type AQP2. When mutant AQP2s were expressed in *Xenopus* oocytes, little increase in osmotic water permeability was observed compared to the wild-type AQP2 control. In parallel with osmotic water permeability, immunofluorescence microscopy and membrane fractionation failed to show the cell surface localization of AQP2, suggesting that trafficking of AQP2 to the plasma membrane is impaired in these mutant AQP2s because of mutation of their C-terminus tails. When mutant AQP2 was coexpressed with wild-type AQP2, a marked reduction of osmotic water permeability and cell surface localization of AQP2 was observed (Kuwahara et al., 2001). Immunoprecipitation showed that mutant AQP2 and wild-type AQP2 formed oligomers (Kamsteeg et al., 1999). The dominant-negative effect is therefore due to the oligomerization of mutant-AQP2 and wild-type AQP2, and the resultant mistargeting of the oligomers by the altered C-terminus tails of mutant AQP2 (Kamsteeg et al., 1999; Kuwahara et al., 2001; Marr et al., 2002b). The error in the targeting of AQP2 was also confirmed by its expression in mammalian cells. When mutant AQP2 was expressed in MDCK cells, it is localized at the basolateral membrane (Asai et al., 2003), or endosomes/lysosomes (Marr et al., 2002b). Coexpression of mutant AQP2 and wild-type AQP2 resulted in their aberrant localization to the basolateral membrane, suggesting a mixed oligomerization of mutant and wild-type AQP2 (Asai et al., 2003). The dominant-negative effect of inherited disorders could be attributed to such oligomerization of wild and mutated

proteins and the misrouting of the oligomers to the aberrant compartment led by the signal inherent in the mutated proteins (Marr et al., 2002b). These findings also indicate the importance of the C-terminus tail in the targeting of AQP2. In addition, mutations in the C-terminus tail do not affect the fundamental three-dimensional structure of AQP2 (Marr et al., 2002b).

7.11.4. AQP2 knock-in model of diabetes insipidus

A single amino acid replacement T126 M in human AQP2 causes the autosomal recessive nephrogenic diabetes insipidus because of the retention of the mutant AQP2 in the endoplasmic reticulum. The targeted replacement with the orthologous mutant gene in mice was carried out using a Cre-loxP method (Yang et al., 2001). In this knock-in model, AQP2 is misrouted to the endoplasmic reticulum as was observed in oocyte and cultured cells, and homozygous animals showed a severe phenotype with a urinary concentration defect along with serum hyperosmolarity and low urine osmolarity. The severe phenotype of the AQP2 mutant mice compared with mice lacking AQP1, AQP3, or AQP4 indicates a critical role of AQP2 in the kidney function (Yang et al., 2001).

7.12. AQP2 in the vas deferens

In addition to the kidney, AQP2 is expressed in the distal portion of vas deferens in the rat (Stevens et al., 2000). By immunofluorescence labeling, AQP2 is localized at the apical plasma membrane in the principal cells of vas deferens epithelium rather than retained in the cytoplasmic compartment as seen in the kidney collecting duct cells. AQP2 did not colocalize with endocytosed Texas Red-dextran showing that AQP2 is not in the apical endosomes. Immunogold electron microscopy of Lowicryl K4M-embedded specimens revealed that AQP2 is abundant along the plasma membrane of the stereocilia at the apical domain, although some labeling in the apical cytoplasmic vesicles is seen (Stevens et al., 2000). The vasopressin receptor is not expressed in the principal cells of vas deferens, and hence the ADH does not affect the localization of AQP2. Since the sequence of AQP2 expressed in this tissue is exactly the same as that in the kidney, the observed differential subcellular localization may be attributed to the differential interpretation of the targeting signal of AQP2 (Stevens et al., 2000).

8. AQP3

8.1. Cloning and characterization of AQP3

AQP3 was cloned from the rat cDNA library (Ishibashi et al., 1994; Ma et al., 1994; Echevarria et al., 1994). AQP3 exhibits a homology to *E. coli* glycerol facilitator GlpF. Expression of AQP3 in *Xenopus* oocytes revealed that it mediates the transport of water in a mercurial-sensitive manner (Ishibashi et al., 1994, 1997c;

Echevarria et al., 1994) as well as small non-ionic molecules such as glycerol (Ishibashi et al., 1994; Ma et al., 1994; Echevarria et al., 1994). Because of its ability to mediate glycerol permeation, AQP3 was also called GLIP (glycerol intrinsic protein) (Ma et al., 1994).

8.2. Localization

AQP3 is expressed in a wide variety of organs such as the kidney and the urinary tract, the digestive tract, the respiratory tract, the epidermis, the eye, and the brain. In most organs, AQP3 is expressed in epithelial cells lining the lumen or their surface.

8.3. AQP3 in the kidney

In the rat kidney, in situ hybridization revealed that AQP3 is expressed in collecting ducts (Ma et al., 1994; Echevarria et al., 1994). Immunohistochemical studies revealed that AQP3 is localized along the basolateral plasma membrane of principal cells of the kidney (Ishibashi et al., 1994; Ma et al., 1994; Echevarria et al., 1994, 1995) (Fig. 7). Collecting ducts are the final portion of the urine concentration process in the kidney and water reabsorption in this region determines the final content of water in the urine. In collaboration with apical aquaporin AQP2, AQP3 at the basolateral membrane may serve in the exit of water from the cell to the interstitium for the transepithelial transfer of water across the collecting duct cell. AQP2 is stored in the cytoplasmic compartments and it translocates to the apical plasma membrane upon stimulation of ADH, whereas AQP3 is constitutively present at the basolateral plasma membrane, and its localization is not affected by ADH (Yamamoto et al., 1995). The thirsting of rats resulted in the increase of AQP3 expression (Ecelbarger et al., 1995; Ishibashi et al., 1997c) but did not change its localization. In addition to AQP3, AQP4 is present at the basolateral membrane of the principal cells of the collecting ducts (Frigeri et al., 1995a). AQP2 also appears at the basolateral membrane upon ADH stimulation in the inner medulla. The roles of AQP3, AQP4 and AQP2 in the efflux of water from the cell has not been fully understood. However, an AQP3 knockout experiment provides a clue (Ma et al., 2000b). The growth and phenotype of AQP3-null mice were grossly normal except for polyuria. The animals consume 10 times more fluid and excrete urine of low osmolarity. Osmotic water permeability of the collecting duct basolateral membrane was reduced by more than 3-fold. AQP3/AQP4 double-knockout mice showed greater impairment of urinary concentrating ability than AQP3 single-knockout mice. These observations show that AQP3 plays an important role in the concentration of urine in the collecting ducts. The significance of AQP3 in the kidney is described and discussed in detail in recent review articles (Nielsen et al., 1999, 2002; Verkman, 1999, 2000).

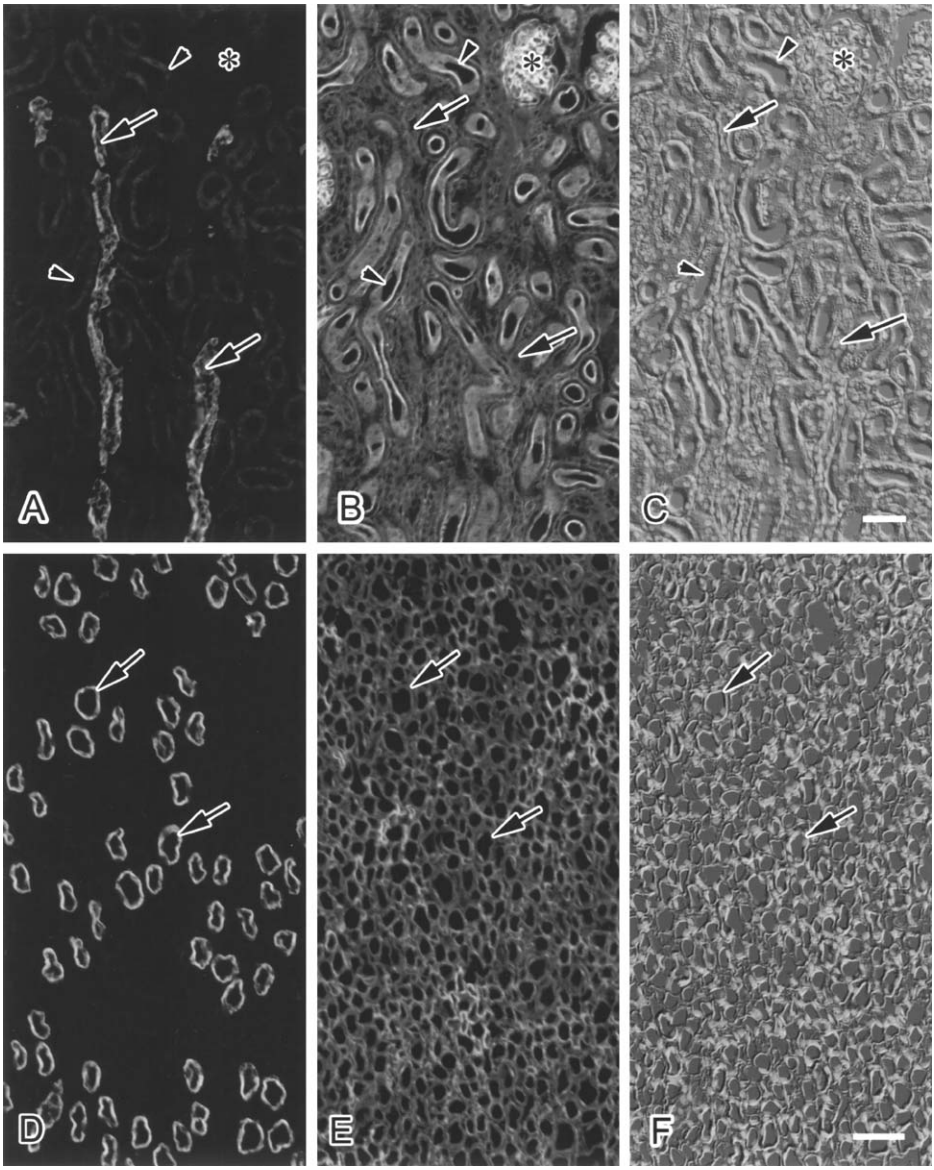


Fig. 7. AQP3 in the rat kidney cortex (A–C) and inner medulla (D–F). AQP3 (A, D), F-actin visualized with fluorescein-phalloidin (B, E), and Nomarski images merged with nuclear DNA images of DAPI fluorescence (C, F) are shown. AQP3 is localized in the collecting ducts (arrows) in the cortex (A–C) and medulla (D–F). Proximal tubules (arrowheads) and glomerulus (*) are negative for AQP3. Bars: 50 μm.

8.4. AQP3 in the urinary tract

AQP3 is present along the epithelial lining of the urinary tract (Matsuzaki et al., 1999a; Spector et al., 2002) (Fig. 8A). The luminal surface of the urinary tract including the renal pelvis, ureter, urinary bladder and the proximal portion of the urethra is covered with transitional epithelium. It is a specialized epithelium found in the urinary tract. At the light microscopic level, it is apparently composed of three layers when the epithelium is in an extended form. Immunogold electron microscopy revealed that AQP3 is localized to the plasma membrane of basal and intermediate cells (Fig. 9). The apical side of large surface cells connected by tight junctions and directly facing the lumen is negative for AQP3. In mammals, the composition of the urine is determined in the kidney and the contribution of the downstream urinary tract is negligible. In fact, the transitional epithelium that is constantly exposed to the highly concentrated urine is impermeable to water. The luminal membrane of the transitional epithelial cells has a specialized membrane structure that serves as an impermeable barrier of water. These observations indicate that water absorption from the urine does not occur in the urinary bladder. Therefore, AQP3 does not serve in the absorption of water from the urine. Rather it may serve in the supply of water from the underlying connective tissues to the epithelial cells which face the high-osmotic urine and tend to lose water (Fig. 8E) (Matsuzaki et al., 1999a). In this context, the flux of water in the transitional epithelial cells is in the direction opposite to that in the collecting duct cells of the kidney. A similar localization of AQP3 is seen in the transitional epithelia covering the renal pelvis, ureter, urinary bladder and the proximal portion of the urethra. The distal portion of the urethra is a transitional zone to the stratified squamous epithelium of the epidermis. AQP3 is expressed and localized in the same way as in the epidermis.

8.5. AQP3 in the digestive tract

AQP3 is expressed in the epithelium along the digestive tract (Matsuzaki et al., 1999a; Ramirez-Lorca et al., 1999). We examined the presence and localization of AQP3 in the rat digestive tract from the oral cavity to the anus, and found that AQP3 expression is prominent in the upper and lower portions of the digestive tract. In the rat, stratified squamous epithelium covers the upper portions, i.e., the oral cavity, esophagus, and forestomach (non-glandular portion of the stomach). As is seen in the epidermis, epithelial cells in the basal and intermediate layers express AQP3. AQP3 in these cells is localized along the plasma membrane. Similar to the epidermis, AQP3 may serve in the replenishment of water from the underlying connective tissues.

