Aquaporin Water Channels in the Kidney

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Aquaporins (AQP) are water channel proteins of cellular membranes serving in the permeation of water across the membrane. AQP families are found virtually in all types of life ranging from bacteria to plant and animal cells. In mammals, at least 13 isoforms of AQP have been identified. They are classified into three subtypes: classical aquaporins, aquaglyceroporins, and superaquaporins. These AQP are differentially expressed in a wide variety of cells and tissues in the body, and play important roles in water metabolism. In the kidney, at least 6 isoforms of AQP, namely AQP1, AQP2, AQP3, AQP4, AQP6, and AQP7, are reported to be expressed.

Water transfer occurs mainly in the proximal tubules and collecting ducts in the kidney. In the proximal tubules, AQP1 and AQP7 are expressed, among which AQP1 plays a major role in water reabsorption. In the collecting ducts, AQP2, AQP3, AQP4, and AQP6 are expressed. AQP3 and AQP4 are localized at the basolateral membrane. AQP2 is stored in the cytoplasmic vesicles and is translocated to the apical plasma membrane in response to antidiuretic hormone. Mutations of AQP2 lead to either loss of channel function or mis trafficking and result in nephrogenic diabetes insipidus, the inability to concentrate urine.

Key words: aquaporin, kidney, water channel, AQP2, diabetes insipidus

I. Water Transport and Water Channels

Water is the most ubiquitous molecule in the cell. The lipid bilayer constitutes the structural basis of the plasma membrane that encloses the cell. Water can penetrate the lipid bilayer to some extent, where osmotic pressure serves as a major driving force. In certain types of organs such as the kidney and exocrine glands, transfer of quantities of water occurs. The presence of water channel proteins in these organs was proposed but their existence proved elusive until Agre’s group identified aquaporin 1 [47].

Water channel protein was first isolated as a novel integral membrane protein of 28 kDa from the human erythrocyte ghost during the purification of the Rh polypeptide [6]. cDNA cloning revealed that this protein has 6 transmembrane domains with N and C termini in the cytoplasmic side and it was named channel-like integral protein of 28 kDa (CHIP28) [46]. It showed strong homology to major intrinsic protein (MIP) of the lens of the eye [12]. Immunohistochemical staining showed that this protein is abundant in the kidney proximal tubules, suggesting its involvement in the water transport function of the kidney [6]. When CHIP28 was expressed in the Xenopus laevis oocytes by the microinjection of the CHIP28 mRNA, oocytes swelled rapidly in the water, which demonstrated that permeability to water at the plasma membrane drastically increased with CHIP28 [47]. Expression of CHIP28 in cultured mammalian cells also increased specific transfer of water at the plasma membrane [28]. These experiments clearly showed that CHIP28 is the long-sought water channel protein.
II. Aquaporins and Their Molecular Structure

Water channel proteins are ubiquitously found in all types of life, from bacteria to plant and animal cells. They were named aquaporins (AQP) [1]. In mammals, more than 10 isoforms of AQP have been identified [2, 3, 35, 45, 57]. The aquaporin family is further divided into three subfamilies: aquaporin family, aquaglyceroporin family, and superaquaporin family (aquaporin superfamily). Aquaporin family is permeable only to water. Aquaglyceroporins, on the other hand, allow passage of water and small solutes such as glycerol and urea. Superaquaporins show less structural similarity to aquaporins whose physiological substrates hence may be different [40]. Recently, CO₂ permeability in certain types of AQP was also reported [60].

AQP is composed of a single polypeptide of approximately 270 amino acids. Highly conserved NPA (asparagine-proline-alanine) motifs (NPA boxes) are found in the first cytoplasmic loop spanning the second and third transmembrane regions, and the third extracellular loop spanning the fifth and sixth transmembrane regions. The presence of two NPA boxes characterizes aquaporins and aquaglyceroporins. In the superaquaporins, only one of the NPA boxes is conserved [40]. Analyses of nucleotide sequences of AQP genes suggest that they originated from the gene duplication.

