Aquaporins in avian kidneys: function and perspectives

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Aquaporins (AQP) are a family of small, hydrophobic proteins (major intrinsic proteins, MIP) that were originally cloned in the mammalian lens as MIP26 (for review, see Refs. 2, 122, 123). AQP are phylogenetically old molecules and are present in all domains of life, including plants, microbial organisms, invertebrates, and vertebrates (for review, see Refs. 11 and 22). AQP form pores selective to water and glycerol but also permeable to a lesser degree to a number of unconventional molecules (125). By the discovery of AQP water channels, it has been elucidated how water crosses biological membranes (2, 95).

Thirteen major mammalian AQP, many isoforms, and two subgroups (AQP and aquaglyceroporins, aquaGLPs) have been identified (38, 44, 57, 123, 126). Based on selective passage, AQP can be classified into three groups: 1) AQP that are primarily selective to water and small molecules, such as polar or unpolar gases, metalloids, etc.; these include AQP 0, 1, 2, 4, 5, 6, 8, and 11. The selectivity of AQP 0, 6, 8, and 11, however, has not been completely elucidated. AQP11 transports water at low capacity but not glycerol (43); 2) aquaGLPs that transport water, and glycerol, including AQP 3, 7, 9, and 10; aquaGLPs also transport small uncharged molecules, urea, and/or ammonia (100, 123); and 3) glyceroporins (GLP) that transport primarily glycerol. GLP has not been found in 13 mammalian AQP but exists in bacteria (10). AQP 10–12 can be classified as unorthodox AQP (7) or super AQP (43). Because function can be misinterpreted by the measuring system, classification on the basis of primary structure and evolutionary basis may be more appropriate (44).

Reptiles, birds, and mammals share a common evolutionary origin. The ancient evolutionary line leading to mammals departed from the reptilian-avian line at an early stage of tetrapod evolution (26, 51, 135). Although birds and mammals represent parallel lines of divergent evolution, both can produce hyperosmotic urine (25). Avian kidneys express AQP1, 2, 3, and 4 (see below). Arginine vasotocin (AVT), an avian antidiuretic hormone, plays an important role in urine concentration via stimulation of trafficking, gene expression, and protein synthesis of AQP2 in the collecting ducts (CDs) (58, 80, 130). In nonmammalian vertebrates, several osmoregulatory organs in addition to the kidney play important roles in water economy as a prerequisite for survival, and the kidney is their major osmoregulatory organ. Birds are the only vertebrates other than mammals that can concentrate urine in adaptation to terrestrial environments. Aquaporin (AQP) and glyceroporin (GLP) are phylogenetically old molecules and have been found in plants, microbial organisms, invertebrates, and vertebrates. Currently, 13 AQP and aquaGLPs and isoforms are known to be present in mammals. AQP 1, 2, 3, 4, 6, 7, 8, and 11 are expressed in the kidney; of these, AQP 1, 2, 3, 4, and 7 are shown to be involved in fluid homeostasis. In avian kidneys, AQP 1, 2, 3, and 4 have been identified and characterized. Also, gene products of AQP5, AQP7, AQP8, AQP9, AQP11, and AQP12 have been reported in birds. AQP 2 and 3 are expressed along cortical and medullary collecting ducts (CDs) and are responsible, respectively, for the water inflow and outflow of CD epithelial cells. While AQP4 plays an important role in water exit in the CD of mammalian kidneys, it is unlikely to participate in water outflow in avian CD. This review summarizes current knowledge on structure and function of avian AQP and compares them to those in mammalian and nonmammalian vertebrates. Also, we aim to provide input into, and perspectives on, the role of renal AQP in body water homeostasis during ontogenic and phylogenetic advancement.

avian aquaporins; water channel; AQP; osmoregulation; AQP development

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body fluid-ion balance; these include the gill, gastrointestinal tract, skin, and more specific organs, such as salt glands in birds and rectal glands in elasmobranchs. The role of AQP's in these osmoregulatory organs remains to be elucidated.

This article will review briefly the current understanding of AQPs, focusing on 1) avian kidneys and 2) the role of AQPs in fluid homeostasis in changing environments and development. Also, AQPs and aquaGLPs in mammals and other vertebrates will be compared to understand the evolutionary impact of AQPs. Readers should refer to several reviews (31, 44, 56, 103, 108, 122) for recent advancements in the field of mammalian AQPs.