The major portion of the digestive tract, i.e., the glandular portion of the stomach to the rectum is covered with the simple columnar epithelium. A relatively low level of AQP3 expression, if any, is seen in this portion compared with the adjacent non-glandular portion of the stomach and the skin. In the glandular stomach, only weak labeling for AQP3 is present in the surface mucous cells. The expression of AQP3 in these cells gradually decreases according to the depth of the gastric pit. Cells in the

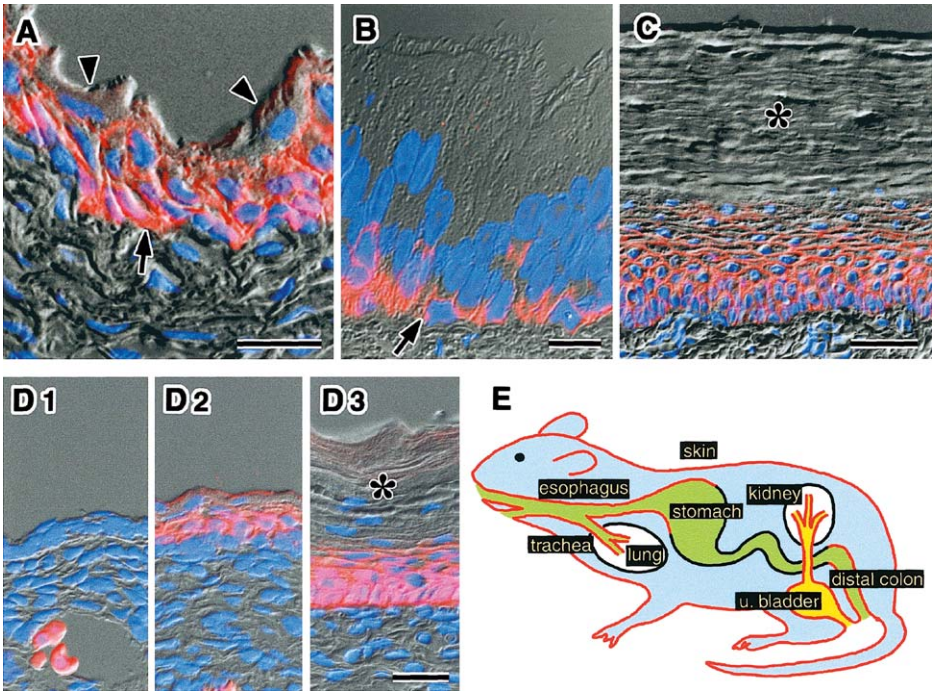


Fig. 8. Localization of AQP3 in rat tissues. (A–D): Fluorescence images are projected onto Nomarski images. AQP3 is shown in red, and nuclei are counter-stained with DAPI (blue). Bars in A, C, and D, 20 μm. Bar in B, 10 μm. (A) Urinary bladder. AQP3 is present in basal and intermediate cell layers (arrow) but not in large superficial cells (arrowheads) of the transitional epithelium. (B) Trachea. AQP3 is present in basal cells (arrow) of pseudostratified ciliated epithelium. (C) Palmar skin. AQP3 is present in basal and intermediate cell layers but not in the stratum corneum (asterisk) of the epidermis. (D) Developmental change of AQP3 in the skin. No label for AQP3 is seen in the epidermis at embryonic Day 15 (D1). AQP3 is present in the epidermis at embryonic Day 18 (D2). AQP3 is present in basal and intermediate cell layers but not in the stratum corneum (asterisk) of the epidermis at postnatal Day 4 (D3). (E) A schematic diagram of the tissue distribution of AQP3 in the rat. AQP3-positive sites are indicated in red. AQP3 is present in epithelia of the urinary tract, digestive tract, respiratory tract, and the skin. Reproduced from Matsuzaki et al. (2002) with permission from Blackwell Publishing.

gastric gland opening to the pit are negative for AQP3. In the duodenum and jejunum, AQP3 was not detected in the lining epithelia including absorptive epithelial cells, goblet cells, or glandular cells in the crypts. In the ileum, a weak expression of AQP3 is noted only in the epithelial cells located at the tip of the villi (Matsuzaki et al., 1999a; Ramirez-Lorca et al., 1999). In the distal colon and rectum, AQP3 is present in the epithelial cells directly lining the central lumen of the tract and of neck of the crypts. Cells deep in the crypts are negative for AQP3. AQP3 is localized along the basolateral membrane, whose role remains elusive. One

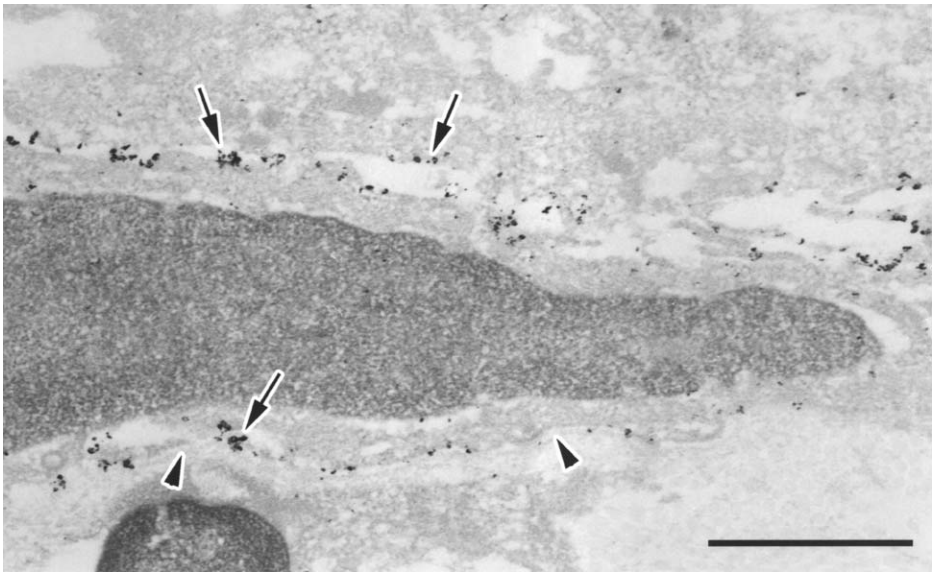


Fig. 9. Ultrastructural localization of AQP3 in the transitional epithelium of the rat urinary bladder. AQP3 is localized along the plasma membrane of the basal and intermediate cells (arrows). Arrowheads indicate basal lamina. Bar: 1 μm. Reproduced from Matsuzaki et al. (1999a) with permission from the Histochemical Society.

possibility is that AQP3 serves in the hydration of the epithelial cells that are directly facing the harsh environment including intestinal contents and feces. Another possibility is its contribution to the transfer of water to the lamina propria, serving as machinery for water absorption in the intestine. At the junction of the rectal and anal epithelia, the simple epithelium abruptly changes to the stratified epithelium and continues to the epidermis. As is seen in the stomach, strong expression of AQP3 is seen in the stratified epithelium.

8.6. AQP3 in the respiratory tract

AQP3 is expressed in the upper portion of the rat respiratory tract (King et al., 1997; Nielsen et al., 1997a; Matsuzaki et al., 1999a). AQP3 is found in the epithelia covering the nasal cavity wall, trachea and bronchus (Fig. 8B). In the pseudostratified epithelia of the nasal cavity, the trachea, and the bronchus, AQP3 is localized primarily to the plasma membrane of the basal cells. These cells are located on the basal lamina but do not face the lumen. Most of ciliated cells that directly face the lumen do not possess AQP3 except that some of the ciliated cells exhibit a basolateral membrane of labeling for AQP3. The luminal surface of the ciliated cells is always negative. AQP3 is not found in mucous secreting goblet cells.

The epithelial lining of the respiratory tract is exposed to the air and tends to lose water by evaporation from its surface. AQP3 in the basal cells may serve as the water

supply machinery from the underlying connective tissue where plenty of water is supplied from the blood stream in the capillaries. Although AQP3 is basically restricted to the basal cells, the gap junctions connecting the cytoplasm of epithelial cells may efficiently facilitate water transfer from the basal cells to the surface ciliated cells and goblet cells.

In the terminal portion of the respiratory tract in the lung, AQP3 is absent in the epithelial cells of the bronchioles. The expression of AQP3 abruptly terminates in the junction of the pseudostratified epithelium and simple columnar epithelium (Matsuzaki et al., 1999a). Simple epithelia lining the bronchioles as well as epithelial cells of the lung alveoli are negative for AQP3.

8.7. AQP3 in the skin

8.7.1. Localization and function in the skin

AQP3 is expressed in the skin covering the body surface in the rat (Matsuzaki et al., 1999a) and human (Sougrat et al., 2002). Its expression is restricted to the epidermis (Matsuzaki et al., 1999a; Sougrat et al., 2002). The labeling of AQP3 is the most intense in the plasma membrane of the basal cells and the adjacent intermediate cells (Figs. 8C and 10). As the epidermal cells differentiate and move upward, the label for AQP3 gradually decreases, and completely disappears in the keratinized layer. Although the thickness of the epidermis varies from part to part, the basic labeling pattern is the same. AQP3 is also present in the associated structures of the epidermis such as hair follicles and glands. AQP3 is found in the external root sheath of the hair follicle and the sebaceous gland including the Meibomian gland of the eyelid.

Since the epidermis is exposed to the air, it is unlikely that AQP3 in the epidermis is involved in transepithelial water absorption. The surface of the epidermis is covered with specialized keratinized layer. The keratinized layer, whose intercellular space is sealed with phospholipids, serves as a barrier layer against the evaporation of water from the skin. In addition, epidermal cells are connected by tight junctions, which play a critical role in the barrier function against water loss (Furuse et al., 2002). We proposed that AQP3 in epidermal cells serves as machinery to supply water to the water-deprived epidermal cells from the underlying dermis where capillaries provide enough water from the blood (Matsuzaki et al., 1999a; 2000). Once water is supplied to the epidermal cells, gap junction intercellular channels connecting them serve as an effective route for the transfer of water among cells including highly differentiated cells in the upper layer of the epidermis. A high level of AQP3 expression is observed in the reconstructed human epidermis in culture (Sougrat et al., 2002) and cultured human keratinocytes (Sugiyama et al., 2001). Measurement of water permeability by stopped-flow light scattering in the stripped human skin and reconstructed epidermis showed that it is inhibited by mercurial and acidic pH, suggesting that water transport in the human epidermis is carried out by the water channel. AQP3-mediated water transport seems to be important to improve the hydration of the viable layers of the epidermis below the keratinized layer (Matsuzaki et al., 1999a; Sougrat et al., 2002).

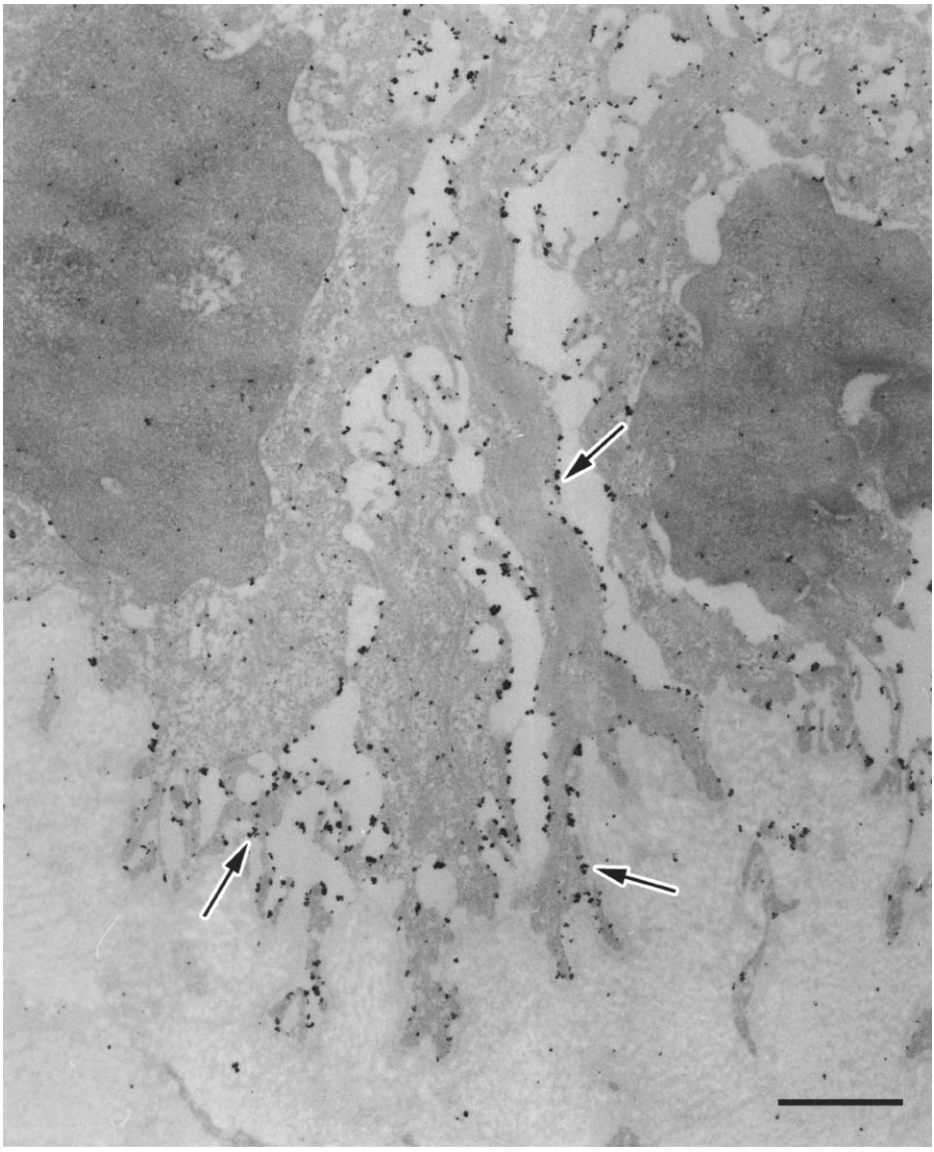


Fig. 10. Ultrastructural localization of AQP3 in the epidermis of the lower lip skin in the rat. AQP3 is localized along the plasma membrane of basal cells of the epidermis (arrows). Bar: 1 μm. Reproduced from Matsuzaki et al. (1999a) with permission from the Histochemical Society.

In the AQP3-null mice, a reduction of water and glycerol permeability in the epidermis was observed (Ma et al., 2002). In addition, surface conductance measurement showed a remarkable reduction of the water content of the keratinized layer. These observations directly show that AQP3 plays a critical role in the

hydration of the epidermis, especially in the keratinized layer. Further analyses revealed that in the keratinized layer, the content of water and glycerol was reduced to 50% of the control wild type (Hara et al., 2002). The content of ions and small solutes other than glycerol such as urea, glucose, and lactic acid was not affected. In addition, skin elasticity was reduced, and basal skin barrier recovery and wound healing were delayed. Since AQP3 is permeable to glycerol as well as water, the selective reduction of glycerol content in AQP3-null mice in the epidermis and keratinized layer may account for these defects. These deficiencies in the epidermis in the AQP3-null mice were fully recovered by the oral administration of sufficient glycerol (Hara and Verkman, 2003). Glycerol could not be replaced by other glycerol-like osmolytes such as xylitol, erythritol and propanediol, showing that glycerol is the key molecule in the maintenance of healthy skin. In fact, reduced transport of glycerol from the blood to the keratinized layer was observed in AQP3-null mice. Taken together, AQP3 is a critical determinant in supplying glycerol to the epidermis and a sufficient amount of glycerol in the epidermis is important in maintaining the hydration and healthy condition of the epidermis. This experiment also provided a scientific basis of glycerol in cosmetic and medical skin formulations (Hara and Verkman, 2003).

8.7.2. AQP3 in the embryonic development of the skin

Expression level of AQP3 in the epidermis is regulated developmentally (Fig. 8D) (Matsuzaki et al., 1999a). In the 15-day fetal rat, AQP3 is not detectable in the skin. AQP3 expression in the epidermal cells is evident at day 18. In postnatal development, the expression level of AQP3 rapidly increases to the adult level. The epidermal barrier to water loss in the rat is rapidly established during embryonic day 20 (Aszterbaum et al., 1992). During fetal development in utero, the fetus is surrounded by amniotic fluid and there is no risk of water loss. Upon delivery, a new-born animal is suddenly transferred from the watery environment to the air, facing possible dry-up of the body. The epidermis is the interface of the animal body to the air and is most susceptible to the water loss. The emergence of AQP3 in fetal rat skin before delivery might be a protective measure to keep the epidermis hydrated after birth.

8.8. AQP3 in the brain

The presence of AQP3 in the ependymal cells was reported (Ma et al., 1994). By immunofluorescence labeling, AQP3 was localized to the basolateral membrane.