The hourglass model of AQP molecules was proposed based on the analysis of amino acid sequences of AQP [19]. The first cytoplasmic loop and the third extracellular loop, both of which contain NPA boxes, are bent and inserted into the membrane. These NPA boxes become close to each other and form the constricted hydrophilic part of the channel, the critical transmembrane pore for the passage of the water molecule. Crystallographic analyses by cryo-electron microscopy and X-ray diffraction later revealed the molecular structure of AQP, and the basic arrangement of the hourglass model was confirmed [43, 49, 53]. AQP is present as tetramers in the membrane and each monomer has a central channel for the passage of water [64]. Each AQP molecule has six tilted alpha-helix transmembrane domains that surround the central pore. The first cytoplasmic loop and the third extracellular loop are folded into this central pore region as suggested by the hourglass model and play an important role in building the critical pore structure. The central channel opens to the extracellular and cytoplasmic sides with funnel-shaped ends in each side. The central filter portion of the channel is composed of hydrophobic pore with minimal solute binding sites and is narrow enough for a single water molecule to pass [53]. The molecular structure of AQP suggests that specificity of the channel to water seems to be mainly determined by the size occluding effect of the pore [22]. In addition, the contribution of amino-acid side chains to hydrogen-bonding interaction to water molecule is important to determine the specificity of the channel. The conserved NPA motif seems to provide such hydrogen-bonding interaction.

III. Distribution of Aquaporins in the Body

AQP1 was originally isolated from human erythrocyte membrane and called CHIP28 [46]. AQP1 is specifically permeable to water. AQP1 is expressed in a variety of tissues and organs such as the kidney, blood vessels, and pancreas. It is usually localized at both the apical and basolateral membranes of epithelial cells and seems to play important roles in the transcellular transfer of water during reabsorption and secretion.

AQP2 was originally identified as an anti-diuretic hormone (ADH)-regulatable water channel in the kidney collecting ducts (WCH-CD) [9]. Expression of AQP2 is mostly confined to the kidney collecting duct cells. AQP2 plays a pivotal role in the regulation of water reabsorption during the course of the urine formation in the kidney; the deficiency of AQP2 results in the nephrogenic diabetes insipidus [24].

AQP3 showed similarity to glycerol facilitator GlpF of E. coli. It is an aquaglyceroporin and transports glycerol as well as water. It was originally called GLIP (glycerol intrinsic protein) [29]. AQP3 is expressed in a wide variety of organs such as the kidney, digestive tract, respiratory tract, skin, eye, mammary gland, and brain [32, 37].

AQP4 is unique in its insensitivity to mercurial compounds, inhibitor of most of water channels, and hence was named as a mercurial-insensitive water channel (MIWC) [13, 19]. Two forms of AQP4, namely M1 and M23 with different initiating methionine, are expressed [27, 66]. AQP4 is abundant in the brain, where it is localized in the endfeet of astrocytes surrounding blood vessels and regulates the water transfer in the blood-brain barrier. Freeze-fracture electron microscopy revealed the presence of orthogonally arranged concentration of intramembranous particles called orthogonal arrays in the astrocyte membrane. Immunogold labeling by the fracture label technique clearly showed that the orthogonal arrays are the condensation of AQP4 molecules [48]. AQP4 is also expressed in the kidney, skeletal muscle, and stomach. In the stomach, AQP4 is specifically expressed in the parietal cells and may play a role in acid secretion.

AQP5 is mostly expressed in secretory glands such as the lacrimal gland, salivary glands [33], sweat gland, and duodenal glands [36], suggesting its role in regulated secretion. AQP5 is also expressed in the lung. Relationship between Sjögren’s syndrome and erroneous subcellular localization of AQP5 in lacrimal gland was reported [59]. AQP6 is expressed in the kidney. It is localized intracellularly and seems to be an anion channel rather than water channel [67]. AQP7 is an aquaglyceroporin, and is expressed in the adipose tissue and testis. AQP7 seems to be involved in the release of glycerol from the adipocyte to maintain blood glycerol level [21]. AQP8 is expressed in many organs such as the pancreas, liver, salivary glands, testis, and the kidney [16]. AQP9 is an aquaglyceroporin. It is expressed in the liver and may be involved in the uptake of plasma glycerol for glyconeogenesis [23]. AQP10 is
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IV. Aquaporins in the Kidney

