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Review

The renal plumbing system: aquaporin water channels

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Abstract. Aquaporins are channels that facilitate movement of water across lipid bilayers. They are expressed in multiple tissues and are essential for regulation of body water homeostasis. The kidney is the main organ responsible for this regulation, and at least seven aquaporins are expressed at distinct sites in the kidney. Aquaporin expression correlates with observed water permeability of each nephron segment: proximal tubule and descending thin limb of Henle have constitutive high water permeability due to expression of AQP1, whereas collecting duct water permeability is tightly regulated by the antidiuretic hormone vasopressin via regulation of AQP2. This review aims at providing insight into renal aquaporins, with special focus on AQP2.

Key words. Kidney; aquaporin; vasopressin; phosphorylation; protein kinase A; diabetes insipidus.

Introduction

The kidney is the main organ regulating body water homeostasis, filtering approximately 180 l of blood per day. Most of the filtered water is reabsorbed in the proximal tubule, thin descending limb of Henle and collecting duct.
Water permeability along these segments and the ascending limb of Henle varies tremendously: water permeability is constitutively high in the proximal tubule and descending limb of Henle, whereas the thin and thick ascending limbs of Henle are water impermeable, and the collecting duct exhibits high water permeability only following stimulation with the antidiuretic hormone vasopressin (ADH). The difference in collecting duct water permeability with and without vasopressin results in production of urine volumes between 0.5 and 20 l a day, respectively. The variation in water permeability between segments of the nephron and the regulation of collecting duct water permeability cannot be accounted for by the low capacity of water diffusion through the plasma membrane. This led to the hypothesis that water crosses plasma membranes through a transport mechanism either selective for water or as a co-transport with other molecules.

In 1992, Agre and associates injected the newly cloned complementary RNA (cDNA) for CHIP28 (channel-like integral membrane protein of 28 kDa) [1, 2] into *Xenopus laevis* oocytes and watched them swell in hypo-osmotic medium [3] (fig. 1). They had serendipitously discovered the first water channel, named aquaporin-1 (AQP1), and the identity of the channels responsible for water transport across lipid bilayers was solved [3]. This led to acceleration in research and knowledge about body water homeostasis and water transport across lipid bilayers that earned Dr Peter Agre the 2003 Nobel Prize in chemistry.
Aquaporins are membrane proteins that mediate facilitated movement of water across the lipid bilayer along osmotic or hydraulic gradients. So far, aquaporins have been found in almost all living organisms: 11 mammalian and several invertebrate, plant, bacteria, microbe and amphibian aquaporins have been cloned. The aquaporin family can be divided into two subgroups: aquaporins and aquaglyceroporins. The aquaporins are considered only to be permeated by water and include AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8. The aquaglyceroporins are, in addition to water, permeable to glycerol and include AQP3, AQP7, AQP9 and AQP10. The aquaporins seem to

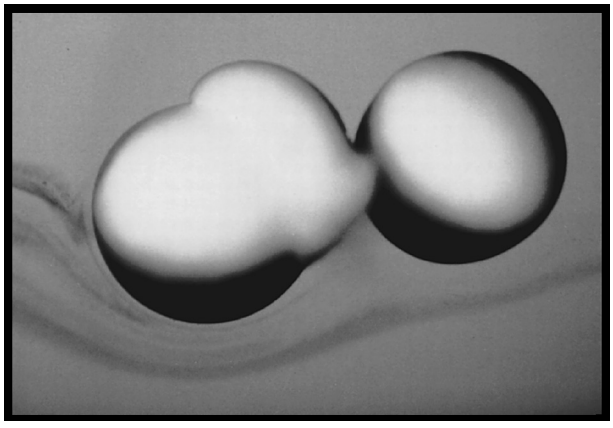

Figure 1. Consequences of AQP1 expression in *Xenopus laevis* oocytes (the original experiment performed by Agre and associates). When shifted to hypoosmotic media, the control oocyte (right) is unaffected, whereas the oocyte injected with AQP1 complementary RNA (left) exhibited high water permeability and exploded. Reprinted with permission from [3] Preston G. M., Carroll T. P., Guggino W. B. and Agre P. (1992) Appearance of water channels in *Xenopus* oocytes expressing red cell CHIP28 protein. Science **256:** 385–387. Copyright 1992 AAAS. www.sciencemag.org.

play different roles in body water homeostasis, and all vary with respect to permeability and localization. This review aims to provide insights into renal aquaporins (AQP1, AQP2, AQP3, AQP4, AQP6, AQP7 and AQP8) by examining their distribution and involvement in regulating body water homeostasis. Special emphasis is on regulation of the vasopressin regulated aquaporin, AQP2.

Aquaporin structure

Aquaporins contain six transmembrane domains [2], and both the carboxy- and amino-termini protrude into the cytosol [4]. The AQP1 sequence revealed that the amino- and carboxy-halves of AQP1 are highly related; however, they are oriented in opposite directions in the membrane [5]. Both the amino- and carboxy-halves contain the signature motif of the aquaporins, Asn-Pro-Ala (NPA), which is located in loops B and E, respectively [6]. These two loops bend into the membrane from opposite directions, forming the pore that allows water to selectively pass [5– 8]. This model is visualized as an hour glass [6] (fig. 2).

The monomeric form of AQP1 migrates as a 28-kDa polypeptide in SDS polyacrylamide gels [1]; hence, the first name for AQP1 was CHIP28 for channel forming integral protein 28 kDa. Although each monomer constitutes a functional water-permeable pore [6], freeze-fracture analysis of AQP1 in membranes revealed that aquaporins form tetramers in the plasma membrane [9].