8.9. AQP3 in the eye

AQP3 is abundant in the stratified squamous epithelia of the bulbar conjunctiva, the continuation of the epidermis (Hamann et al., 1998). Since the surface of the eye tends to be dry under certain circumstances, the role of AQP3 may be similar to that in the skin.

8.10. AQP3 in the ear

In the mouse ear, AQP3 is abundant in the spiral ligament near the area where the basilar membrane anchors in the cochlea, fibrocytes in the spiral limbus, and cells bordering the inner spiral tunnel (Huang et al., 2002).In the vestibular system, AQP3 is localized in the fibrocytes in the subepithelial connective tissues of the saccule. Aquaporins in the inner ear may be involved in the homeostasis of endolymph.

8.11. Regulation of the expression of AQP3

As the possible role of AQP3 in the protection against water loss was suggested (Fig. 8E) (Matsuzaki et al., 1999a, 2001), its expression may be regulated by the osmotic environment. In fact, the expression of AQP3 increases when MDCK cells are cultured in the hypertonic medium (Fig. 11) (Matsuzaki et al., 2001). In cultured human keratinocytes, an increase in the osmotic pressure with sorbitol, sodium chloride, mannitol, and sugars resulted in the increased AQP3 mRNA expression (Sugiyama et al., 2001). This observation also shows that osmotic stress upregulates AQP3 gene expression.

9. AQP4

9.1. Cloning

AQP4 was originally cloned in rats as a new aquaporin abundantly expressed in the brain by homology cloning (Jung et al., 1994; Hasegawa et al., 1994b). When expressed in *Xenopus* oocytes, it exhibited mercurial-insensitive osmotic water permeability and was named the mercurial-insensitive water channel (MIWC). The AQP4 gene encodes two distinct mRNA with different translation initiating methionines, M1 or M23, and two corresponding forms of AQP4 proteins are synthesized. Human AQP4 was also cloned from the fetal brain cDNA library (Yang et al., 1995; Lu et al., 1996). Immunogold electron microscopy employing antibodies that distinguish these two forms of AQP4 revealed that they are colocalized in the astrocytes (Neely et al., 1999). Velocity centrifugation, cross-linking, and immunoprecipitation analyses of solubilized AQP4 revealed that they form heterotetramers (Neely et al., 1999). These heterotetramers exhibited similar water permeability, when expressed in *Xenopus* oocytes, suggesting that the N-terminus of AQP4 had little effect on the activity of AQP4.

9.2. Orthogonal arrays in the membrane

Freeze-fracture replica electron microscopy revealed arrays of orthogonally arranged intramembranous particles in the plasma membrane of astrocytes (Landis and Reese, 1974) and skeletal muscle cells (Ellisman et al., 1976) and were named

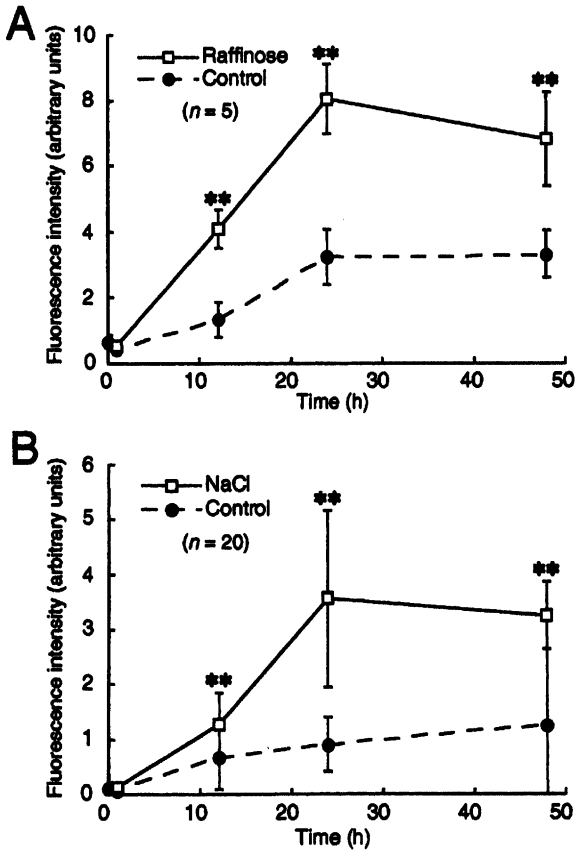


Fig. 11. Hypertonicity-induced expression of AQP3 in MDCK cells. Cells cultured on coverslips were maintained in isotonic medium (control) or exposed to hypertonic medium containing either raffinose (A) or NaCl (B) for the indicated times. AQP3 in cells was immunofluorescently labeled and examined with a fluorescence microscope. Fluorescence images of 5 (A) or 20 (B) fields for each time point were recorded with a cooled-CCD camera under identical conditions, and the fluorescence intensity of individual images was measured. Data are means $\pm$SD. $^{**}P < 0.01$ versus corresponding value for control cells. Reproduced from Matsuzaki et al. (2001) with permission from the American Physiological Society.

orthogonal arrays. The localization of orthogonal arrays is parallel with that of AQP4. In addition, a 28-kDa protein identified by antibodies against sarcolemmal antigen immunolabeled the plasma membrane with orthogonal arrays, suggesting that the arrays may represent AQP4 (Verbavatz et al., 1994). When AQP4 is expressed in CHO cells, orthogonal arrays are formed (Yang et al., 1996; van Hoek et al., 1998). Fracture label analysis showed that these orthogonal arrays found in AQP4-transfected CHO cells are labeled with anti-AQP4 antibody. Fracture labeling of the rat brain and spinal cord also showed the specific labeling of the orthogonal array with AQP4 (Rash et al., 1998). Moreover, these orthogonal arrays disappear in

the skeletal muscle, collecting duct cells in the kidney, and the brain of the AQP4-null mice, showing the identity of orthogonal arrays as AQP4 (Verbavatz et al., 1997). These observations lead to the conclusion that the orthogonal array is a specialized membrane domain composed of AQP4.

9.3. AQP4 in the kidney

AQP4, together with AQP3, is localized at the basolateral membrane of the principal cells of the collecting ducts in the rat (Frigeri et al., 1995a) (Fig. 12). Immunogold electron microscopy revealed that AQP4 and AQP3 are colocalized in the basolateral membrane of principal cells (Frigeri et al., 1995b). In the mouse, AQP4 is present not only in the basolateral membrane of collecting duct cells but also in the S3 proximal tubule cells (van Hoek et al., 2000). The significance of species differences is not clear but could be due to the physiological differences between mice and rats.

AQP4 may serve as a water channel for the exit of water from the basolateral membrane for the concentration of the urine. AQP4 deletion has little or no effect on development, survival, growth, and neuromuscular function, but produces a small defect in urinary concentrating ability (Ma et al., 1997a). Transepithelial osmotic water permeability is reduced by 4-fold in the initial inner medullary collecting duct, suggesting that AQP4 is responsible for the majority of basolateral water transfer but its deletion is associated with a very mild defect in urinary concentrating ability (Chou et al., 1998). Deletion of both AQP1 and AQP4 results in the further reduction of urinary concentrating ability compared with that of AQP1- or AQP4-null mice, suggesting an additive effect of AQP1 and AQP4 in the urine concentration (van Hoek et al., 2000). AQP3/AQP4 double-knockout mice show greater impairment of urinary concentrating ability compared with AQP3 single-knockout mice (Ma et al., 2000b). These observations show that AQP4 plays an important role in the concentration of urine in the collecting ducts. The significance of AQP4 in the kidney is described and discussed in detail in recent review articles (Verkman, 1999, 2000; Nielsen et al., 2002).

9.4. AQP4 in the skeletal muscle

Immunofluorescence labeling of rat skeletal muscle revealed that AQP4 is selectively expressed in the fast-twitch extensor digitorum longus muscle (Frigeri et al., 1998) (Fig. 13). AQP4 is not found in the slow-twitch soleus muscle. AQP4, by facilitating a faster response in osmotic water permeability, seems to play an important role in the rapid volume change associated with contraction in the fast-twitch muscle. Immunogold electron microscopic labeling revealed that AQP4 is localized at the sarcolemma (Frigeri et al., 1998). AQP4 is absent in the neuromuscular junction (Crosbie et al., 2002).

AQP4 is markedly reduced in the skeletal muscle of dystrophin-deficient mdx mice (Frigeri et al., 1998). In addition, similar results were obtained in various model animals of muscular dystrophy and cardiomyopathy such as transgenic mice with a

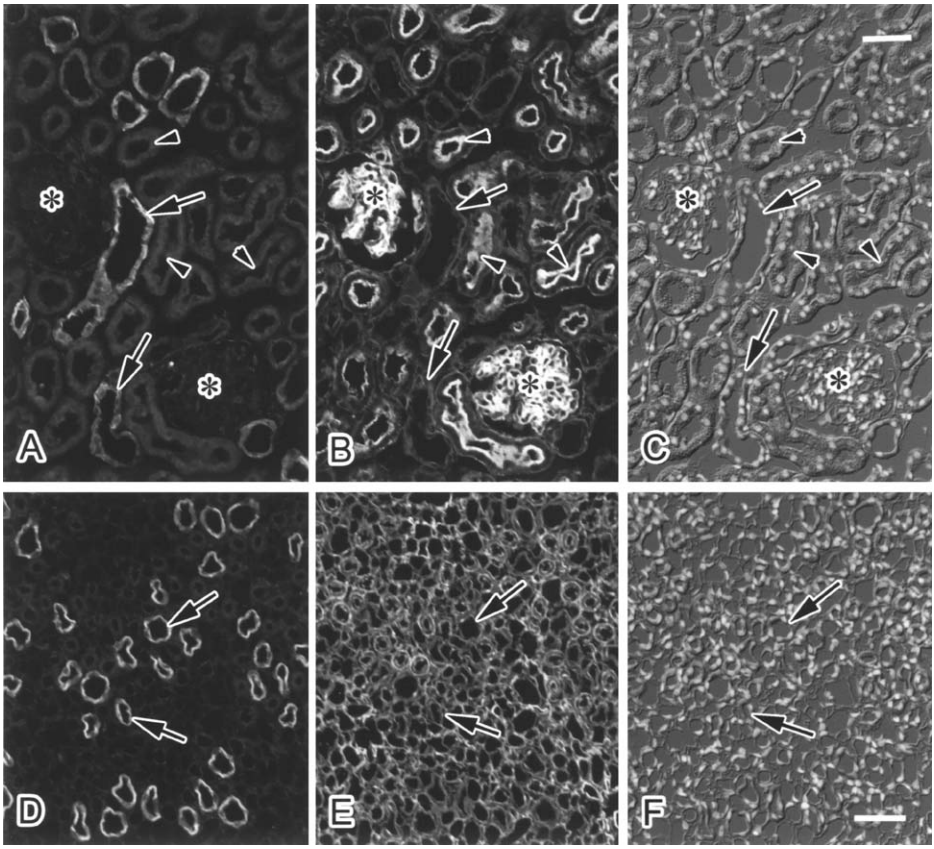


Fig. 12. AQP4 in the rat kidney cortex (A–C) and inner medulla (D–F). AQP4 (A, D), F-actin visualized with fluorescein-phalloidin (B, E), and Nomarski images merged with nuclear DNA images of DAPI fluorescence (C, F) are shown. AQP4 is localized in the collecting ducts (arrows) in the cortex (A–C) and medulla (D–F). Proximal tubules (arrowheads) and glomeruli (*) are negative for AQP4. Bars: 50 μm.

truncated dystrophin gene on an mdx background, sarcoglycan-deficient mice, and BIO 14.6 cardiomyopathic hamsters representing δ-sarcoglycan-deficient limb girdle muscular dystrophy-2F. These results indicate that the loss of AQP4 in skeletal muscle correlates with muscular dystrophy and may be a common feature of pathogenesis (Crosbie et al., 2002).

Expression of AQP4 is severely reduced in Duchenne muscular dystrophy, and in 50% cases of Becker muscular dystrophy when examined by immunofluorescence labeling and immunoblotting (Frigeri et al., 2002). The marked reduction of AQP4 expression was also shown in Duchenne muscular dystrophy, and Fukuyama-type congenital muscular dystrophy by immunohistochemisty, immunoblotting, and RT-PCR (Wakayama et al., 2002, 2003). The expression of AQP4 does not change in

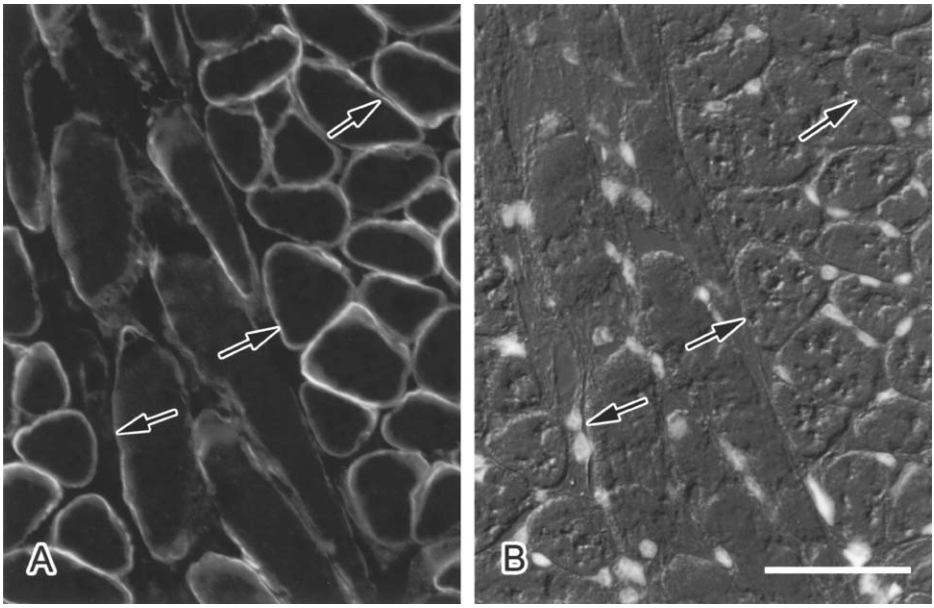


Fig. 13. AQP4 in the skeletal muscle in the rat tongue. AQP4 (A), and Nomarski image merged with nuclear DNA image of DAPI fluorescence (B) are shown. AQP4 is localized along the plasma membrane of skeletal muscle fibers (arrows). Bar: 50 μm.

limb–girdle muscular dystrophy and facioscapulhumeral muscular dystrophy biopsy specimens (Frigeri et al., 2002). These examinations indicate that reduction of AQP4 occurs in human muscular dystrophy and may be an important feature of Duchenne and Becker muscular dystrophy.