At least 6 isoforms of AQPs, namely AQP1, AQP2, AQP3, AQP4, AQP6, and AQP7, are reported to be expressed in the kidney (Fig. 1) [45, 57]. AQP1 is expressed in the proximal tubules, descending thin limbs of the Henle’s loops, and vasa recta. AQP1 is localized at both the apical and basolateral membranes of these epithelial and endothelial cells, and participates in the transcellular transfer of water. AQP1-null mice revealed decreased transepithelial water permeability, showing that water transfer in the proximal tubules is carried out transcellularly via AQP1 water channel rather than paracellularly [51]. The knockout mice became dehydrated after deprivation of water due to impaired urine concentrating ability although the phenotype is not as severe as that in AQP2-null mice.

AQP2 is expressed in the collecting ducts (Fig. 2a) [9, 50]. AQP2 is unique in that it is not localized at the plasma membrane but resides in the intracellular vesicles in the basal condition (Fig. 2b). Upon stimulation with antidiuretic hormone (ADH), AQP2-bearing vesicles fuse with the apical plasma membrane by the exocytic process and AQP2 becomes exposed to the lumen of the collecting duct (Fig. 2c) [5, 45, 57, 58, 63]. Hence water is allowed to

Fig. 1. Schema showing the localization of AQPs in the kidney. APM, apical membrane; BLM, basolateral membrane; VES, intracellular vesicles.

Fig. 2. AQP2 and AQP3 in the collecting ducts of the rat kidney. a. AQP2 and AQP3 are present in the collecting ducts. A cryostat section was labeled for AQP2 (red) and AQP3 (green) using Quantum Dot 655 and Quantum Dot 525 as fluorochromes, respectively. Nuclei were stained with DAPI. Specimens were examined with a confocal microscope. b, c. AQP2 (red) is localized in intracellular vesicles in the resting state (b). Administration of ADH results in the translocation of AQP2 (red) to the apical membrane (c). AQP3 (green) is localized in the basolateral membrane. Semithin frozen sections were labeled for AQP2 and AQP3 using Rhodamine Red X and FITC as fluorochromes, respectively. Nuclei were stained with DAPI. Micrographs were recorded with a cooled-CCD camera. Bars=10 μm.
pass through the collecting ducts transcellularly via apical AQP2 and basolateral AQP3/AQP4 (Fig. 2). Termination of the stimuli results in the rapid retrieval of the surface AQP2 to the intracellular compartments via endocytic process.

Both AQP3 and AQP4 are also expressed in the principal cells of the collecting ducts (Fig. 3) [8, 15, 29]. They are localized at the basolateral membrane (Figs. 2, 4). AQP3 and AQP4 constitute a part of the transcellular route for the transcellular reabsorption of water in coordination with apical AQP2. Knockout mice experiments suggest that AQP3 is more important than AQP4 in the transcellular transfer of water in the collecting ducts [30]. Why two isoforms of AQP3, albeit one an aquaglyceroporin and the other an aquaporin, are present in the basolateral membrane of the same cells remains elusive. Immunohistochemical labeling revealed the differential expression of AQP3 and AQP4 along the collecting ducts in the mouse (Fig. 3). AQP3 is more abundant in the cortical collecting ducts, whereas AQP4 has an opposite tendency. The significance of such differential localization is not clear. In addition to collecting ducts, a small amount of AQP3 is detected in the basolateral membrane of proximal tubules in the cortex. AQP4 is also expressed in the S3 proximal tubules.

AQP3 is expressed in the epithelia covering the surface of the renal pelvis and the downstream urinary tract [32]. In these transitional epithelia, AQP3 is localized at the plasma membrane of basal and intermediate cells. In the cells covering the surface, the apical membrane facing the lumen is negative for AQP3 labeling. These epithelial cells directly face the hypertonic urine. This seems to make the epithelial cells lose water and tend to become hypertonic. Expression of AQP3 is upregulated under hypertonic condition [34]. AQP3 in the urinary tract thus seems to be involved in the supply of water from the lamina propria to these epithelial cells.