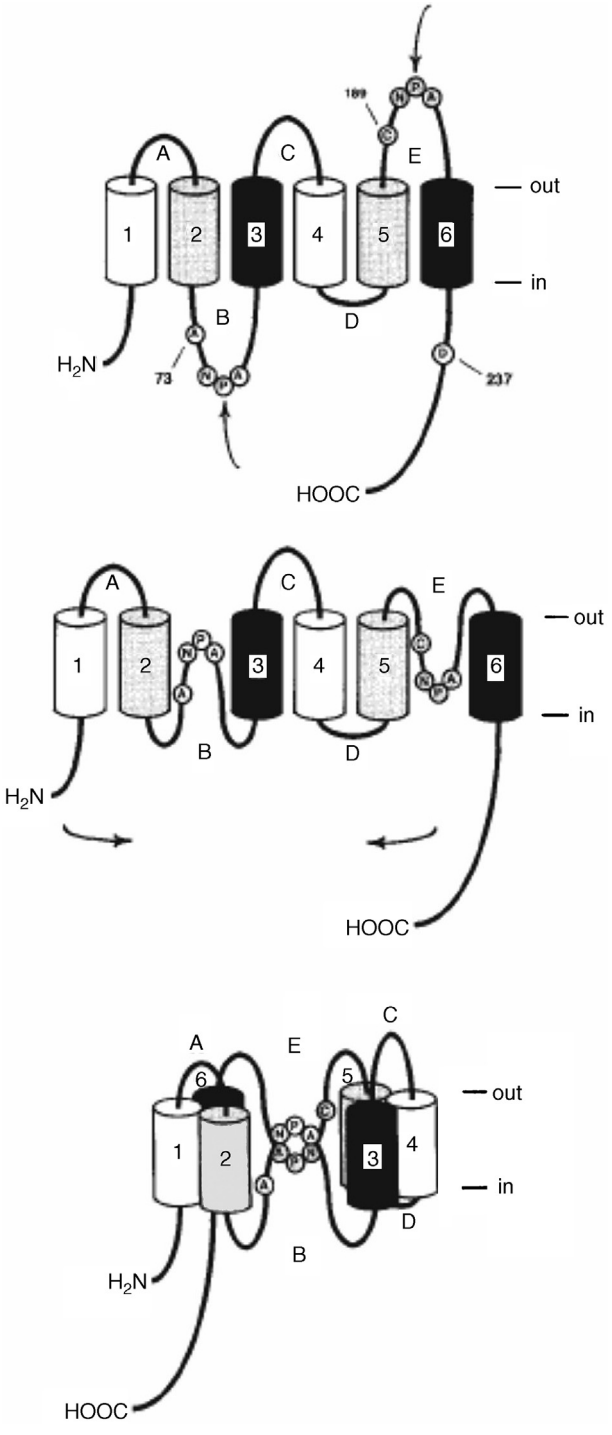

Figure 2. Schematic illustration of AQP1 membrane topology. AQP1 consists of six transmembrane domains. Loops B and E protrude into the lipid bilayer, forming the water-permeable pore structure. Reprinted with permission from [6] Jung J. S., Preston G. M., Smith B. L., Guggino W. B. and Agre P. (1994) Molecular structure of the water channel through Aquaporin CHIP: the hourglass model. J. Biol. Chem. **269:** 14648–14654. Copyright 2003 The American Society for Biochemistry and Molecular Biology.

Pore structure

The pore of human AQP1 is ~3 Å in diameter at its narrowest place [8], and a water molecule is approximately 2.8 Å. Thus, a single water molecule can pass through the pore at a time at a rate of approximately 3×10^9 water molecules per monomer per second [10–12]. Studies of the *Escherichia coli* glycerol facilitator (GlpF) show that the pore region of aquaglyceroporins is slightly larger than that of aquaporins, allowing the larger glycerol molecule to pass [13].

Computer simulations of human AQP1 revealed that the unique selectivity to water molecules of AQP1 is due to electrostatic fields created on each side of the pore that repel hydronium and hydroxide and block proton transfer [14]. In the pore, the two asparagines of the NPA motifs create a hydrophilic environment in which water forms hydrogen bonds, turns 180° and passes to the other side [8, 15]. Cysteine 189, which is conserved in many of the aquaporins, is localized in the pore region (fig. 2) and is the site responsible for the observed mercurial inhibition of AQP1 [16].

For further information regarding the unique structure of aquaporins, the reader is referred to the following reviews [17, 18].

Pore properties

AQP1 potentially facilitates transport of additional molecules besides water. AQP1 has been reported to function as a guanosine cyclic monophosphate (cGMP)-gated ion channel when expressed in oocytes [19], but only 1 in a million AQP1 molecules shows ion conductivity in purified AQP1 reconstituted in lipid bilayers [20]. Later studies showed that AQP1 does not display ion conductivity in mammalian cells [21], leaving the potential ion conductivity of AQP1 unsettled. Also, human AQP1 has been reported to facilitate CO_2 transport in oocytes [22, 23] and proteoliposomes [24], and that transport was inhibited by mercurial compounds [23, 24]. However, studies of CO_2 permeability in lung, kidney and erythrocytes from AQP1-null and wild-type mice [25, 26] did not reveal any difference in CO_2 permeability, leaving the physiological importance of AQP1-mediated CO_2 permeability also unresolved.

Other aquaporins have pore properties different from those of AQP1. Interestingly, AQP3 permeability seems to be blocked by nickel [27], copper [28] and acidification [27] in tissue culture cells, and protein kinase (PKC) phosphorylation of Ser180 inhibits AQP4 water permeability via a dopamine-mediated pathway in tissue culture cells [29]. AQP6 also seems to function as a nitrate [30] channel in oocytes and an anion [31] channel in tissue culture cells. AQP6 ion permeability is gated by Hg^{2+} [32] in oocytes, and AQP6 water permeability is Hg^{2+} inducible [31] in oocytes. Although AQP6 shares greatest sequence homology with AQP2 and AQP0, Hg^{2+} activation also seems to activate AQP6 permeability of glycerol and urea [33] in oocytes, perhaps placing AQP6 in the aquaglyceroporin family. AQP7 facilitates transport of arsenite [34] in tissue culture cells, indicating that the aquaporins and aquaglyceroporins may serve much more complex functions than 'just' water and glycerol transport.

Renal aquaporin localization and regulation

In the kidney, aquaporins are essential for maintaining body water homeostasis. Several aquaporins are expressed in the kidney along the water-permeable segments (fig. 3).