The recruitment of AQP4 to the sarcolemma seems to be mediated by the binding of the C-terminus of AQP4 to the PDZ domain of sarcoglycan in the dystrophin-associated protein (DAP) complex that seems to play important roles in the integrity and stability of the sarcolemma (Frigeri et al., 2001; Adams et al., 2001).

In the AQP4-null mice, the fast-twitch muscle of the extensor digitorum longus showed no significant change compared with that in the wild ones in osmotic water permeability and force generation (Yang et al., 2000b). Similar results were obtained in mdx mice (Yang et al., 2000b). These observations indicate that AQP4 does not play a significant role in the skeletal muscle. Further studies are needed to clarify the relationship between AQP4 and the pathogenesis of human muscular dystrophy.

9.5. AQP4 in the stomach

Immunohisotochemically, AQP4 was reported to be expressed in parietal cells in the human stomach (Misaka et al., 1996). In the rat stomach, immunoperoxidase labeling showed that AQP4 is present in the basolateral membrane of the parietal cells of the gastric gland (Koyama et al., 1999). Double immunofluorescence labeling

with H^+/K^+-ATPase clearly showed that AQP4 is expressed only in the parietal cells (Fujita et al., 1999; Wang et al., 2000; Carmosino et al., 2001) (Fig. 14). Quantitative immunogold electron microscopy demonstrated that AQP4 is preferentially localized at the basal membrane where plasma membrane is in contact with the basal lamina (Fujita et al., 1999). The apical and lateral membranes were barely labeled with AQP4 antibodies. The mechanism of preferential localization to the basal membrane is not clear, but AQP4 might be interacting with the PDZ-containing proteins through its C-terminus sequence of LSSV (Fujita et al., 1999).

The effect of histamine was examined in the human gastric cell line (HGT-1) stably transfected with rat AQP4 (Carmosino et al., 2001). Expression of AQP4 at the basolateral plasma membrane was accompanied by distinct orthogonal arrays of intramembranous particles by freeze-fracture replica electron microscopy. After histamine stimulation for 20 min, the density of orthogonal arrays decreased by 50%

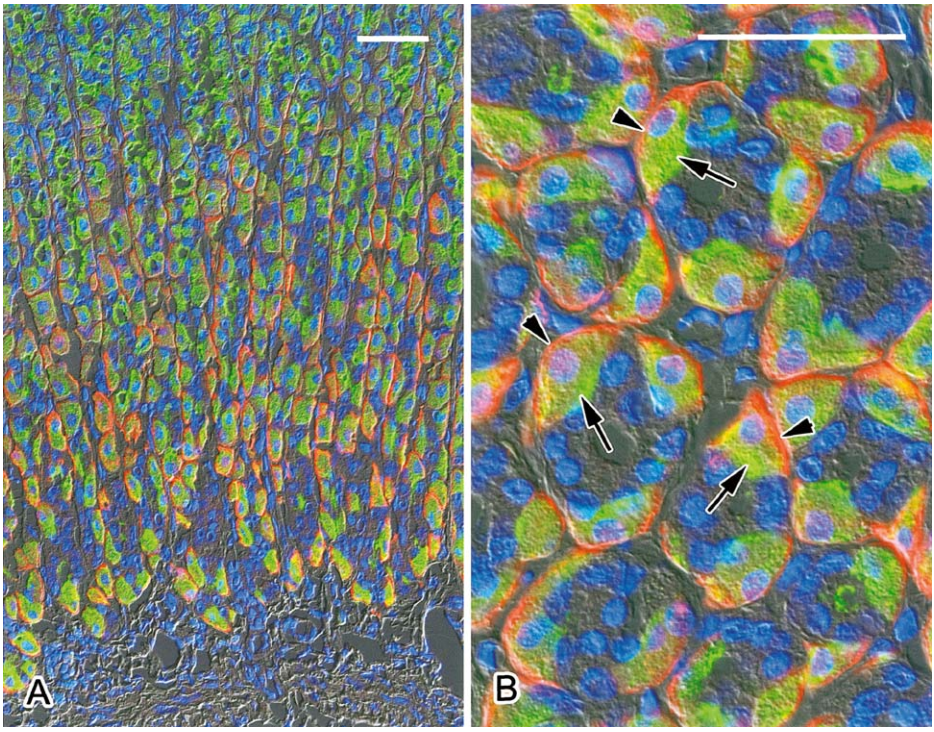


Fig. 14. AQP4 in the rat stomach. Immunofluorescence images of AQP4 (red) and H^+/K^+-ATPase (green) are projected onto Nomarski images. Nuclei are counterstained with DAPI (blue). (A) A survey view. AQP4 is seen in the cells of gastric gland. (B) AQP4 (red) is coexpressed in parietal cells with H^+/K^+-ATPase (green). AQP4 is localized in the basolateral region (arrowheads), whereas H^+/K^+-ATPase labeling is seen in the supranuclear to apical regions (arrows). Bars: 50 μm.

and internalization of the surface membrane was seen by cell surface biotinylation. This observation indicates that AQP4 may undergo acute rearrangement by endocytosis (Carmosino et al., 2001).

Localization of AQP4 in the parietal cells suggests its role in the transcellular transfer of water accompanying HCl secretion. However, no significant difference is seen in baseline gastric acid secretion, pentagastrin-stimulated, histamine plus carbachol-stimulated acid secretion, gastric pH, and gastric fluid secretion between the AQP4-null mice and wild ones (Wang et al., 2000). AQP4-null mice showed normal morphology in the gastric epithelium at least at the light microscopic level. Further studies are needed to elucidate the role of AQP4 in acid secretion in parietal cells.

9.6. AQP4 in the lung and airway epithelium

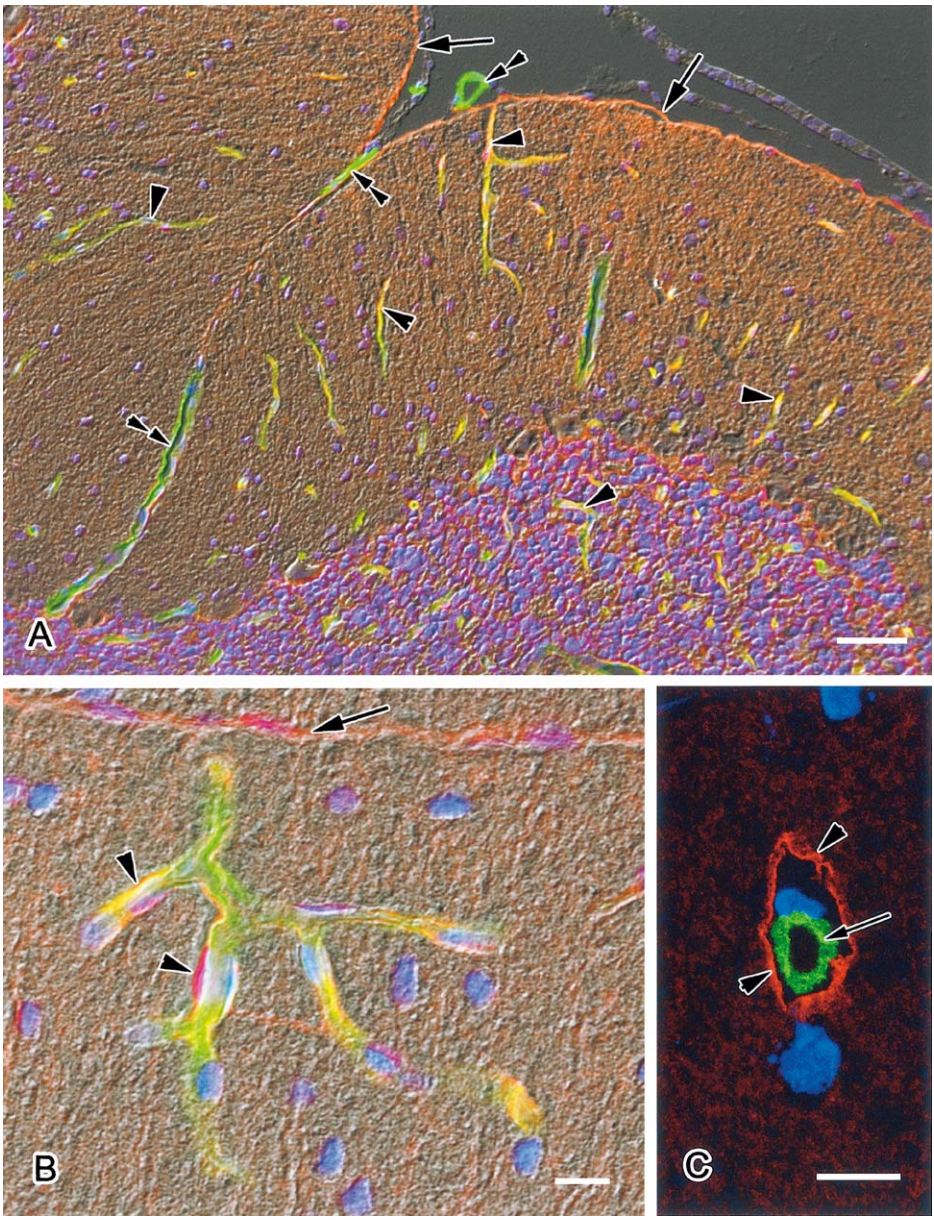
Osmotic water permeability is high in the lung and airway. Water transporting activity in the lung and airway is important in maintaining the humidification of the air, secretion, water clearance at birth, maintenance of the volume and composition of airway surface liquid, and the formation of edema (Verkman et al., 2000a). AQP1, AQP3, AQP4, and AQP5 are major expressed aquaporin isoforms. AQP4 is expressed mainly in the epithelium lining the airway whereas AQP1 is expressed in microvascular endothelia, AQP3 in large airway epithelium, and AQP5 in the type I pneumocytes of alveoli and secretory glands (Verkman et al., 2000a).

RNase protection assay showed that AQP4 is expressed in the respiratory tract including the lung and trachea (Umenishi et al., 1996). Localization of AQP4 in the rat lung and airway epithelium was studied by an immunoperoxidase method and immunogold electron microscopy (Nielsen et al., 1997a). AQP4 is localized in the basolateral membrane of columnar cells of bronchial, tracheal, and nasopharyngeal epithelium (Nielsen et al., 1997a; Kreda et al., 2001). In nasal conchus, AQP4 resides in the basolateral membrane of intra- and sub-epithelial glands. AQP4 in the lung and airway epithelium may be involved in alveolar fluid clearance in adult and neonatal lungs, and the formation of edema after lung injury. However, examination of the AQP-null mice revealed that AQP4 is not essential in these processes (Song et al., 2000a).

9.7. AQP4 in the brain

Northern blot analysis and RNase protection assay showed that AQP4 is abundantly expressed in the brain (Jung et al., 1994) (Fig. 15). AQP4 is present not in the neurons but in the astrocytes where it is preferentially concentrated in the endfeet and glia limitans (glial limiting membrane) (Nielsen et al., 1997b). Detailed localization of AQP4 was carried out by immunoperoxidase labeling and immunogold electron microscopy (Nielsen et al., 1997b). AQP4 is abundant in astrocytes bordering the subarachnoidal space, ventricles, and blood vessels. AQP4 is localized in the plasma membrane of astrocytes, and is particularly concentrated in the area of direct contact with capillaries, ependymal layer and pia. In addition, the

basolateral membrane of the ependymal cells that line the subfornical organ is positive for AQP4, whereas the ependyma of the choroid plexus is devoid of AQP4 (Nielsen et al., 1997b). The polarized distribution of AQP4 in the perivasucular endfeet and glia limitans indicates the presence of a specialized membrane domain for water flux by AQP4. Colocalization of AQP4 and cholinergic muscarinic



receptor was reported in the rat astrocytes and ependymal cells, suggesting a close relationship between water and electrolyte movement (Badaut et al., 2000). Concentrated AQP4 may be responsible for water transport to compensate for the change of osmolarity in response to K^+ siphoning by astrocytes accompanying the high level of neuronal activity as is characteristic in Müller cells in the retina (Nielsen et al., 1997b).

The importance of the di-leucine-like motif and tyrosine-based motif in the polarized targeting of AQP4 was suggested in MDCK cells expressing AQP4 (Madrid et al., 2001). Also the importance of the PDZ binding motif was suggested in cultured astrocytes (Nakahama et al., 2002).

AQP4 has the C-terminus sequence of SSV, potentially capable of binding to PDZ domains. AQP4 seems to be tethered at the endfeet of astrocytes by binding to the PDZ domain of syntrophin through its C-terminus (Neely et al., 2001). Syntrophin is a scaffolding adaptor protein that forms the dystrophin protein complex. Chemical crosslinking and immunoprecipitation in the rat cerebellum showed that AQP4 forms a complex with dystrophin, β-dystroglycan and syntrophin (Neely et al., 2001). In α-syntrophin-null mice, immunoblotting showed that the total expression level of AQP4 is normal. Immunogold electron microscopy, however, revealed that the polarized distribution to the endfeet is reversed, showing that α-syntrophin is required for the perivascular localization of AQP4 (Neely et al., 2001). Double labeling immunogold electron microscopy also confirmed that AQP4 and α1-syntrophin are colocalized at the endfeet of astrocytes (Inoue et al., 2002). In dystrophin-null mice (mdx-βgeo), mislocalization of AQP4 proteins occurs suggesting that dystrophin-associated protein complex is important in its targeting to astroglial endfeet surrounding capillaries and glia limitans (Vajda et al., 2002).

AQP4 is also abundant in osmosensory areas including the supraoptic nucleus and subfornical organ (Jung et al., 1994; Nielsen et al., 1997b). Heavy labeling is localized in the stacks of glial lamellae, suggesting that the lamellae may serve as a transducer or amplifier in the osmoregulatory responses.

Fig. 15. AQP4 and glucose transporter GLUT1 in the rat cerebellum. Cryostat sections were immunofluorescently labeled for AQP4 (red) and GLUT1 (green). Nuclei were counterstained with DAPI (A, B) or TO-PRO-3 (C) and shown in blue. Fluorescence images are projected onto Nomarski images in A and B. GLUT1 is present in the endothelial cells in the brain and serves in the transfer of glucose across the blood–brain barrier (Takata et al., 1997). (A) A survey view. AQP4 (red) is abundant in the outermost edge of the cerebellum corresponding to the glia limitans (arrows). Blood vessels inside the brain are positive for both AQP4 and GLUT1 and seen in yellow (arrowheads). Note that blood vessels outside the brain are positive for GLUT1 but negative for AQP4 (double arrowheads). (B) Enlarged view. AQP4 is abundant in glia limitans (arrow). AQP4 is also abundant around GLUT1-positive blood vessels (arrowheads). (C) Confocal image of the blood vessel. GLUT1 is localized in the endothelial cells of the blood vessel (arrow), and AQP4 is localized in the surrounding astroglia endfeet (arrowheads). Bars: 50 μm (A) and 5 μm (B, C).