AQP6 is suggested to be an anion channel, and is expressed in the acid-secreting intercalated cells of the collecting ducts [14]. Double-labeling with AQP3 clearly demonstrates that AQP6 is expressed in distinct cells from AQP3-expressing principal cells. AQP6 is localized intracellularly and is distributed throughout the cytoplasm [67]. Immunoelectron microscopic examination showed that AQP6 is localized in the membrane of vesicles and tubules, although the characterization of such intracellular storage sites remains to be clarified. Whether AQP6 functions in the intracellular vesicles or at the cell surface after being translocated to the plasma membrane is not known.

AQP7 is expressed in the proximal tubules, where it is localized in the brush borders [17, 44]. In addition, AQP8 was reported to be expressed in the proximal tubules [7]. The physiological role of AQP7 and AQP8 in the kidney function is not clear.

V. Intracellular Trafficking of AQP2

Signaling pathway of AQP2 translocation

In the principal cells of the collecting ducts, the binding of ADH to V2 receptor at the basolateral membrane and the subsequent activation of adenylate cyclase result in the elevation of cellular cAMP level (Fig. 5). Protein kinase A (PKA) is then activated and phosphorylates AQP2. Elevation of the cytoplasmic cAMP level is sufficient for the translocation of AQP2 from the intracellular pool to the
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Fig. 4. Ultrastructural localization of AQP3 in the dog kidney. Dog kidney was labeled with the preembedding Nanogold method. AQP3 is localized along the basolateral membrane (arrows) of principal cells. Apical membrane facing the lumen (L) is negative for AQP3 (arrowheads). The inset shows the enlargement of the area indicated with a rectangle. Bars=1 μm.

Fig. 5. A schema showing the signal transduction of ADH, trafficking of AQP2, and transcellular water transfer. ADH, anti-diuretic hormone; AC, adenylate cyclase; PKA, protein kinase A.

plasma membrane [23]. In the experimental protocols for translocation, forskolin is often used to increase cytoplasmic cAMP level and induce AQP2 trafficking to the plasma membrane. Among possible phosphorylation sites of AQP2 molecules, phosphorylation of serine 256 is necessary and sufficient for the translocation of AQP2 to the plasma membrane [61].

AQP2 resides in two distinct compartments inside the cell

We examined the intracellular storage compartments of AQP2 in the principal cells of the collecting ducts in the kidney [54]. Double-labeling with various organelle markers revealed that AQP2 does not colocalize with markers for the Golgi apparatus, endoplasmic reticulum, trans-Golgi network, or lysosomes. Some of AQP2 is colocalized with early endosome antigen 1 (EEA1), an early endosome marker, suggesting that early endosomes are involved in the trafficking of AQP2.

Further analyses were carried out in cultured epithelial cells expressing AQP2 for the AQP2-bearing vesicles and their trafficking [55]. In MDCK cells transfected with human AQP2, intracellular localization of AQP2 is the same as that in principal cells of the kidney. It is translocated to the apical plasma membrane by elevating the cAMP level by forskolin. To track the trafficking of AQP2, cytoplasmic AQP2 was first translocated to the apical plasma membrane by forskolin stimulation (Fig. 6a). After
the complete translocation of AQP2 to the plasma membrane was achieved. forskolin was washed out and the subsequent retrieval of AQP2 to the intracellular storage sites was visualized. AQP2 is endocytosed and first seen in the EEA1-positive larger vesicles located in the supranuclear cytoplasm (Fig. 6b). It then moves to the smaller subapical vesicles located just below the apical membrane (Fig. 6c). Most of these subapical vesicles are positive for Rab11, an apical recycling endosome marker [55]. Responsiveness to forskolin-stimulation is restored when the endocytosed AQP2 is transferred to the subapical small vesicles, showing that these subapical Rab11 vesicles are the storage compartment of AQP2 from the EEA1-positive compartment to the subapical storage compartment since Rab11 enhances the formation of PI3-kinase inhibitors such as wortmannin and LY294002 strongly inhibit this transition (Fig. 6d) [55]. The subapical storage vesicles are not homogeneous, however, since not all the subapical storage vesicles are positive for Rab11. Trafficking of AQP2 is summarized in Figure 5. Further characterization of the subapical storage compartment will shed light on the regulatory mechanism in the trafficking of AQP2.