AQP1

AQP1 constitutes approximately 1% of total cortical protein [35] and has an important function in the kidney. AQP1 is abundantly expressed in the proximal tubule and descending thin limb of Henle (fig. 3) [35], the nephron segments with constitutive high water permeability and in the epithelium of the descending vasa recta [36]. AQP1 is localized to the apical and basolateral plasma membranes of proximal tubule epithelial cells and cells of the descending thin limb of the loop of Henle (fig. 3) [35]. Expression levels of AQP1 seem not to be regulated in response to either water restriction or vasopressin infusion [37], consistent with the constitutive high water permeability of proximal tubule and descending thin limb.

Important information regarding the function of AQP1 has been obtained from AQP1-null mice. These mice display reduced urine osmolality and inability to concentrate urine in response to water deprivation [38]. Consistent with this, micropunctural studies on AQP1-null mice show reduced water reabsorption in proximal tubules and descending thin limbs of Henle compared with wild-type mice [39]. Water permeability of the descending vasa recta is also significantly decreased in AQP1-null mice compared with wild-type mice [40]. These results reveal the functionally important role of AQP1 in the kidney.

In humans, AQP1 seems to be less important for the renal concentrating mechanism than in mice. AQP1 encodes the minor blood-group-antigen Colton [41], and Colton-null individuals appear to be clinically normal, but are unable to concentrate urine when challenged by water deprivation [42].

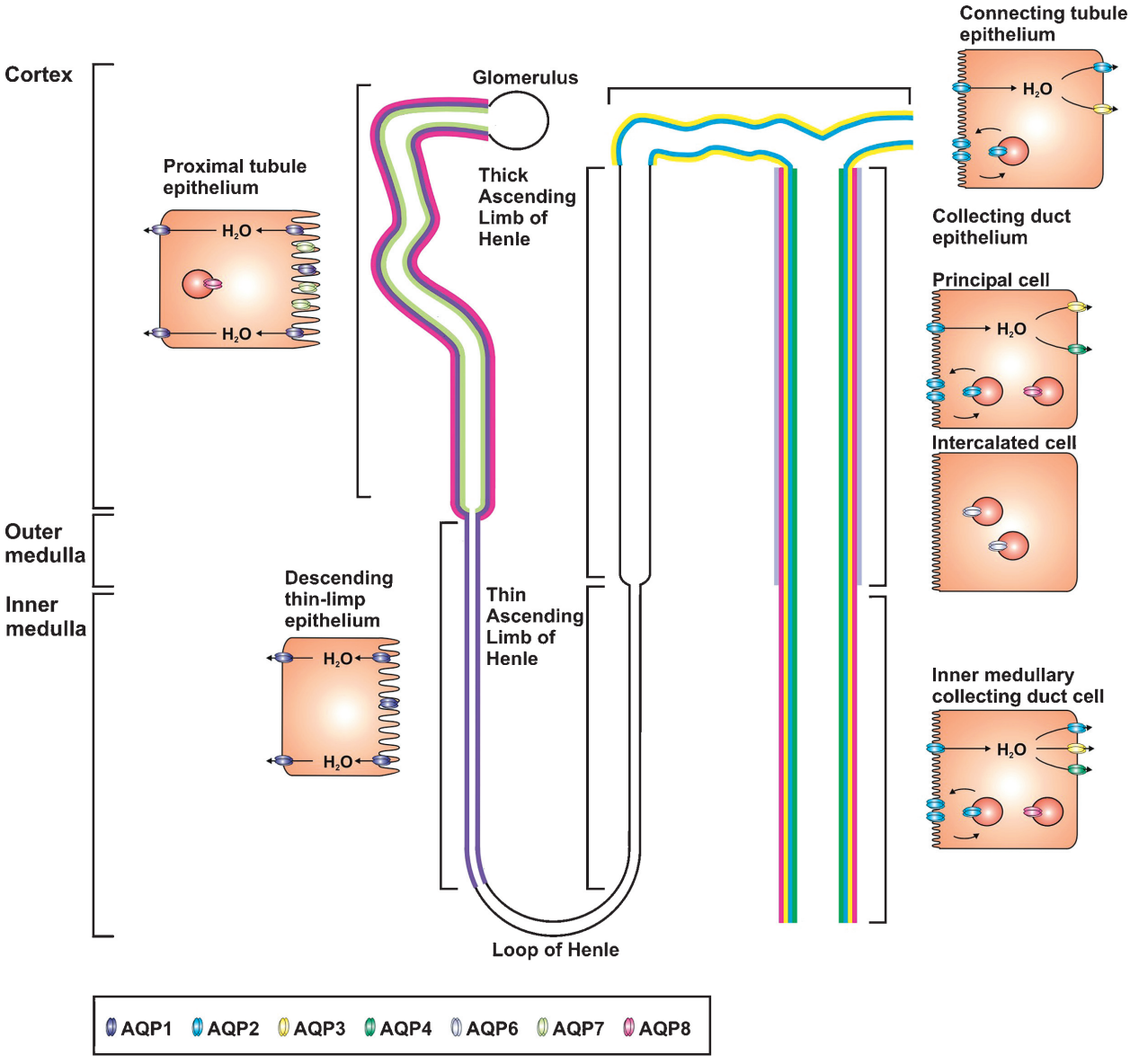

Figure 3. Schematic drawing of the renal nephron and collecting duct showing the sites of aquaporin localization. AQP1 is present in the apical and basolateral plasma membranes of the proximal tubule and thin descending limb of Henle. AQP2 is present in the apical plasma membrane and subapical intracellular vesicles in the connecting tubule and collecting duct, and in the basolateral plasma membrane of the connecting tubule and inner medullary collecting duct. AQP3 is present in the basolateral plasma membrane of the connecting tubule and collecting duct principal cells. AQP4 is present in the basolateral plasma membrane of the collecting duct principal cells. AQP6 is present in intracellular vesicles of acid-secreting intercalated cells. AQP7 is present in the apical plasma membrane of proximal tubule. AQP8 is present in intracellular vesicles of the proximal tubule and collecting duct. Modified with permission [170]. King L. S., Kozono D. and Agre P. (2004) From structure to disease: the evolving tale of aquaporin biology. Nat. Rev. Mol. Cell Biol. **5:** 687–698. Copyright 2000 Nature Publishing Group. www.nature.com

AQP2

The collecting duct is the major site for vasopressin-mediated regulation of body water homeostasis. AQP2 [43] is the predominant vasopressin-regulated aquaporin of the renal collecting duct where it is localized in principal cells in the apical plasma membrane, subapical vesicles [43–45] and the basolateral plasma membrane (fig. 3) [45, 46]. The expression level and localization are tightly regulated, and this regulation is essential for both short-term and long-term renal regulation of body water balance.