9.8. AQP4 and brain edema

Because of its abundance and localization, the participation of AQP4 in the formation of edema in the brain was suggested (King and Agre, 1996; Nielsen et al., 1997b). Upregulation of the AQP4 level was found in brain injuries and tumors and is related to the disruption of the blood–brain barrier suggesting the contribution of AQP4 in edema formation (Vizuete et al., 1999; Saadoun et al., 2002).

The AQP4-null mice showed much better survival than wild-type mice in a model of brain edema caused by acute water intoxication, or focal ischemic stroke by cerebral artery occlusion (Manley et al., 2000). When water intoxication was tested by injecting water and an anti-diuretic hormone, delayed onset of brain edema was observed in dystrophin-null mice whose AQP4 is mislocalized (Vajda et al., 2002). These results indicate that AQP4 plays a key role in the modulation of brain water transport, and suggests that AQP4 inhibitors could serve as a possible therapeutic measure to reduce brain edema associated with a wide variety of cerebral disorders (Manley et al., 2000; Vajda et al., 2002; Papadopoulos et al., 2002). Precautious care should be taken in the therapeutic manipulations of AQP4 since it facilitates the bidirectional water flux (Amiry-Moghaddam et al., 2003).

9.9. AQP4 in the eye

Physiological fluid movement and regulation of the ionic environment and volume is crucial in the eye function. Multiple isoforms of AQPs are shown to be expressed in the eye (Nagelhus et al., 1998; Hamann et al., 1998). Among them, in situ hybridization, immunoperoxidase labeling, and immunogold electron microscopy showed that abundant AQP4 is present in the retinal Müller cells and astrocytes (Nagelhus et al., 1998; Hamann et al., 1998). Detailed quantitative immunogold electron microscopic analysis revealed that in the Müller cells, AQP4 is preferentially concentrated in the endfeet membranes (Nagelhus et al., 1998). This observation suggests that AQP4 plays a prominent role in the water handling in the retina by directing osmotically driven water flux to the vitreous body (Nagelhus et al., 1998). Marked enrichment of AQP4 in the vitreal and perivascular endfeet matches the preferential localization of K^+ channels and indicates that AQP4 may be involved in the water flux associated with K^+ siphoning in the retina. In fact, quantitative immunogold labeling for inwardly rectifying K^+ channel Kir4.1 in the retinal Müller cells in the rat revealed that both AQP4 and Kir4.1 are enriched in the plasma membrane domains that face the vitreous body and blood vessels (Nagelhus et al., 1999). These observations suggest that Müller cells may be able to direct the water flux to the vitreous and the blood vessels via specialized membrane domains where Kir4.1 and AQP4 are colocalized. Reduced electroretinogram β-wave potentials were recorded in 10-month-old AQP4-null mice, showing that deletion of AQP4 results in mildly abnormal retinal function (Li et al., 2002).

AQP4 is also expressed in the non-pigmented epithelial cells of the ciliary body (Hamann et al., 1998). Measurement of the aqueous humor production in the mice

AQP1 and/or AQP4 revealed that AQP1 and AQP4 play a modest role in the intraocular pressure regulation by facilitating the water transfer (Zhang et al., 2002).

9.10. AQP4 in the ear

Multiple isoforms of AQPs are expressed in the inner ear and seem to have roles in the delicate regulation of volume and the ionic environment in a confined compartment for the acoustic signal transduction. In situ hybridization and immunofluorescence labeling revealed that AQP4 is expressed and localized in the selected types of supporting cells in the organ of Corti (Minami et al., 1998; Takumi et al., 1998; Li et al., 2001). AQP4 is also expressed in supporting cells in the vestibular sensory organs and glial cells surrounding the auditory nerves in the rat and mouse (Mhatre et al., 2002b). Immunogold electron microscopy showed that AQP4 is localized at the basolateral plasma membrane of Hensen's cells, Claudius cells, and inner sulcus cells (Takumi et al., 1998). Hair cells were not labeled.

Roles of aquaporins in hearing were assessed in AQP-null mice lacking either AQP1, AQP3, AQP4 or AQP5 (Li and Verkman, 2001). Although a light microscopic examination of the cochlea showed no detectable differences, AQP4-null mice elicited impaired hearing of the frequency-independent type. Impaired hearing but normal neural conduction time in the auditory nerve was also reported in AQP4-null mice, suggesting that cochlear dysfunction may be responsible for the hearing impairment (Mhatre et al., 2002b). AQP4 in the supporting cells in the organ or Corti may be involved in maintaining osmotic balance during K^+ recycling (Li and Verkman, 2001). The uptake of K^+ by Hensen's cells drives AQP4-mediated water influx and water exits the supporting cell syncytium through AQP4-expressing basal membranes of Claudius cells that face the root of the spiral ligament. AQP4 thus seems to be involved in facilitating the K^+ flux in supporting cells as is proposed in the brain and the eye. The deafness may be caused by the altered basal ionic composition of the endolymph and/or outer hair cell volume (Li and Verkman, 2001).

In the middle ear, AQP4 is present in the basolateral membrane of ciliated cells as in the airway epithelium (Minami et al., 2001).

10. AQP5

10.1. Cloning and characterization

cDNA of AQP5 was identified from the rat submandibular gland (Raina et al., 1995) and from the human salivary gland (Lee et al., 1996) by homology cloning. Expression of rat AQP5 cRNA in *Xenopus* oocytes conferred a marked increase in mercurial-sensitive osmotic water permeability (Raina et al., 1995).

10.2. AQP5 in the salivary gland

In situ hybridization showed that AQP5 is expressed in the secretory lobules in the submandibular gland (Raina et al., 1995). By immunohistochemistry, AQP5 is present in the major salivary glands, i.e., the submandibular gland, parotid gland, and sublingual gland (Nielsen et al., 1997a; He et al., 1997; Funaki et al., 1998; Matsuzaki et al., 1999b) (Fig. 16). In addition, small submucosal salivary glands in the tongue also express AQP5 (Matsuzaki et al., 2003). AQP5 was detected in the secretory acinar cells of submandibular, parotid, and sublingual glands in the rat (Matsuzaki et al., 1999b). Expression of AQP5 is restricted to the lobuli; the interlobular tissues including large ducts and blood vessels are negative for AQP5. In the submandibular gland, AQP5 is present in the acinar secretory cells where it is localized at the apical membrane including the intercellular secretory canaliculi (Matsuzaki et al., 1999b). Detailed examination of semithin frozen sections that were doubly labeled with tight junction protein occludin and AQP5 clearly showed that positive labeling of AQP5 along the lateral aspects of acinar cells is not the lateral plasma membrane but in fact the intercellular secretory canaliculi (Matsuzaki et al., 1999b). The basolateral membrane is negative for AQP5. The abundance of AQP5 at the apical membrane of the secretory acinar cells suggests that the secretion of water is carried out transcellularly rather than paracellularly. In the submandibular gland of the rat, much more AQP5 was detected along the apical membrane of the intercalated duct cells in addition to acinar cells (Matsuzaki et al., 1999b). This observation suggests that the intercalated ducts may participate in the secretion of water. In the parotid and sublingual glands, AQP5 is restricted to the acinar cells.

The apparent homology of AQP5 amino acid sequence to AQP2 suggests the possibility that water permeation in the cells of the salivary gland is controlled by a translocation mechanism as seen in AQP2 in the collecting duct cells in the kidney; AQP5 stored in the intracellular compartment moves to the plasma membrane upon stimulation. In the parotid, a fractionation study suggested that cholinergic stimulation induced the translocation of AQP5 from the intracellular membrane fraction to the apical membrane fraction (Ishikawa et al., 1998). The translocation was rapid and transient, and within 5 min the retrieval of AQP5 from the apical membrane to the intracellular pool occurred. However, immunohistochemical examination showed that AQP5 is restricted to the plasma membrane, and no intracellular pool of AQP5 has so far been identified. The inability to detect a cytoplasmic AQP5 pool may be due to the possible masking of C-terminus antigenic sites of AQP5 with other proteins. Similar translocation of AQP5 to the plasma membrane triggered by cAMP was reported in the murine lung epithelial cell line and mouse lung tissue slices (Yang et al., 2003).

In human parotid, submandibular, sublingual, and labial glands, northern blot analysis showed the abundance of AQP5 transcript (Gresz et al., 2001). Immunoperoxidase labeling of paraffin sections revealed that AQP5 is localized to the apical membrane of both mucous and serous acinar cells. In combination with AQP3 localized along the basolateral membrane, it may provide a pathway for the transcellular osmotic water flow in the formation of the primary saliva (Gresz et al., 2001).

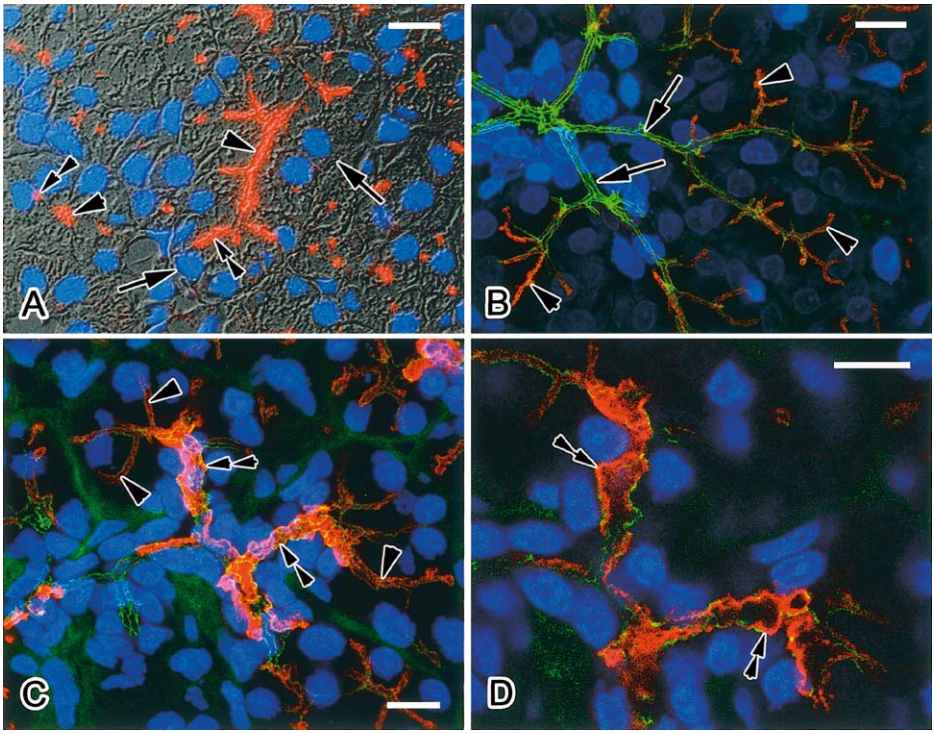


Fig. 16. AQP5 in the rat salivary glands. (A) Parotid gland. Immunofluorescence image of AQP5 (red) are projected onto Nomarski image. Nuclei are counterstained with DAPI (blue). AQP5 is localized at apical membranes of acinar cells (arrowheads) and scattered in the lateral aspects of acinar cells (double arrowheads). Basal membranes (arrows) and cytoplasm are not labeled for AQP5. (B) Parotid gland. Triple-labeling for AQP5(red), tight junction protein occludin (green), and DNA (TO-PRO-3; blue). Sixteen consecutive optical-section images obtained with a laser confocal microscope were projected to generate a single image. AQP5 is localized at the apical side along with occludin in acinar cells (arrowheads). Basal aspects and cytoplasm are not labeled for AQP5. No label for AQP5 is seen at intercalated ducts (arrows). (C) Submandibular gland. Triple-labeling for AQP5(red), occludin (green), and DNA (TO-PRO-3; blue). Twelve consecutive optical-section images were projected to generate a single image. AQP5 is localized at the apical side along with occludin in the acinar cells (arrowheads). Basal aspects and cytoplasm are not labeled for AQP5. Intense label for AQP5 is localized along intercalated ducts (double arrowheads). (D) Enlarged view of an intercalated duct region in the submandibular gland. A single optical-section from the 12 images used in C. Abundant label for AQP5 in intercalated ducts is restricted to the apical aspects (double arrowheads). Bars: 10 μm. Reproduced from Matsuzaki et al. (1999b) with permission from Springer.

To explore the role of AQP5 in saliva secretion, AQP5-null mice were generated (Ma et al., 1999; Krane et al., 2001b). The knockout mice have a grossly normal appearance but pilocarpine-stimulated secretion of saliva was reduced by more than

60% (Ma et al., 1999). Marked reduction of osmotic water permeability was seen in the parotid and sublingual acinar cells isolated from AQP5-null mice (Krane et al., 2001b). The saliva of AQP5-null mice was hypertonic and more viscous, whereas the secretion of amylase and protein secretion were not affected (Ma et al., 1999; Krane et al., 2001b). Although AQP1 and AQP4 are expressed in the salivary glands, no apparent defect was observed in saliva secretion in AQP1-null or AQP4-null mice (Ma et al., 1999). These observations show that AQP5 plays an important role in the saliva secretion.

10.3. AQP5 in the gastrointestinal tract

AQP5 is expressed in some of the mucus-secreting glands in the gastrointestinal tract in the rat (Parvin et al., 2002; Matsuzaki et al., 2003). In the stomach, AQP5 is expressed in the pyloric gland cells. In the intestine, AQP5 is present in the duodenal gland cells (Parvin et al., 2002; Matsuzaki et al., 2003). AQP5 was reported to be localized to both the apical and lateral membranes (Parvin et al., 2002). However, double-labeling with tight junction protein occludin revealed that AQP5 is mostly localized to the apical membrane (Matsuzaki et al., 2003). AQP5 is absent in goblet cells, absorptive epithelial cells of villi, and cells of intestinal glands.

Most pancreatic juice is isotonic and is secreted through the duct system. RT-PCR and immunohistochemistry revealed that AQP5 is expressed in the human pancreas (Burghardt et al., 2003). AQP5, together with AQP1, is expressed in the apical membrane of the intercalated duct cells. Localization of AQP5 and AQP1 is similar to that of the cystic fibrosis transmembrane conductance regulator (CFTR), a marker of ductal electrolyte secretion, suggesting that AQP5 may play a role in the water transfer in the pancreatic secretion. In the rat pancreas, however, AQP5 was not detected in the acinar, ductal or vascular tissues (Hurley et al., 2001). The inconsistent results could be due to species differences.

10.4. AQP5 in the airway glands

AQP5 is localized at the apical membrane of the glandular cells of the airway submucosal glands (Nielsen et al., 1997a). AQP4 is localized at the basolateral membrane (Nielsen et al., 1997a). Transepithelial transfer of water may be carried out by the combined action of AQP4 and AQP5. To assess the role of aquaporins, knockout mice lacking AQP4 or AQP5 were analyzed (Song and Verkman, 2001). Pilocarpine-induced secretion was markedly reduced in AQP5-null mice, but little effect was observed in AQP4-null animals. Since protein and salt secretion did not change in these animals, reduced fluid secretion could be attributed to the lack of AQP5 at the apical membrane of the secretory cells, suggesting that apical AQP5 is the rate-limiting step in the secretion of the airway glands. AQP5 in the airway glands may play an important role in determining the nature of the surface liquid and in the homeostasis as well as pathogenesis of cystic fibrosis (Song and Verkman, 2001). AQP5 is also present in the apical membrane of columnar epithelial cells of

the trachea, bronchi, and extrapulmonary lobar bronchioles in humans (Kreda et al., 2001) and mouse (Krane et al., 2001a).