Comparison of AQP2 with glucose transporter GLUT4

Glucose transporter GLUT4 is an isoform of facilitative glucose transporters of the GLUT family and is specifically expressed in adipocytes and muscle cells [65]. GLUT4 is localized in the intracellular compartment under the basal condition. Stimulation with insulin induces the recruitment of the intracellular GLUT4 to the plasma membrane and drastically increases the ability to take up glucose, which contributes to lowering the blood glucose level. Once the normal glucose level is restored, the insulin signal is turned off and GLUT4 at the cell surface is rapidly endocytosed back to the cytoplasmic storage compartment for another round of translocation to the cell surface. AQP2 and GLUT4 therefore exhibit apparently similar mechanisms to regulate the water permeability and glucose transport activity of the cell, respectively. Both of them have cytoplasmic pools of channel or transporter proteins which are translocated to the plasma membrane by vesicular transport and exocytosis, in a process controlled by the hormones. In skeletal muscle cells and 3T3-L1 adipocytes, ample GLUT4 is present in the perinuclear region. Immunofluorescence and immunoelectron microscopy showed that GLUT4 is abundant in the Golgi apparatus and the trans-Golgi network [56]. Only a portion of intracellular GLUT4 is found in other vesicular compartments. Moreover, translocation of GLUT4 to the plasma membrane is far from complete even under maximal insulin stimulation.

To further compare their intracellular storage sites, GLUT4 was coexpressed with AQP2 in MDCK cells. As expected from the observation of muscle cells and kidney cells, GLUT4 and AQP2 barely colocalized [55]. The majority of GLUT4 is present in the perinuclear regions, whereas AQP2 resides in the subapical vesicles. The apparent differential localization may be due to the differential regulatory mechanism of the signal transduction pathway: AQP2 relies on adenylate cyclase and PKA, whereas GLUT4 is under the control of protein tyrosine-kinase insulin receptor [58]. Another possibility is that most of the intracellular AQP2 is retained in the subapical "ready-to-go" compartment, whereas only a limited amount of GLUT4 is stored in such "ready-to-go" compartments, and the rest is sequestered in other "not-ready-to-go" compartments such as the trans-Golgi network and the Golgi [58].

Congenital nephrogenic diabetes insipidus and mutation of AQP2

One of the most severe disorders in water handling in the kidney is diabetes insipidus, the condition of inability to
concentrate urine [39]. Diabetes insipidus is classified into two types: central and nephrogenic. Central diabetes insipidus is caused by the disorder in the secretion of ADH in the posterior lobe of the hypophysis. Nephrogenic diabetes insipidus, on the other hand, is due to the mutations in the vasopressin V2 receptor or in AQP2 in the kidney. The importance of AQP2 in renal function is further demonstrated in the knock-in mouse model of mutated AQP2. The targeted replacement with mutated AQP2 resulted in severe phenotype with urinary concentration defect [66]. The key role of AQP2 in urine concentration is evident since the knockout mice lacking AQP1, AQP3, or AQP4 do not show such severe phenotype.

Dominant and recessive mutations of AQP2 are known [10, 24, 62]. In the recessive mutations, mutation of AQP2 results in the loss of water transport function [41]. Another type of recessive mutation occurs when the mutated AQP2 fails to form the tetramer and is retained inside the cell [20]. In either case, wild-type AQP2 still serves as a functional water channel in the heterozygous cells. Dominant nephrogenic insipidus is found in AQP2 mutants with mutated C-terminus [20, 25, 42]. C-terminus tail seems to play an important role in targeting AQP2 but does not affect the fundamental three-dimensional structure of the AQP2 molecule [31]. The mutated AQP2 has aberrant localization such as to the intracellular organelles or to the basolateral membrane, and thereby is unable to contribute to water transfer through the apical membrane [4, 31]. In addition, this type of mutation allows the mutated AQP2 to form tetramers with wild-type AQP2, thereby conveying the dominant negative effect.

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VII. References


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