It has not been possible to study AQP2-null mice, which die by day 6 after birth [47]. However, studies of other animal models and humans with AQP2 mutations (discussed below) prove the essential role of AQP2 in renal function. The following section aims to describe the

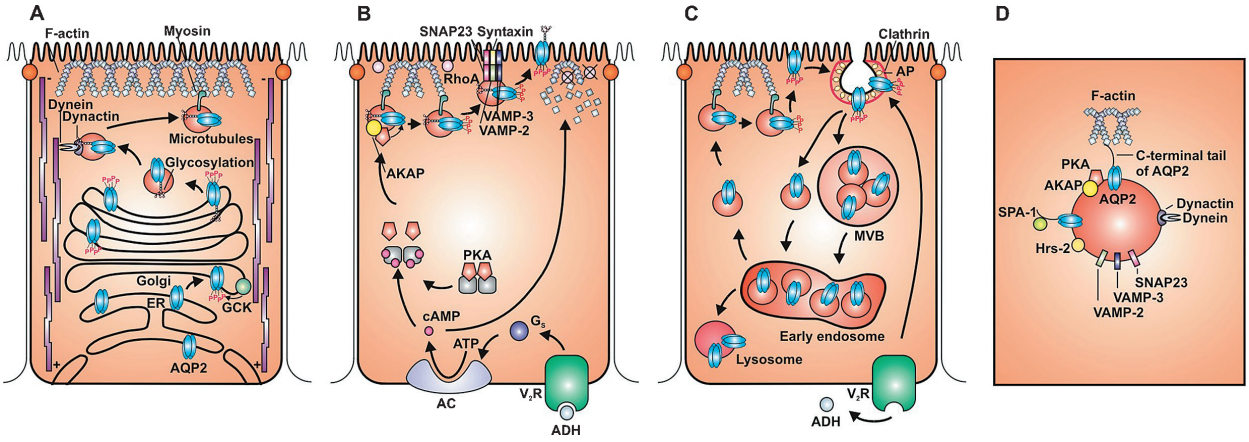

Figure 4. AQP2 – from synthesis to degradation. Schematic illustration of the molecular apparatus involved in AQP2 regulation and the signaling cascade following vasopressin binding to the V_2 receptor. See text for details. (*A*) AQP2 synthesis to subapical intracellular vesicles. (*B*) Vasopressin-mediated exocytosis of AQP2. (*C*) AQP2 endocytosis initiated by vasopressin removal and the potential recycling of AQP2. (*D*) AQP2 vesicle showing proteins that co-localize with vesicular AQP2. AC, adenylate cyclase; ADH, antidiuretic hormone; AKAP, PKA-anchoring proteins; AP, adaptor protein; ATP, adenosine triphosphate; cAMP, adenosine 3′,5′-cyclic monophosphate; ER, endoplasmic reticulum; GCK, Golgi casein kinase; Gs, GTP-binding protein; MVB, multivesicular body; P, indicates S256 phosphorylation; PKA, protein kinase A; SNAP23, synaptosomal associated protein 23; SPA-1, Signal-induced proliferation-associated gene-1, VAMP-2/3, synaptobrevin-2/3; VAMP-2, synaptobrevin-2; V_2R, vasopressin receptor.

known cellular regulatory and sorting mechanisms for AQP2 (fig. 4) and the involvement of AQP2 in regulation of body water homeostasis.

Short-term regulation of AQP2 – exo-/endocytosis (vasopressin-dependent)

Short-term regulation of collecting duct water permeability is controlled by the antidiuretic hormone vasopressin, which regulates the quantity of AQP2 in the apical plasma membrane. At basal conditions, isolated perfused collecting ducts exhibit low water permeability coinciding with AQP2 localizing to intracellular vesicles [48], but upon vasopressin stimulation AQP2 is rapidly inserted into the apical plasma membrane (fig. 4B), instantly increasing water reabsorption and urine concentration [48]. Removal of vasopressin initiates AQP2 endocytosis (fig. 4C), thus restoring low collecting duct water permeability [48]. This regulation confirms the classic collecting duct 'shuttle hypothesis' proposed from studies of toad bladder (the amphibian equivalent to the mammalian collecting duct) that described particles moving to and from the plasma membrane upon vasopressin stimulation and washout, respectively [49].

The molecular apparatus involved in vasopressin-regulated AQP2 distribution is quite well understood. Vasopressin binds to the V_2-receptor in the basolateral plasma membrane, leading to activation of adenylate cyclase through V_2-receptor-coupled heterotrimeric guanine triphosphate (GTP)-binding protein G_s, which increases cyclic adenosine 3′,5′-cyclic monophosphate (cAMP) levels, leading to activation of protein kinase A (PKA) (fig. 4B). Following activation, PKA is recruited to AQP2-containing vesicles by PKA-anchoring proteins (AKAPs) (fig. 4B) [50], probably the AKAP 18 delta isoform (AKAP18delta), which co-localizes with vesicular AQP2 (fig. 4B) [51]. Besides a rise in cAMP levels, vasopressin binding to the V_2 receptor also leads to a rise in intracellular calcium levels in isolated inner medullary collecting ducts (IMCDs) [52, 53], probably released from ryanodine-sensitive stores via a calmodulin-dependent mechanism [54]. Blocking this rise in calcium in isolated IMCDs does not affect vasopressin-mediated increase in cAMP but does inhibit cAMP-mediated AQP2 trafficking to the plasma membrane [54]. However, patch-clamp analysis and water flux measurements in primary cultures of IMCD cells showed that cAMP alone was sufficient to induce AQP2 localization to the plasma membrane without a simultaneous rise in intracellular calcium [55], although cytosolic calcium levels below 25 nM inhibited exocytosis. This indicates that calcium is necessary for AQP2 exocytosis but that the rise in intracellular calcium observed in isolated IMCDs upon vasopressin stimulation may not be a requirement for exocytosis. More studies are needed to elucidate the exact role of calcium in AQP2 exocytosis.