Phylogenic Advancement of the Kidney and Its Role in Osmoregulation

Equilibration between extracellular and intracellular fluid compartments is maintained by movement of water across the lipid bilayer in cell membranes. Water movement is due to passive diffusion driven by osmotic gradients that are formed by ionic and nonionic solutes. Water channels that transport water are thus essential for all living forms. Although water handling by the kidney is important for both aquatic and terrestrial life in maintaining body fluid osmolality and volume homeostasis, the role of the kidney differs, depending on habitats and the phylogenetic stage of the vertebrates (25). However, when we compare structures and functions of renal corpuscles and renal tubules among animals that belong to various stages of phylogeny, we find a number of similarities in glomerular filtration and tubular transport properties, including cellular mechanisms of filtration, active ion pumps, cotransporters, and exchangers.

The kidneys of the most primitive living vertebrates, marine cyclostomes, have large glomeruli and archinephric ducts, and solute and water absorptions from renal tubules are only minimal. Their internal osmolality largely depends on that of the external media, and their urine-to-plasma (U/P) osmolal ratio is one (25, 79). During an early stage of vertebrate phylogeny, the evolution of distal nephrons, particularly the diluting segment (82, 89, 113), enabled vertebrates to adapt to phylogeny, the evolution of distal nephrons, particularly the diluting segment (82, 89, 113), enabled vertebrates to adapt to terrestrial life in maintaining body fluid osmolality and volume homeostasis. Their internal osmolality largely depends on that of the external media, and their urine-to-plasma (U/P) osmolal ratio is one (25, 79). During an early stage of vertebrate phylogeny, the evolution of distal nephrons, particularly the diluting segment (82, 89, 113), enabled vertebrates to adapt to terrestrial life in maintaining body fluid osmolality and volume homeostasis. Their internal osmolality largely depends on that of the external media, and their urine-to-plasma (U/P) osmolal ratio is one (25, 79). During an early stage of vertebrate phylogeny, the evolution of distal nephrons, particularly the diluting segment (82, 89, 113), enabled vertebrates to adapt to terrestrial life in maintaining body fluid osmolality and volume homeostasis.

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A 271-amino acid homologue to AQP1 has been isolated from the kidney and lower intestine of the house sparrow *Passer domesticus* (19). Sparrow AQP1 protein is expressed in both the cortex and medulla at the proximal and distal tubules and in podocytes of the glomerulus. In the lower intestine, AQP1 protein was demonstrated in the proximal and distal rectum and in the coprodeum (19). Its expression in water-permeable epithelial tissues suggests that AQP1 may have a role in osmoregulation in sparrows, although the biological function of AQP1 has not been tested.

**AQP1 IN MAMMALS AND OTHER VERTEBRATES.** AQP1 appears to be phylogenetically old. Notable features include: 1) a homotetrameric complex with one subunit bearing a polylactosaminoglycan, 2) minimal polypeptide mass extending above or below the lipid bilayer, and 3) possible individual water pores within each subunit (2, 3). AQP1 is mercury sensitive and is expressed widely in mammalian water-permeable tissues (77). It is permeability selective to water but not to urea, protons, hydroxyl ions, ammonium ions, or salt. In the presence of carbonic anhydrase, AQP1 expression significantly increases.
the CO$_2$ permeability of oocyte membranes (73). It remains to be determined whether this CO$_2$ movement is physiologically important. The oocyte membrane also has significant NH$_3$ permeability, which is enhanced by AQP1 (74).

Phylogenetic analysis of 17 anuran AQP mRNA sequences deposited in public databases has revealed six classes of anuran AQPs, two of which are distinct to anurans (reviewed in Refs. 56 and 115). “FA-CHIP” in Rana esculenta (1), “AQP-t1” in Bufo marinus (64), AQPh1 in Hyla japonica (116), and HC-1 in Hyla chrysoscelis (139) resemble each other in both sequence and wide tissue distribution patterns (56). These proteins are also similar to those of mammalian AQP1 (76%-98% sequence identity), and expression cloning has confirmed that AQP-t1 (64), AQPh1 (116), and HC-1(139) function as water channels but not as glycerol channels. Temperature-sensitive regulation of HC-1 expression was seen in the brain, kidney, and liver. A zebrafish homologue of AQP1 is permeable for both CO$_2$ and NH$_3$ (20).