10.5. AQP5 in the lung

AQP5 is specifically localized in the type I pneumocytes that cover the lung alveoli (Nielsen et al., 1997a; Funaki et al., 1998; Kreda et al., 2001; Krane et al., 2001a) (Fig. 17A). Immunoelectron microscopic examination clearly showed that the localization of AQP5 is restricted to the apical plasma membrane (Nielsen et al., 1997a). In the AQP5-null mouse, the lung was morphologically normal, and did not affect the expression of other aquaporins (AQP1, AQP3, and AQP4) (Ma et al., 2000a). Although the airspace–capillary osmotic water permeability was reduced by 10-fold by AQP5 deletion, apparently no significant changes were observed in the hydrostatic lung edema formation induced by an increase in pulmonary artery pressure, and in the active alveolar fluid absorption (Ma et al., 2000a). Deletion of AQP5 did not affect the lung fluid clearance in the neonatal mice, the lung fluid clearance in ventilated adult mice, or the lung fluid accumulation in a lung injury

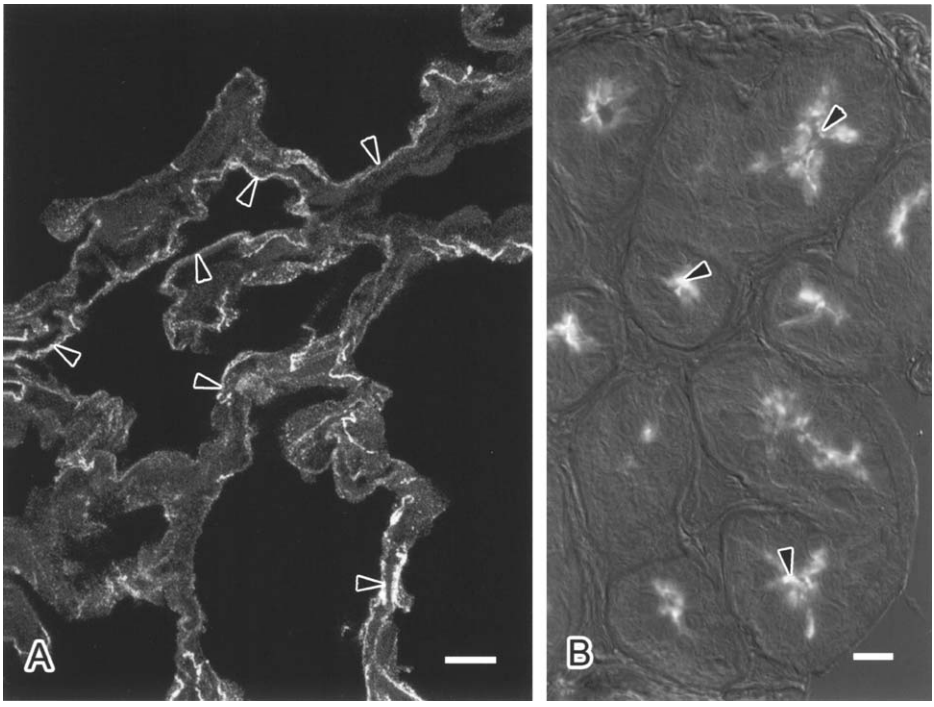


Fig. 17. AQP5 in the lung and sweat gland in the rat. (A) Lung. AQP5 is localized in the type I pneumocytes (arrowheads). A projection of 14 consecutive confocal images. (B) Sweat gland. AQP5 is localized to the apical membrane of the secretory cells (arrowheads). Fluorescence image for AQP5 is projected onto Nomarski image. Bars: 5 μm.

model (Song et al., 2000a). Deletion of both AQP5 and AQP1 had little effect on lower airway humidification (Song et al., 2001). These unexpected results suggest that AQP5 mediates osmotic water permeability in type I alveolar cells but is not indispensable in the normal function of the lung. Recently, AQP5-null mouse lung was shown to be hyperresponsive to cholinergic stimulation, suggesting that AQP5 influences bronchoconstriction (Krane et al., 2001a). Further studies are needed to see the relationship with asthma and AQP5.

In addition to strong expression of AQP5 in type I cells, the presence of AQP5 in type II cells was reported (Kreda et al., 2001; Krane et al., 2001a).

10.6. AQP5 in the sweat gland

The eccrine sweat gland secretes salts and water and plays important roles in thermoregulation and the pathophysiology of hydrosis. AQP5 was detected in mouse, rat, and human sweat glands by RT-PCR and immunoblotting (Nejsum et al., 2002). Immunoperoxidase and immunofluorescence labeling revealed that AQP5 is abundant in the rat and mouse sweat glands (Fig. 17B). In the secretory portion, AQP5 is localized at the apical and basolateral membrane, whereas it is restricted to the apical membrane in the initial portion of the excretory ducts. No AQP5 is seen in the last portion of the excretory ducts. Immunogold electron microscopy showed that abundant AQP5 is localized at the apical plasma membrane of secretory cells with minor labeling along the basolateral membrane. No intracellular storage of AQP5 has been found, suggesting that AQP5 does not undergo regulation of vesicular trafficking as seen in the case of AQP2 in the kidney collecting duct cells. The knockout of AQP5 did not affect the number or morphology of sweat gland in mice (Song et al., 2002). The effect of pilocarpine injection was examined in wild and AQP5-null mice. The number of active sweat glands became drastically reduced in AQP5-null mice, indicating that AQP5 is essential for sweat secretion (Nejsum et al., 2002). However, detailed analyses of sweat secretion in AQP5-null mice by proton nuclear magnetic resonance, real-time video imaging of sweat droplet formation as well as direct counting of droplets indicate that AQP5 is not physiologically involved in fluid secretion in mice sweat glands (Song et al., 2002).

10.7. AQP5 in the eye

In situ hybridization showed that AQP5 is expressed in the cornea (Raina et al., 1995). It is localized in the plasma membrane of the stratified squamous epithelial cells of the cornea (Hamann et al., 1998). AQP5 may play a role in maintaining the transparency of the corneal epithelium as well as underlying stroma.

10.8. AQP5 in the lacrimal gland

AQP5 is localized in the apical membrane of the secretory acini of the lacrimal gland in the mouse (Ishida et al., 1997) and the rat (Matsuzaki et al., 1999b).

Transcellular transfer of water may occur via AQP5 in the apical membrane and AQP4 in the basolateral membrane (Ishida et al., 1997). In the mouse lacrimal gland, tear secretion occurs by the pilocarpine treatment, and immunoreactivity for AQP5 at the apical membrane of the acinar cells increased when the antibody recognizing the C-terminus domain was used. Such an increase of reactivity was not observed when the antibody to the extracellular domain was used, suggesting that there was some modification of the AQP5 molecule in its cytoplasmic C-terminus upon stimulation (Ishida et al., 1997). In addition to possible regulation of AQP5 activity by its C terminus, a translocation mechanism could be responsible as seen in AQP2.

The role of AQP5 in the tear secretion was assessed in the knockout animals (Moore et al., 2000). Pilocarpine-stimulated tear secretion was measured in mice lacking AQP1, AQP3, AQP4, or AQP5. No significant differences were observed when compared with wild animals in the volume of tear fluid and the concentration of chloride ion. These results indicate that aquaporins including AQP5 are not essential in the lacrimal gland fluid secretion. AQP1 in the capillary and AQP4 at the basolateral membrane of acinar cells are not essential either. Since the knockout of AQP5 results in the defective saliva production (Ma et al., 1999), these unexpected results in tear production may be attributed to a much slower fluid secretion rate in the tear compared to the saliva secretion (Moore et al., 2000).

10.9. AQP5 in the inner ear

AQP5 is localized in the external sulcus cells and the cells of the spiral prominence in the lateral wall of the cochlear duct (Mhatre et al., 1999). The restricted expression of AQP5 in the apical turns of the cochlea suggests its potential role in low-frequency hearing (Mhatre et al. 1999). However, AQP5-null mice showed normal inner ear structure and hearing, suggesting that AQP5 is not critical in the inner ear auditory function (Merves et al., 2003).

10.10. Relationship to Sjögren's syndrome

Patients with Sjögren's syndrome suffer from dry mouth and dry eye due to deficient secretion of saliva and tear. Abnormal distribution of AQP5 in the acinar cells of the salivary gland was reported (Steinfeld et al., 2001). By immunoperoxidase staining of biopsy specimens, AQP5 was shown to be localized to the basal membrane of acinar cells in Sjögren's syndrome, suggesting that abnormal distribution of AQP5 in acinar cells and the resultant inability to pass water contribute to the deficiency of fluid secretion in this syndrome.

A similar aberrant distribution of AQP5 was reported in the lacrimal glands in Sjögren's syndrome (Tsubota et al., 2001). AQP5 was distributed diffusely in the cytoplasm rather than at the apical membrane in acinar cells of the lacrimal gland in Sjögren's syndrome. The AQP5 protein amount measured by ELISA did not change in Sjögren's syndrome suggesting that the selective defect can be attributed to intracellular trafficking rather than synthesis of AQP5. On the other hand, immunofluorescence and immunoperoxidase labeling of the paraffin sections of

the labial salivary gland showed that localization of AQP5 is confined to the apical membrane of acinar cells in Sjögren's syndrome in the same way as in the controls when immunostaining was carried out after antigen retrieval by microwave irradiation (Beroukas et al., 2001). This observation indicates that AQP5 is not responsible for the pathogenesis in Sjögren's syndrome. The inconsistent results may be attributed to the different antibodies used and/or staining procedures. AQP5 does not seem to play a major role in tear secretion in the lacrimal gland by the measurement of tear secretion in AQP5-null mouse (Moore et al., 2000). The roles of AQP5 in tear secretion in humans remain to be clarified.

Selective down-regulation of AQP1 in the myoepithelial cells surrounding acinar cells of the salivary gland was reported in Sjögren's syndrome. This observation suggests that the dysfunction of myoepithelial cells may play a crucial role in the reduction of saliva secretion (Beroukas et al., 2002). Further studies are needed to clarify the role of aquaporins in the secretion of saliva and tear and pathogenesis of this syndrome.

11. AQP6

11.1. Cloning and characterization

AQP6 was cloned as a homologue of aquaporins from the rat and human kidney cDNA library and was originally called as WCH3 and hKID, respectively (Ma et al., 1993a, 1996). When expressed in the *Xenopus* oocyte, an increase of glycerol or urea transport was not seen but an increase of mercurial-sensitive osmotic water permeability was observed (Ma et al., 1996). On the other hand, low basal water permeability and its rapid increase by the addition of mercury ion, a known water channel inhibitor to other aquaporins, was observed (Yasui et al., 1999a). AQP6 showed gated anion channel characteristics activated by low pH or HgCl2 when expressed in the *Xenopus* oocyte (Yasui et al., 1999a; Hazama et al., 2002). Detailed analysis in mammalian cells had been restricted because of its intracellular localization. Fusion protein of AQP6 with green fluorescent protein resulted in its delivery to the plasma membrane, where it exhibited nitrate channel activity (Ikeda et al., 2002). These unusual observations suggest that AQP6 is a vacuolar-type aquaporin not engaged in the water transfer at the cell surface.

11.2. Localization of AQP6

Immunohistochemical labeling of AQP6 with H^+ATPase revealed that AQP6 is expressed in the acid-secreting intercalated cells of collecting ducts in the rat kidney (Yasui et al., 1999a, b; Ohshiro et al., 2001) (Fig. 18). Immunoperoxidase and immungold electron microscopic examination and membrane fractionation demonstrated that AQP6 is localized not at the cell surface but resides in the intracellular vesicles in kidney epithelial cells of collecting ducts (Yasui et al., 1999b). Similar results were obtained by in situ hybridization and immunohistochemical

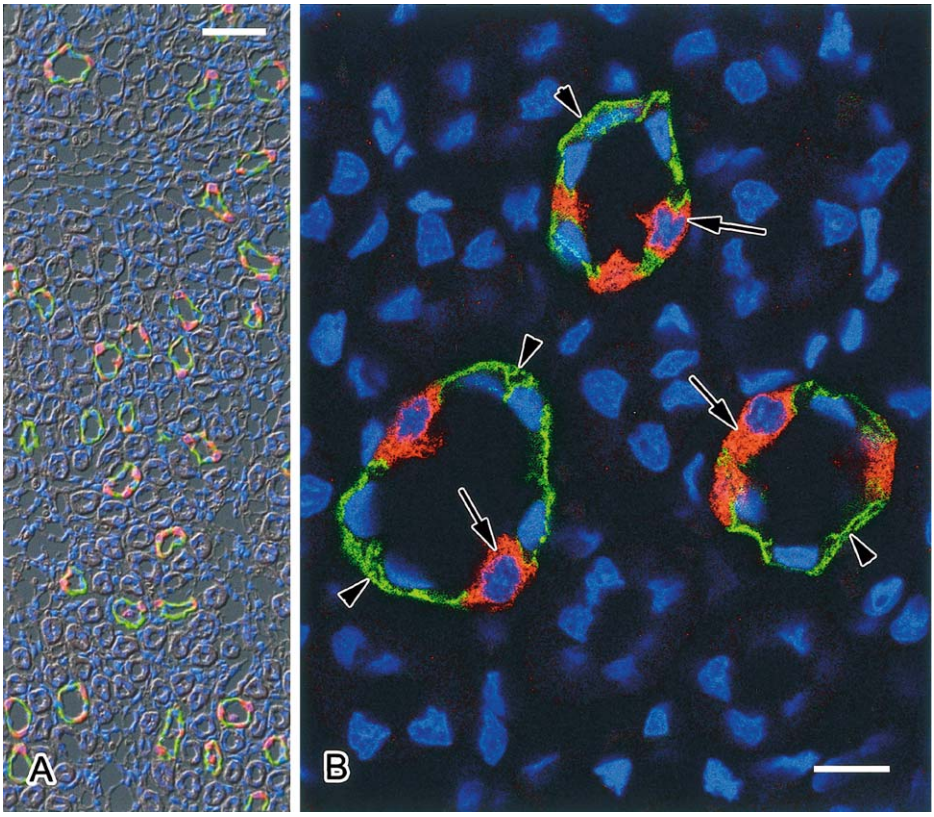


Fig. 18. AQP6 in the rat kidney. Immunofluorescence images of AQP6 (red) and AQP3 (green). Nuclei are counterstained with DAPI (A) or TO-PRO-3 (B) (blue). (A) A survey view of AQP6 and AQP3 projected onto Nomarski images in the kidney medulla. AQP6 is seen in the cells of AQP3-positive collecting ducts. Bar: 50 μm. (B) AQP6 (red) is expressed in AQP3-negative intercalated cells (arrows). Note that AQP6 is localized intracellularly. AQP3-positive principal cells (green) are negative for AQP6 (arrowheads). Bar: 10 μm.

examination of the rat kidney. AQP6 is concentrated in the outer and inner medullary collecting ducts. These observations indicate that AQP6 may play an important role inside the cell.