AQP2 phosphorylation in short-term regulation

PKA phosphorylation of AQP2 on Serine 256 (Ser256) [56] initiates exocytosis of AQP2 containing vesicles to the apical plasma membrane (fig. 4B) [45, 46, 48], instantly increasing water permeability and urine-concentrating ability. At least three out of the four AQP2 monomers must be phosphorylated [57] for AQP2 plasma membrane insertion.

It has generally been believed that non-phosphorylated AQP2 resides in intracellular vesicles and that AQP2 is dephosphorylated upon initiation of endocytosis

[58]. However, recent studies show this may not be the case. An AQP2 mutant mimicking constitutively non-phosphorylated AQP2 (AQP2-Ser256Ala) is mostly targeted to lysosomes rather than to transport vesicles [59], although this non-phosphorylated mutant does traffic to the plasma membrane, as blocking of endocytosis results in accumulation of AQP2 at the plasma membrane [60]. However, in steady state or upon vasopressin stimulation this mutant is not localized to the plasma membrane [61], indicating a rapid endocytosis of non-phosphorylated AQP2.

The AQP2 mutant mimicking constitutively phosphorylated AQP2 (AQP2-Ser256Asp) is localized in the plasma membrane at steady state [57], but blocking PKA results in intracellular localization of AQP2-Ser256Asp in tissue culture cells [62], and AQP2 can be endocytosed in inner medullary (IM) slices independent of dephosphorylation [62, 63]. These studies show that PKA activity is a requirement for AQP2 plasma membrane localization, but that phosphorylation is not sufficient for AQP2 plasma membrane targeting. This is further supported by studies of the Brattleboro diabetes insipidus (DI) rat, which suffers from congenital central DI (explained below) due to a lack of circulating vasopressin [64] and is profoundly polyuric. The Brattleboro DI rat has decreased AQP2 expression levels [65–67], with the majority of AQP2 being intracellular [65, 67]. Immunohistochemical studies of IM show that although localized intracellularly, the Brattleboro DI rat has significant amounts of phosphorylated AQP2 [58] and that treatment with 1-desamino-8-D-argenine vasopressin (dDAVP) induces AQP2 exocytosis [68] but does not increase AQP2 phosphorylation [58].

Together, these results show that phosphorylation may be a requirement for AQP2 targeting to the plasma membrane, but other regulatory steps besides phosphorylation are necessary for initiation of AQP2 plasma membrane targeting.

From endoplasmic reticulum to transport vesicles

In addition to regulating AQP2 exocytosis, Ser256 in the AQP2 carboxy-terminus regulates protein sorting into transport vesicles in the Golgi complex and delivery to the plasma membrane. In the endoplasmic reticulum (ER), AQP2 is only weakly phosphorylated at Ser256, but AQP2 becomes highly phosphorylated in the Golgi complex, probably by Golgi casein kinase (fig. 4A) [59]. After exiting the Golgi complex, AQP2 is phosphorylated at low levels, rendering Ser256 available for further regulation by phosphorylation at later steps [59]. The sites for dephosphorylation are unknown. A mutant mimicking constitutively phosphorylated AQP2 at Ser256 is mainly localized in the plasma membrane [57], indicating that dephosphorylation of AQP2 is not required for correct AQP2 exit from the Golgi complex. Glycosylation of AQP2 is also important for AQP2 exit of the Golgi complex (fig. 4A), as a non-glycosylated AQP2 mutant (AQP2-Asn123Gln) seems to fold correctly, but is retained in the Golgi complex [69].

AQP2 plasma membrane targeting

Transport and targeting of AQP2 to the apical plasma membrane seem to involve microtubules, actin and a docking system. In polarized cells, microtubule minus ends are organized towards the apical plasma membrane and plus ends towards the basal plasma membrane. The microtubule minus end-directed motor protein dynein has been localized to AQP2-containing vesicles (fig. 4A) [70], indicating AQP2 transport along microtubules to the apical plasma membrane. Dynactin, a protein complex believed to mediate interaction between the motor protein dynactin and transport vesicles [71], is also found on AQP2 vesicles (fig. 4A) [70], further supporting the idea that AQP2-containing vesicles are transported along microtubules. Also, the actin cytoskeleton seems to be involved in AQP2 transport. Myosins are actin-based molecular motors that interact with actin microfilaments and the myosin regulatory light chain. The IIA and IIB isoforms of non-muscle myosin heavy chain are present in rat kidney IMCD cells where they are involved in vasopressin-mediated transport of AQP2 to the plasma membrane [72]. These data indicate that transport of AQP2 to the plasma membrane is most likely motor-driven along both microtubule and actin filaments.

Organization of the actin cytoskeleton is also important for AQP2 trafficking to the plasma membrane. Inhibition of the RhoA GTPase in tissue culture cells causes F-actin depolymerization and AQP2 targeting to the apical plasma membrane (fig. 4B) [73] even without vasopressin stimulation [74], indicating a significant role of actin in regulation of AQP2 trafficking. Interestingly, the β- and γ-isoforms of actin bind directly to the carboxy-terminus of AQP2 (fig. 4D) [75], indicating a direct role of actin in AQP2 trafficking.

Recently, the GTPase Rap1 has been shown to be involved in regulating AQP2 trafficking to the plasma membrane, since activation of Rap1 inhibits AQP2 plasma membrane targeting [76]. This may be due to an effect of Rap1 on F-actin, as inhibition of Rap1 decreases F-actin polymerization in tissue culture cells [77]. The effect of Rap1 on AQP2 is most likely executed by the signal-induced proliferation-associated gene-1 (SPA-1), which inhibits Rap1 activity and binds directly to AQP2 via a PDZ domain (fig. 4D) [76]. Interestingly, AQP2 is not targeted to the plasma membrane in transgenic SPA-1-null mice challenged by water deprivation [76].

The docking/fusion machinery involved in docking and fusion of AQP2-containing vesicles to the plasma membrane is poorly understood. The vesicle soluble N-ethylmaleimide-sensitive factor attachment protein receptor (v-SNARE) synaptobrevin-2 (VAMP-2) is associated with AQP2 vesicles (fig. 4B, D) [78–80], and cleavage of

VAMP-2 completely abolishes cAMP-induced AQP2 trafficking to the plasma membrane in tissue culture cells [81]. VAMP-3 (cellubrevin) and the synaptosomal associated protein 23 (SNAP23) is also localized to AQP2 vesicles and the apical plasma membrane (fig. 4B, D) [82, 83], as is the SNAP23-associated ATPase Hrs-2 (fig. 4B, D) [84, 85], which inhibits SNAP23 [86]. However, the importance of VAMP-3, SNAP23 and Hrs-2 in AQP2 targeting is still unresolved. Also, a target-SNARE (t-SNARE) is present in the apical plasma membrane of kidney collecting duct (fig. 4B), although there are conflicting reports whether it is syntaxin-3 [87] or syntaxin-4 [88], leaving the identity of the t-SNARE involved in AQP2 targeting unresolved. These results indicate a potential role of VAMP-2, VAMP-3, SNAP-23, and syntaxin-3 or syntaxin-4 in correct delivery of AQP2 to the apical plasma membrane. Members of the Rab GTPase family are involved in all stages of vesicle formation to fusion (for recent review, see [89]). Rab3 and Rab5a [79] have been localized to IMCD vesicles and may therefore also be involved in AQP2 docking and fusion.

AQP2 endocytosis and recycling

AQP2 endocytosis (fig. 4C) is initiated by removal of vasopressin [48] or blocking the V_2-receptor [90], which results in restoration of low collecting duct water permeability [48]. AQP2 endocytosis is mediated through clathrin-coated pits (fig. 4C) [91]. Inhibition of clathrin-mediated endocytosis by expression of the GTPase-deficient Lys44Ala mutants of dynamin 1 and 2 [91] results in accumulation of AQP2 at the plasma membrane. After vasopressin washout, AQP2 appears to be localized in multivesicular bodies and vesicles in isolated perfused IMCD [48]. Upon endocytosis, AQP2 appears to transiently traffic to early endosomes (fig. 4C), as shown by co-localization with early endosome antigen (EEA1) [92]. Subsequently, AQP2 returns to a pool of subapical vesicles [92], from where it again can be targeted to the apical plasma membrane (fig. 4C) [93].

Besides vasopressin removal, prostaglandin E_2 (PGE2) and dopamine also decrease collecting duct water permeability. PGE2 inhibits cAMP-induced water permeability of rat terminal IMCDs [94], and counteracts vasopressin-induced AQP2 translocation to the plasma membrane in IM slice preparations and in tissue culture cells [62, 63], possibly via E-prostanoid-3 (EP_3) receptors and RhoA activation [95]. Dopamine counteracts vasopressin-induced water permeability in rat cortical collecting duct, probably via D_4-like receptors [96] and in the IMCD via α_2-adrenoceptors [97]. Dopamine also induces AQP2 endocytosis in IM slices and tissue culture cells [62].

Polarized distribution of AQP2

The polarized distribution of AQP2 is as complex as its subcellular localization. AQP2 is present in the apical plasma membrane and in subapical vesicles all along the connecting tubule (CNT) to the IMCD (fig. 3) [43–45, 98, 99].

Basolateral plasma membrane-localized AQP2 is present in the CNT and IMCD (fig. 3) [45, 46], but absent in cortex and outer medullary collecting duct cells [100]. How this difference in polarization is regulated is presently unknown. Long-term (6 days), but not short-term (2 h) stimulation with the vasopressin homolog dDAVP increases basolateral plasma membrane expression of AQP2 in CNT and IMCD [100], indicating that the basolateral plasma membrane pool of AQP2 is regulated differently than that in the apical plasma membrane. Hypertonicity may play a role in the polarized distribution of AQP2, as hypertonicity seems to be involved in vasopressin-mediated redirection of AQP2 from the apical to the basolateral plasma membrane in tissue culture cells [101].

Both the amino- and carboxy-termini of AQP2 are involved in correct polarized plasma membrane sorting of AQP2 [102]; however, the intracellular sorting machinery responsible for segment-specific organization of AQP2 along the CNT and collecting duct is at present unknown.

Long-term regulation of AQP2

In addition to short-term regulation of AQP2 shuttling between intracellular vesicles and the plasma membrane, AQP2 protein expression is also regulated.

AQP2 protein expression is regulated by the state of hydration. Water deprivation increases AQP2 expression levels [45, 103, 104], thereby increasing urine concentration, whereas water loading decreases AQP2 expression levels [46, 103], resulting in urine dilution. This regulation may in part be facilitated by the action of vasopressin. Continuous vasopressin infusion in the Brattleboro DI rat reverses polyuria and increases AQP2 expression levels to that of normal rats [37, 65]. Also, prolonged vasopressin stimulation in normal rats increases AQP2 messenger RNA (mRNA) and protein expression levels [37, 65, 105], and prolonged infusion with a V_2-receptor antagonist decreases AQP2 protein expression levels [106, 107]. These results show that the long-term regulation of AQP2 protein expression is due to the action of vasopressin and, like the vasopressin-mediated short-term regulation of AQP2, long-term regulation also acts via the V_2-receptor. This is further supported by studies in DI +/+ severe mice, which have a constitutively active cAMP phosphodiesterase and hence permanently low cAMP levels. DI +/+ severe mice are unable to concentrate urine in response to vasopressin and have significantly reduced AQP2 protein expression levels [108]. This indicates that the long-term regulatory effect of vasopressin acts via the same pathway as the short-term regulatory effect of vasopressin and via increase in mRNA transcription. The

increase in AQP2 mRNA upon vasopressin stimulation [105] is most likely mediated by PKA via the cAMP-responsive element binding protein (CREB) through a dual effect on the cAMP-responsive element (CRE) and the AP1 element in the promoter region of the AQP2 gene [109, 110].