12. AQP7

12.1. Cloning and characterization

AQP7 was identified and cloned from the rat testis and showed the highest homology to AQP3 (Ishibashi et al., 1997b). cDNA of AQP7 encode a 26-kDa

protein. When expressed in *Xenopus* oocyte, it exhibited osmotic water permeability as well as stimulated facilitated transport of urea and glycerol (Ishibashi et al., 1997b; Kuriyama et al., 1997). The permeation is sensitive to mercurial. In addition, AQP7, together with AQP9, is proposed to be responsible for arsenite transport (Liu et al., 2002). Northern blot analysis and in situ hybridization showed its expression in the testis. The mouse and human homologue of rat AQP7 was cloned (Ishibashi et al., 1998b). Human AQP7 had been independently cloned as a gene uniquely expressed in adipose tissues and hence named aquaporin adipose (AQPap or AQP7L) (Kuriyama et al., 1997; Kishida et al., 2000, 2001a). Conservation of AQP7, or the sequence identity of AQP7 among species is low, which is unusual in the aquaporin family (Ishibashi et al., 1998b), suggesting its possible species-specific roles.

12.2. AQP7 in the kidney

RT-PCR of the microdissected nephron segments showed that the transcript of AQP7 is present in the proximal convoluted and straight tubules in the rat and mouse kidney (Nejsum et al., 2000). Immunohistochemically, AQP7 is localized in the brush border membrane of proximal straight tubules (S3) (Ishibashi et al., 2000a; Nejsum et al., 2000) (Fig. 19). These observations suggest that AQP7 may serve in the transcellular water reabsorption in concert with AQP1 and/or secretion of urea.

12.3. AQP7 in the adipose tissue

AQP7 is highly expressed in adipocytes and its contribution to glycerol release from adipocytes is proposed (Kishida et al., 2000). In 3T3-L1 adipocytes, expression of AQP7 is found upon differentiation of the cells from the fibroblast phenotype to an adipocyte one. The release of glycerol from 3T3-L1 adipocytes is mercurial sensitive, suggesting that AQP7 is instrumental in the transfer of glycerol. Confocal immunofluorescence microscopy revealed that in the non-stimulated state, AQP7 is present in the perinuclear cytoplasm with a scattered distribution. Upon epinephrine stimulation, AQP7-labeling became evident at the cell surface in 20 min, suggesting translocation of AQP7-bearing vesicles from the intracellular pool to the plasma membrane (Kishida et al., 2000). The epinephrine signal is mediated via protein kinase A. A similar mechanism as is seen in the translocation of AQP2 in the kidney collecting duct cells by ADH may work in the possible translocation of AQP7 in adipocytes. Considering that AQP7 is the only aquaglyceroporin expressed in adipocytes, it may play a pivotal role in the release of glycerol and the regulation of the plasma glycerol level. Coordinated work of AQP7 in the release of glycerol in the adipocytes, and its uptake in hepatocytes by another aquaglyceroporin AQP9 is proposed (Kuriyama et al., 2002). Expression of AQP7 is regulated by peroxisome proliferators-activated receptor gamma (PPAR-γ), a regulator of adipocyte differentiation (Kishida et al., 2001b).

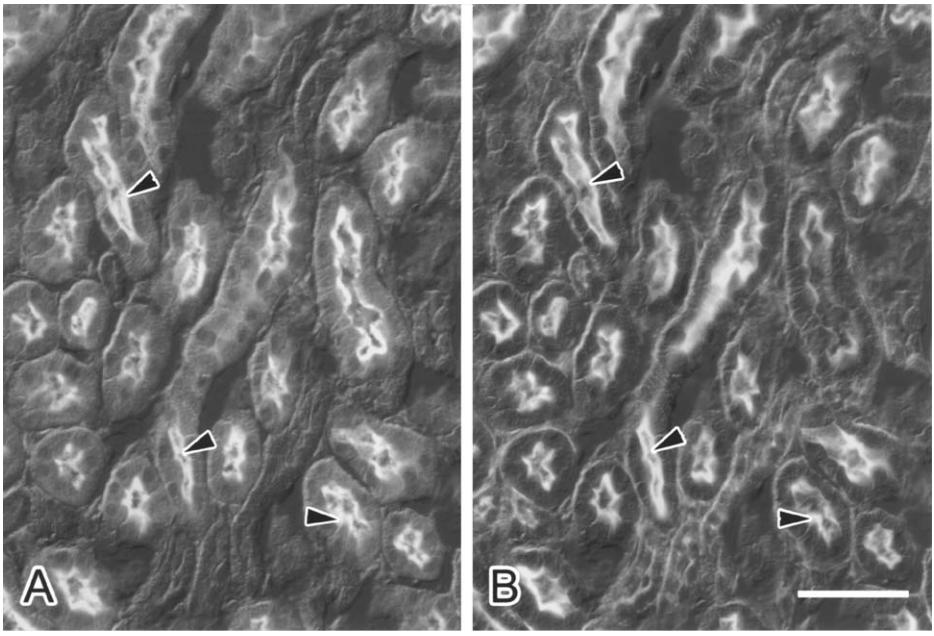


Fig. 19. AQP7 in the outer medulla of the rat kidney. Fluorescence image for AQP7 (A) or F-actin with fluorescein-phalloidin (B) is projected onto the Nomarski image. Note that brush borders of proximal tubules are clearly seen in F-actin labeling. AQP7 is expressed in the proximal tubules, where it is localized in the apical brush borders (arrowheads). Bar: 5 μm.

12.4. AQP7 in the immune system

Immature dendritic cells constitutively take up large volumes of fluid by macropinocytosis and concentrate macromolecules in the endocytic compartment. AQP3 and AQP7 are expressed in immature human dendritic cells, whose expression level coincidentally decreases in the maturation of dendritic cells in coincidence with a down-regulation of macropinocytosis (de Baey and Lanzavecchia, 2000). Treatment of cells with a mercurial drug p-chloromercuribenzenesulfonate inhibited the uptake and concentration of macrosolutes taken up by fluid phase endocytosis, and lead to the swelling of dendritic cells. These observations indicate that AQP3 and/or AQP7 may play an important role in the regulation of cell volume control that is essential in concentrating macromolecules taken up by cells.

12.5. AQP7 in the testis

In situ hybridization showed that AQP7 is expressed in round spermatids in the seminiferous tubules (Ishibashi et al., 1997b). Immunohistochemical staining showed that AQP7 protein is present in late to maturing spermatids (Ishibashi et al., 1997b) (Fig. 20). The role of AQP7 in the transfer of water and/or glycerol in sperm

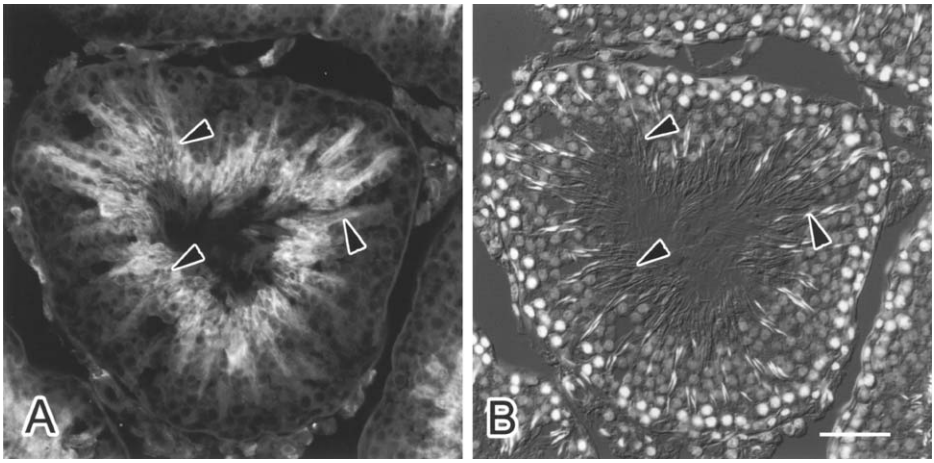


Fig. 20. AQP7 in the seminiferous tubules of the rat testis. AQP7 (A), and Nomarski images merged with nuclear DNA images of DAPI fluorescence (B) are shown. AQP7 is present in the spermatids (arrowheads). Bar: 5 μm.

differentiation and function is not clear. Localization of AQP7 in seminiferous tubules was analyzed in detail in the rat testis. AQP7 is localized in the elongated spermatids, testicular spermatozoa, and residual bodies remaining in the seminiferous epithelium (Suzuki-Toyota et al., 1999; Calamita et al., 2001b, c). The immunoreactivity for AQP7 is mostly concentrated at the middle piece where condensation of the cytoplasmic mass of elongated spermatids occurs. This observation suggests that AQP7 may be involved in volume reduction in developing spermatids where the seminiferous tubule fluid is hypertonic (Suzuki-Toyota et al., 1999).

12.6. AQP7 in the ear

AQP7 is localized in the Reissner's membrane (Huang et al., 2002). It is also present in the supporting cells of the organ of Corti such as Deiter's cells, Hensen's cells and inner phallangeal cells in the cochlea. In the saccule and utricle, AQP7 is present in the supporting cells and their wall.

13. AQP8

13.1. Cloning and characterization

AQP8 was originally cloned in the rat (Koyama et al., 1997; Ishibashi et al., 1997a), and the mouse (Ma et al., 1997b). AQP8 is a 28-kDa protein with a long N-terminus and a short C-terminus. It is expressed in many organs and tissues

including the pancreas, liver, colon, salivary gland, kidney, testis, epididymis, stomach, duodenum, jejunum, lung, trachea, and placenta (Ishibashi et al., 1997a; Koyama et al., 1997; Ma et al., 1997b; Elkjaer et al., 2001). These features are found in plant aquaporin, gamma-tonoplast intrinsic protein (γ-TIP) (Koyama et al., 1997). When expressed in *Xenopus* oocytes, a marked enhancement of water permeability in a mercurial-sensitive manner was observed. In addition, AQP8 is permeable to urea but not to glycerol (Ma et al., 1997b).

13.2. AQP8 in the kidney

AQP8 is localized in the proximal tubules and less in the collecting ducts in the cortex and medulla in the rat kidney (Elkjaer et al., 2001). In both cases, AQP8 labeling was seen scattered all over the cytoplasm as was seen in other tissues. Brush borders and basolateral membrane were not labeled. Cell fractionation studies also confirmed that AQP8 immunoreactivity was enriched in the intracellular membrane vesicle fractions compared to the plasma membrane fractions. Such intracellular localization was also observed in the localization of AQP8-transfected LLC-PK_1 cells. AQP8 in the cytoplasmic vesicles suggests that AQP8 may be involved in the osmotic equilibration between the cytoplasm and vesicles (Elkjaer et al., 2001). A homology of AQP8 to plant γ-TIP also suggests this hypothesis. Another possibility is its involvement in the translocation to the plasma membrane as is seen in AQP2 in collecting ducts.

13.3. AQP8 in the salivary glands

In situ hybridization showed the expression of AQP8 in the rat salivary gland (Koyama et al., 1997). Binding of anti-AQP8 antibody to the basolateral membrane was reported in the rat submandibular gland (Wellner et al., 2000). It was not detected in the ductal cells. This finding suggests a possibility that water crosses the epithelial cells of the acini through basolateral AQP8 and apical AQP5 transcellularly. On the other hand, immunoperoxidase and immunofluorescence labeling of rat parotid, submandibular, and sublingual glands showed that AQP8 is expressed not in the acinar cells but in the myoepithelial cells (Elkjaer et al., 2001). AQP8 was found intracellularly as was the case in other tissues. The physiological role of AQP8 in myoepithelial cells remains unknown.

13.4. AQP8 in the gastrointestinal tract

In situ hybridization showed that AQP8 is expressed in the rat liver and pancreas (Ishibashi et al., 1997a; Koyama et al., 1997). Immunohistochemical examination revealed that AQP8 protein is present in hepatocytes in the rat liver and is localized intracellularly as is seen in other tissues and organs. AQP8 is also localized at the plasma membrane of the bile canaliculi (Calamita et al., 2001a; Tani et al., 2001) and intracellular vesicles (Calamita et al., 2001a). In isolated rat hepatocytes, AQP8 is localized in the intracellular microsomal fraction, and is redistributed to the plasma

membrane with the increase of water permeability of the plasma membrane by dibutyryl cAMP in a microtubule-dependent manner (Garcia et al., 2001). When glucagon was administered, protein kinase A- and microtubule-dependent translocation of AQP8 to the bile canalicular membrane was observed (Gradilone et al., 2003). This finding indicates that translocation of AQP8 from the intracellular pool to the canalicular membrane may play an important role in glucagon-induced bile secretion.

In the rat exocrine pancreas, AQP8 is expressed and confined to the apical membrane of acinar cells (Hurley et al., 2001; Tani et al., 2001). In addition, small intracellular apical vesicles were labeled with AQP8 antibody, suggesting the regulation by translocation from the intracellular storage pool.

In situ hybridization and RNase protection assay showed that AQP8 is expressed in the colon (Koyama et al., 1997). Immunohistochemical labeling revealed that AQP8 protein is present in the absorptive epithelial cells in the duodenum, jejunum, and colon (Elkjaer et al., 2001). In the small intestine, AQP8 is localized in the apical part of the cytoplasm, just beneath the brush border, suggesting that AQP8 is not involved in the transcellular absorption of water in the intestinal epithelium, although the possibility of the trafficking to the membrane from the intracellular vesicles remains. Localization to the apical membrane of mucosal epithelial cells was also reported (Tani et al., 2001).

13.5. AQP8 in the airway

In the rat bronchial and tracheal glands, AQP8 is present in the myoepithelial cells surrounding the secretory acini as is seen in the salivary glands (Elkjaer et al., 2001).

13.6. AQP8 in the testis

Rat AQP8 was originally cloned from the testis (Ishibashi et al., 1997a). In situ hybridization revealed that AQP8 is expressed at all stages of sperm formation from primary spermatocytes to spermatids (Ishibashi et al., 1997a). Immunohistochemically AQP8 is present in the spermatogenic cells (Elkjaer et al., 2001; Calamita et al., 2001b) and Sertoli cells (Tani et al., 2001). Possible involvement of AQP8 in cytoplasmic condensation during the differentiation of spermatids into spermatozoa was suggested (Calamita et al., 2001b). AQP8 is also present intracellularly in the basal cells in the epididymal epithelium (Elkjaer et al., 2001).