Vasopressin-independent regulation of AQP2

Vasopressin-independent regulatory mechanisms are involved in both short- and long-term regulation of AQP2. Elucidating these mechanisms should be the focus of intense interest due to the potential impact on treatment of diseases associated with AQP2 dysregulation and conditions that cannot be treated by altering vasopressin levels.

Short-term regulation. In addition to vasopressin, the hormone oxytocin also has an antidiuretic effect and increases collecting duct water permeability in isolated perfused tubules [111]. This probably occurs through activation of V_2-receptors [112], which results in increased cAMP levels, followed by targeting of AQP2 to the apical plasma membrane [113]. Modulation of the cGMP pathway also stimulates AQP2-Ser256 phosphorylation and AQP2 plasma membrane insertion in tissue culture cells [114] without a rise in cAMP levels. In vitro, PKG can phosphorylate AQP2 on the carboxy-terminus, and similar to the effect of PKA, the AQP2-Ser256Ala mutant fails to be targeted to the plasma membrane when stimulated with cGMP [114]. The effect of cGMP on AQP2 targeting is further supported by the finding that atrial natriuretic peptide (ANP) and nitric oxide, which increase cGMP but not cAMP levels, induce AQP2 insertion into the plasma membrane in tissue culture cells [114]. However, it is not yet known whether the effect of cGMP acts through PKA or whether an alternative phosphorylation mechanism exists.

Long-term regulation. The most obvious evidence for the existence of vasopressin-independent regulation is vasopressin escape. In some clinical circumstances such as the syndrome of inappropriate ADH secretion (SIADH), vasopressin levels are elevated, resulting in free water retention. However, the degree of water retention is limited by a phenomenon called vasopressin escape, which is characterized by a sudden increase in urine volume independent of circulating vasopressin levels [115]. Interestingly, vasopressin escape is associated with a decrease in whole kidney AQP2 protein levels, but maintains targeting of the remaining AQP2 to the apical plasma membrane [116], indicating that the escape mechanism is at the transcriptional level and/or at the level of protein turnover. Thus, vasopressin escape provides a counteracting mechanism for vasopressin-mediated collecting duct water reabsorption. PGE2 and dopamine could be involved in the vasopressin escape mechanism, as both are known to counteract vasopressin-mediated collecting duct water reabsorption [94, 96] and to induce AQP2 endocytosis in the continuous presence of vasopressin in IM slice preparations and tissue culture cells [62, 63].

The Brattleboro DI rat provides a useful model for studying vasopressin-independent regulation of AQP2. Surprisingly, the Brattleboro DI rat displays high expression levels of AQP2 (approximately half those of controls) [65], although the protein is localized intracellularly [65, 67]. The Brattleboro DI rat has a slight ability to concentrate urine in response to water deprivation [117], probably by increasing the amount of AQP2 (~1% to ~3.5% of total AQP2) in the apical plasma membrane [67]. Interestingly, treatment with a V_2-receptor antagonist induces a decrease in AQP2 expression levels in Brattleboro DI rats [58, 67], indicating that AQP2 expression is regulated via V_2-receptor signaling but that a signal molecule other than vasopressin must be able to activate the receptor; a likely candidate is oxytocin, since its levels increase in dehydrated Brattleboro DI rats [118] and it induces AQP2 targeting to the apical plasma membrane [113].

Additional evidence for vasopressin-independent regulation of AQP2 has been obtained by studying lithium-induced nephrogenic DI (NDI). Lithium, a drug used worldwide for treatment of bipolar disorders, often causes polyuria. Rats treated with lithium display dramatic downregulation of AQP2 to ~4% of the level of controls [119]. Interestingly, challenging the rats by water deprivation for 48 h increases AQP2 expression levels to an even greater extent than dDAVP infusion, but fails to target AQP2 to the plasma membrane [119]. Thus, dDAVP and water deprivation may regulate AQP2 expression and targeting through different pathways, indicating the existence of vasopressin-independent pathways of AQP2 regulation associated with water deprivation. Hypertonicity may also play a role in vasopressin-independent regulation of AQP2. Hypertonicity can reverse downregulation of AQP2 synthesis in primary cultures of IM cells [120] independent of CREB [121], probably through the tonicity responsive element in the AQP2 promoter [121].

Insulin may also regulate AQP2 expression and targeting. Insulin increases AQP2 mRNA and protein levels in tissue culture cells and potentiates the effect of vasopressin on AQP2 expression levels [122]. Also, insulin increases water permeability of isolated perfused collecting ducts [123]. This indicates that the effect of insulin on collecting duct water permeability ocurs via regulation of AQP2. Combined, these results show that pathways other than those mediated by vasopressin are involved in regulating AQP2 expression.

AQP2 in water balance disorders

Diabetes insipidus (DI) is a condition associated with excretion of a large volume of urine. There are two types of diabetes insipidus, central and nephrogenic. Central DI

(CDI) is characterized by an inability to secrete the required amount of vasopressin, whereas NDI is characterized by an inability to concentrate urine in response to vasopressin. CDI and NDI can either be inherited or acquired.

Inherited CDI is an autosomal dominant defect caused by mutations in the vasopressin gene [124]. Aquired CDI usually occurs as a consequence of head trauma or disease of the hypothalamus or pituitary. Patients suffering from CDI can be treated with the vasopressin analog desmopressin, which restores their ability to concentrate urine.

Congenital NDI is a rare but severe condition in which patients produce up to 20 l of urine per day. X-linked dominant NDI, the most common form of congenital NDI, has been identified as mutations in the V_2-receptor gene [125–127]. Autosomal-linked NDI has been identified as mutations in the AQP2 gene [128] and exists both in a dominant and recessive form. In the dominant form, mutated AQP2 seems to form heterotetramers with normal AQP2, leading to retention of the tetramers in the Golgi complex [129, 130]. In the recessive form, mutated AQP2 seems to be retained in either the ER or Golgi complex, but unlike dominant NDI, mutated AQP2, does not form heterotetramers with normal AQP2, resulting in a normal phenotype of heterozygotes [128, 131–133].

Acquired NDI is far more common than inherited NDI. Acquired NDI can be a side effect of drug treatment, such as lithium [119], and is seen in multiple pathophysiological conditions, including hypokalemia [134], ureteral post-obstruction [135], hypercalcemia [136], nocturnal enuresis [137], and acute [138] and chronic [139] renal failure. A shared characteristic of acquired NDI investigated in animal models is a decrease in AQP2 expression levels.

Dysregulation of renal aquaporins has also been observed in several conditions associated with expanded extracellular fluid volume such as occurs in congestive heart failure [140], liver cirrhosis [141] and pregnancy [142]. A characteristic for conditions investigated in animal models so far is an increase in AQP2 expression levels.

In contrast to the above, diabetes mellitus type I (DM-I) is associated with diuresis but rats have increased AQP2 protein expression levels [143]. This is most likely due to the fact that the diuresis is osmotic, hence the DM-I rats are dehydrated and increase AQP2 protein expression as a compensatory response to substantial water excretion.

Thus, AQP2 dysregulation is involved in a variety of conditions associated with altered water balance, emphasizing the importance of unraveling cellular regulation.

AQP3 and AQP4

AQP3 [144, 145] is localized to the basolateral plasma membranes of collecting duct principal cells [144–147] and the connecting tubule (fig. 3) [99]. AQP4 [148] is localized to the basal plasma membranes of collecting duct principal cells (fig. 3), with strongest staining intensity in IM, declining towards cortex [149]. In mice, in contrast to rat and human, AQP4 is also found in the basolateral plasma membrane of proximal tubule S3 segments where it participates in the concentrating mechanism [150]. AQP3 and AQP4 mediate potential exit pathways of water entering the principal cells through AQP2 in the apical plasma membrane [151, 152].

AQP3 and AQP4 are not found in significant amounts in the cytoplasm, and so far there is no evidence for short-term regulation of AQP3 and AQP4 similar to that of AQP2. In contrast, AQP3 is also regulated by vasopressin at the long-term level, and the regulation of AQP3 seems to occur with AQP2. Renal AQP3 mRNA and protein levels are, like those of AQP2, increased during water deprivation [37, 146, 147, 153]. Like AQP2, vasopressin infusion in Brattleboro DI rats increases AQP3 protein expression [37]. Also AQP3, like AQP2 [121], is regulated by osmolality as hypertonicity increases the expression of AQP3 in tissue culture cells [154]. AQP3-null mice display severe polyuria and reduced ability to concentrate urine in response to dDAVP infusion [151], indicating an essential role for AQP3 in the concentration mechanism. AQP3-null individuals have recently been identified and are defined as the GIL blood group [155]. They display reduced glycerol permeability of erythrocytes [155], but data regarding their urinary concentrating mechanism are not available.

Regulation of AQP4 seems to be different from that of AQP3. The protein expression level of kidney AQP4 is unchanged in response to hydration state [37]; however, water deprivation seems to increase IM AQP4 mRNA [153]. Urine osmolality does not differ between wild-type mice and AQP4-null mice; however, AQP4-null mice have a mild concentrating defect in response to water deprivation [152] and display a fourfold reduction in water permeability in isolated IMCD [156]. Although AQP4-null mice seem relatively normal with respect to the renal concentrating mechanism, mice lacking both AQP3 and AQP4 have an even greater increase in urine production than AQP3-null mice [151], indicating a compensatory role of AQP4 in AQP3-null mice.

AQP6

AQP6 is localized with H^+-ATPase in membranes of intracellular vesicle of acid-secreting α-intercalated collecting duct cells (fig. 3) [31, 157, 158]. AQP6 may be found in podocytes and straight proximal tubule late segments 2 and 3 [158], but preliminary studies of AQP6-

null mice [159] showed this result may be an artifact of antibody cross-reactivity.

AQP6 protein expression is regulated by changes in body water balance and chronic acid load, increasing with water loading and acid loading [160]. Based on these results, AQP6 is speculated to play a role in body acid/base balance maintenance, but more studies are needed to understand the physiological role of AQP6.

AQP7

AQP7 [161] is localized to the apical plasma membrane of proximal convoluted and straight tubule cells in rat and mouse kidney (fig. 3), with strongest labeling intensity of proximal tubule segment 3 declining towards segment 1 [162, 163]. The importance of AQP7 in kidney water regulation remains unknown.

AQP8

AQP8 was cloned from different rat [164, 165], mouse [166, 167] and human [168] tissues and shows species differences with respect to transport selectivity; only mouse AQP8 is capable of urea transport [166]. In the kidney, AQP8 is distributed in intracellular vesicles throughout the cytoplasm in proximal tubule and collecting duct cells (fig. 3) [169]. AQP8 is speculated to play a role in osmoequilibration between the cytoplasmic and vesicular compartments [169], but functional studies regarding renal AQP8 expression have not been published.

Conclusions

Following the discovery of aquaporins, research regarding transport of water through the lipid bilayer expanded exponentially and led to greatly increased knowledge regarding regulation of body water homeostasis and cellular events in pathophysiological conditions associated with water balance disorders. The increased knowledge of the cellular localization, function, structure and channel specificities of the aquaporins renders them obvious therapeutic targets for channel-modulating agents. The next decades may feature a new understanding of aquaporin manipulation and targeting to the benefit of patients.

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