14. AQP9

14.1. Cloning and characterization

AQP9 was identified from human leukocytes and showed similarity to aquaglyceroporins AQP3 and AQP7 (Ishibashi et al., 1998a). AQP9 was also

identified as a neutral solute channel with broad selectivity (Ishibashi et al., 1998a; Tsukaguchi et al., 1998). When expressed in *Xenopus* oocytes, AQP9 exhibited high permeability to both water and non-charged small solutes such as glycerol, urea, purines and pyrimidines. AQP9 did not facilitate the transport of amino acids, cyclic sugars, or inorganic ions. The activity of AQP9 was sensitive to mercurial as is the case in other aquaporins as well as to phloretin. Similar results were reported in human AQP9 (Tsukaguchi et al., 1999). It was proposed that AQP9 enables the coordinated movement of water and solutes across the cell membrane, allowing the rapid cellular uptake and exit of metabolites with minimal osmotic perturbation (Tsukaguchi et al., 1998). Measurement of permeability in the proteoliposomes reconstituted with expressed and purified rat AQP9 revealed that AQP9 mediates the effective transport of glycerol and urea in a mercurial-sensitive manner but has only minimal osmotic water permeability (Carbrey et al., 2003). In addition, the possible involvement of AQP9 as well as AQP7 in the uptake of arsenite was suggested (Liu et al., 2002).

14.2. Localization and roles of AQP9

Northern blot analysis revealed that AQP9 transcripts are present in the rat liver, testis and brain (Tsukaguchi et al., 1998). In humans, AQP9 is strongly expressed in leukocytes, and less abundantly in the liver, lung, and spleen (Ishibashi et al., 1998a). A similar expression pattern of AQP9 was also reported in the human liver, peripheral leukocytes, spleen, lung, and bone marrow (Tsukaguchi et al., 1999).

In situ hybridization showed that AQP9 transcript is present in hepatocytes in the liver (Tsukaguchi et al., 1998). In the testis, AQP9 was expressed in spermatocytes in early developmental stages and interstitial Leydig cells. By immunohistochemistry, localization of AQP9 in the plasma membrane of Leydig cells was reported (Nicchia et al., 2001). In the male reproductive tract, AQP9 is enriched in the apical membrane of non-ciliated cells in the efferent duct and principal cells of the epididymis and vas deferens (Pastor-Soler et al., 2001). The expression level of AQP9 is upregulated by androgen (Pastor-Soler et al., 2002). Active reabsorption and secretion transport are important in the maturation, concentration, and storage of the sperm, suggesting that AQP9 may play an important role in this process.

In the rat brain, transcript of AQP9 is found in the astrocytes (Tsukaguchi et al., 1998). It is upregulated by ischemia in the mouse, suggesting it may be involved in the regulation of postischemic edema in the brain (Badaut et al., 2001). Immunohistochemical staining also showed that AQP9 is present in tanycytes localized in the area lacking the blood–brain barrier such as the circumventricular organs of the third ventricle and hypothalamic regions, suggesting its possible role in the osmoreception (Nicchia et al., 2001).

During the maturation of rat oocytes, correlation of the AQP9 expression level and water and small solute permeability was reported, suggesting that AQP9 may be responsible for the membrane permeability (Ford et al., 2000).

Expression of AQP9 in the basolateral membrane of mucus-secreting goblet cells was reported in the duodenum, jejunum, ileum, and colon, suggesting its involvement in the synthesis and/or secretion of mucin (Okada et al., 2003).

In the ear, AQP9 is present in the interdental cells of the inner spiral tunnel in the cochlea (Huang et al., 2002). It is also present in the saccular wall.

AQP9, together with AQP3, is localized in the apical membrane of the syncytiotrophoblast in the human term placenta (Damiano et al., 2001). Both aquaporins are members of the aquaglyceroporin subfamily and especially, AQP9 allows the passage of a wide variety of small solutes in addition to water. They may be involved in the transfer of water and solutes across the placental barrier.

Plasma glycerol is a major substrate for hepatic gluconeogenesis. AQP7 expressed in adipocytes mediates the release of glycerol into the plasma derived from stored fat. It may be taken up with AQP9 into the liver for gluconeogenesis. In fact, expression of hepatic AQP9 is upregulated by fasting and down-regulated by refeeding in the mouse (Kuriyama et al., 2002). Increase of AQP9 expression is also seen in insulin deficiency induced by streptozotocin. The increase of hepatic AQP9 is accompanied by the increase of hepatic gluconeogenic mRNA, suggesting that AQP9 is involved in the uptake of glycerol into the hepatocyte for gluconeogenesis (Kuriyama et al., 2002). These observations were also confirmed in the rat (Carbrey et al., 2003). In hepatoytes, AQP9 is localized at the sinusoidal membrane (Carbrey et al., 2003) that is easily accessed by the blood constituents (Fig. 21). A sex-linked difference in the expression level of AQP9 in the rat hepatocytes was reported; there were higher levels of AQP9 in males (Nicchia et al., 2001), although its significance is elusive.

15. AQP10

AQP10 was cloned in human and showed sequence similarity to aquaglyceroporins such as AQP3, AQP7, and AQP9 (Ishibashi et al., 2002). When expressed in *Xenopus* oocytes, it exhibited mercurial-sensitive osmotic water permeability. In addition, as expected from the primary structure, AQP10 stimulated the glycerol and urea transport (Ishibashi et al., 2002). Although human AQP10 was originally reported to code a much smaller molecule lacking urea and glycerol permeability (Hatakeyama et al., 2001), this may be due to the loss of the 6th transmembrane domain during the course of cloning (Ishibashi et al., 2002). No transcript corresponding to this smaller form was detected (Ishibashi et al., 2002).

AQP10 is expressed exclusively in the small intestine (Ishibashi et al., 2002). In situ hybridization revealed that AQP10 is present in the absorptive epithelial cells (Hatakeyama et al., 2001). In the mouse, AQP10 is a pseudogene and no transcript was detected in the jejunum (Morinaga et al., 2002).

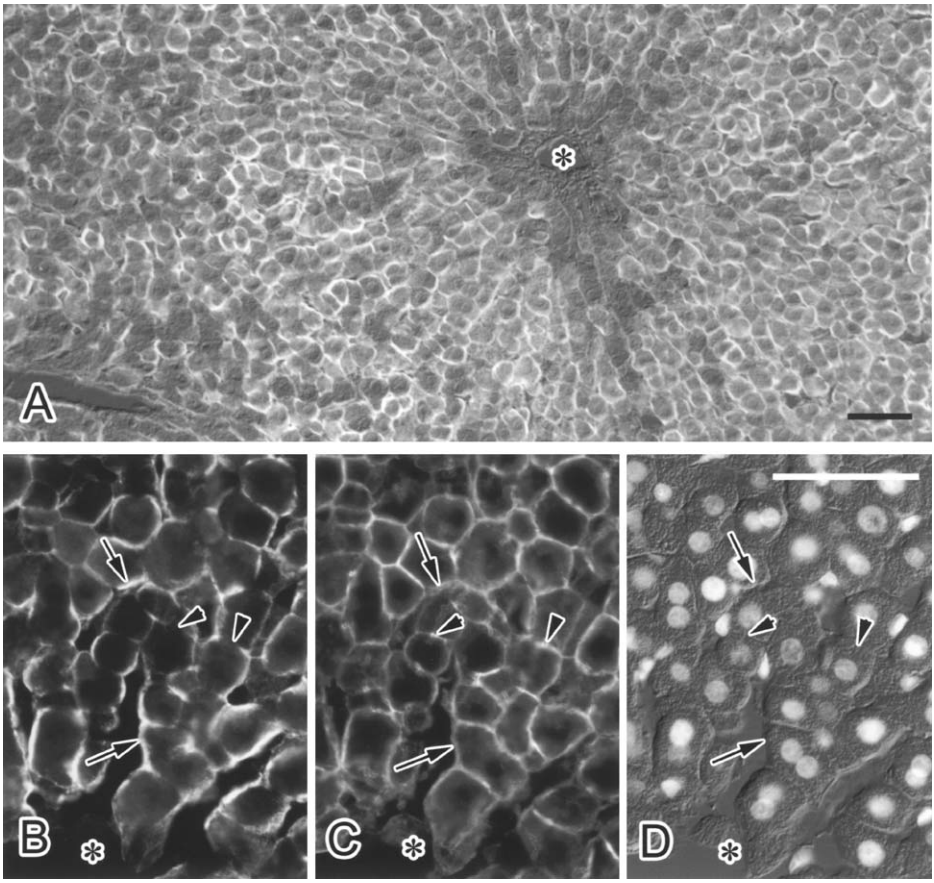


Fig. 21. AQP9 in the rat liver. (A) A survey view. AQP9 is expressed in hepatocytes. Fluorescence for AQP9 is projected onto Nomarski image. (B–D) AQP9 (B), F-actin visualized with fluorescein-phalloidin (C), and Nomarski image merged with nuclear DNA image of DAPI fluorescence (D) are shown. AQP9 is localized along the sinusoidal and lateral regions (arrows in B). F-actin staining with fluorescein-phalloidin (arrowheads in C) clearly shows the bile canalicules where AQP9 is absent (arrowheads in B). *, central vein. Bars: 50 μm (A–C); 10 μm (D).

16. Aquaporins and molecular water pump of cotransporters

Transepithelial transport of water is considered to be driven by the local osmotic gradient. Aquaporins at the plasma membrane provide ideal channels for efficient and rapid water transfer across the membrane. Cotransporters such as Na^+-dependent glucose transporter SGLT1 transport water when expressed in *Xenopus* oocytes (Loo et al., 1996; Zeuthen et al., 2001). An apparent lack of local osmotic gradient, absence of appropriate aquaporins in the small intestine, and low water

permeability of the brush border membrane in the small intestine suggest that SGLT1 may be responsible for the absorption of water in the intestine (Loo et al., 1996). SGLT1 is localized at the microvillous plasma membrane of the intestinal epithelial cells and kidney proximal tubule cells (Fig. 22) (Takata et al., 1991, 1992, 1993; Takata, 1996). Further examinations revealed that many cotransporters transport water. A molecular water pump hypothesis was proposed stating that cotransporter is involved in the transfer of water (Loo et al., 1999a, 2002; Zeuthen et al., 2002). Although the physiological role of cotransporters in the water absorption and secretion in epithelial cells remains to be thoroughly tested, cotransporters and aquaporins could serve in concert in the transfer of water across the membrane. In fact, the distribution of aquaporins does not always account for the water transport mechanism (Hamann, 2002). Further studies are needed to fully elucidate the water transport mechanism across the membrane (Spring, 1999; Loo et al., 1999b).

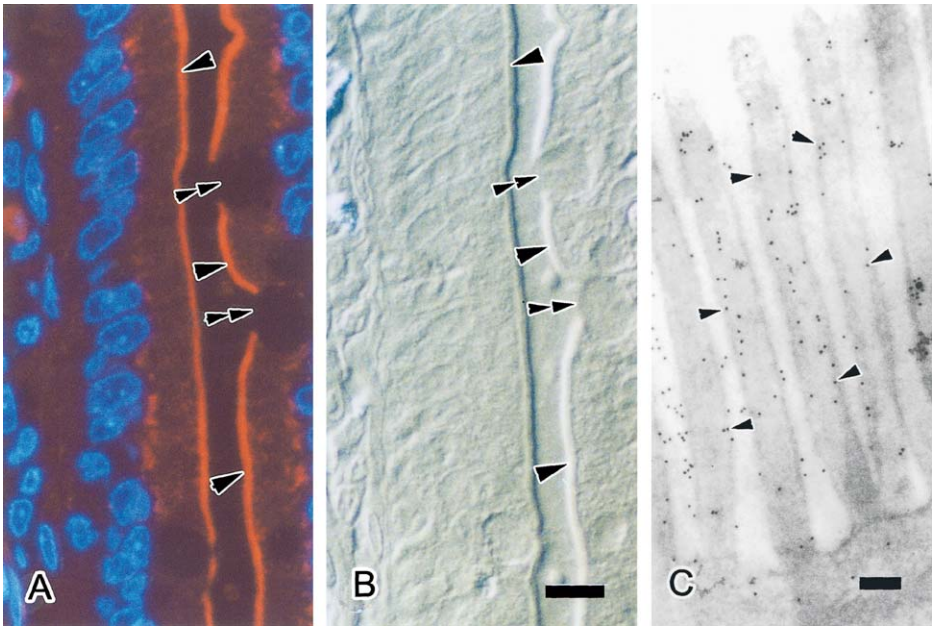


Fig. 22. Localization of Na^+-dependent glucose cotransporter SGLT1 in the rat jejunum. (A, B) Immunofluorescence localization of SGLT1 (A) and the corresponding Nomarski image (B). Nuclei are counterstained with DAPI (blue). Bar: 10 μm. SGLT1 (red) is localized along the brush border of the absorptive epithelial cells (arrowheads). Mucus-secreting goblet cells are negative for SGLT1 (double arrowheads). (C) Ultrastructural localization of SGLT1. Colloidal gold particles representing SGLT1 is localized in the microvillous membrane of the absorptive epithelial cells (arrowheads). Bar: 0.1 μm. A, B was reproduced from Takata et al. (1993).

17. Concluding remarks

Water is a ubiquitous and indispensable molecule of the life. The search of isoforms for the aquaporin family has revealed more than 10 members in mammalian cells so far. They transport water and some of them additionally transport small solutes such as glycerol and urea. Histochemical examination by in situ hybridization and immunostaining has revealed their differential localization in cells and tissues (Table 1). Many of them are present at the plasma membrane of the epithelial cells where water permeability is high, suggesting that aquaporins is important in the transcellular transfer of water across the epithelial layer. Knockout experiments have revealed that some of them are critical but many of them seem to be non-essential (Verkman, 1999, 2000; Verkman et al., 2000c). Aquaporin may not be rate-limiting in many tissues (Marples, 2000). A detailed evaluation of these AQP-null animals remains to be done. In addition, the contribution of molecules other than aquaporins to the water transfer should be assessed.

Most of the work in aquaporins has been concentrated in the kidney. Aquaporins are differentially localized in the cells of the proximal tubule, Henle's loop, and collecting duct, where they play a pivotal role in the reabsorption of water (Verkman, 1999; Nielsen et al., 2002). The role of aquaporins in the intestine in the water absorption is elusive and remains to be thoroughly tested. This is also the case in the glands in secretion. Since aquaporins are found in virtually all types of cells from bacteria to plant and animal cells, comparative studies of aquaporins will reveal how this molecule has evolved during evolutional changes. Combined molecular, physiological, and morphological examinations will reveal the mechanism of water transfer in the body and role of aquaporins in the life. In addition, knockout experiments suggest that drugs directed to aquaporins may be effective as therapeutic measures.

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