AQP2. BIRDS. A full-length qAQP2 cDNA encodes a 274 amino acid sequence with 76% overall identity with rat AQP2 (130). qAQP2 shows six transmembrane domains, two NPA motifs, and a putative N-linked glycosylation site (aspar-
agine-124) and phosphorylation sites (serine-257) for cAMP-dependent protein kinase. The deduced site of HgCl2-inhibitory action in qAQP2 is cysteine. The relative density of the ~29-kDa protein band detected by immunoblot from the medullary cones is modestly higher in water-deprived/AVT-treated quail. In situ hybridization (ISH) of qAQP2 mRNA prepared from mature quail by using a digoxigenin-labeled riboprobe shows strong hybridization signals along the CDs and their branches in the medulla and superficial (cortical) regions (Fig. 4). The qAQP2 mRNA level in the medullary cone (looped nephron) is higher than in the superficial (loopless nephron) area, but both were significantly increased (real-time PCR, ratio to 18S) by water deprivation (58), suggesting that responsiveness to antidiuretic hormone (ADH) has evolved independently of the development of Henle’s loop and countercurrent urine concentration.

As in mammalian AQP2, qAQP2 molecules appear to be located, when inactive, in subapical vesicles. After dehydration or AVT injection, increases in qAQP2 mRNA (58) and immunoreactive protein at apical regions (130) are seen. In isolated medullary CDs from adult quail (34 ± 2 wk old), basal diffusional water permeability (tritiated water flux coefficient) is considerable and is slightly increased by AVT and more clearly by forskolin (FSK), which stimulates adenylate cyclase, whereas a FSK analogue (no adenylate cyclase stimulation) shows no effect (85). This response to AVT is much lower than the response to ADH in rat and hamster CDs. In quail, the basal cAMP levels (41 wk old) are also higher in the medullary regions and are increased slightly by AVT and markedly by FSK (85), suggesting that the low permeability response to AVT is not due to insufficient adenylate cyclase activity in the avian medulla. Furthermore, in isolated quail medullary CDs, net volume flux increases in hyperosmotic media in the absence of AVT (85). This finding and the relatively high basal diffusional permeability without AVT suggest that diffusional water movement across the CDs may occur without direct control by AVT. Water deprivation increases AQP2 mRNA levels in both loopless and looped nephrons, however, suggesting that the evolution of the urine-concentrating mechanism and the evolution of the role of AQP2 in water transport may differ.

Bonilla-Felix and coworkers (9) examined in vitro cAMP production by arginine vasopressin (AVP) in newborn (1–7 days old) and adult (8–10 wk) rabbit cortical CDs. Basal cAMP productions are similar in both, but stimulation by AVP is lower in CDs from newborns, suggesting that the immature kidney is less responsive to ADH. Likewise, in newborn rat kidneys, the increase in water permeability of the CDs evoked by AVP is less marked than in adults (59). It remains to be determined whether this lower responsiveness to ADH in newborn mammals and birds is due to immature AVP/AVT receptors or signal pathways. It has been shown that AQP2 promoter includes a region sensitive to changes in osmolality that may directly influence AQP2 expression (104). The tonicity-responsive enhancer (TonE) is observed in the AQP2 gene, which implies that TonE and its transcription factor TonE-binding protein (TonEBP) are important for AQP2 transcription (30, 104).

AQP2 IN MAMMALS AND OTHER VERTEBRATES. AQP2 protein is present at the apical membrane of kidney CD cells from the rat (33) and plays a key role in urine-concentrating ability (104, 123, 126). The so-called “shuttle hypothesis” has been proposed for an AQP cellular mechanism (for review, see Refs. 87, 103, 120). In the inactive state, AQP2 is located in subapical vesicles endosomes. Upon AVP binding to the basolateral receptor and activation of the G protein-mediated adenylate cyclase-cAMP-protein kinase A (PKA) pathway, vesicles containing AQP phosphorylated at serine-256 are translocated to the apical membrane, where AQP2 are inserted and act as a water channel (47, 76). To initiate trafficking, at least three of the four monomers in AQP2 tetramers need to be phosphorylated (50). Furthermore, AQP2-containing vesicles

Fig. 4. In situ hybridization (ISH) of digoxigenin-labeled riboprobe of qAQP3 and qAQP2. Left: medullary cone from 10-wk-old quail showing positive ISH signal of qAQP3 along the medullary CD and branches (unpublished observations). Right: kidney from 3-wk-old quail showing strong hybridization signals in the collecting ducts (CDs) and their branches of the medullary cone and in superficial (cortical) CDs. In contrast to qAQP2 protein that is localized in apical/subapical regions of CD epithelial cells, epithelial cells are diffusely labeled for qAQP2 mRNA (from Ref. 86 and Yang Y, Yamamoto K, Nishimura H; unpublished observations).
appear to be translocated via the microtubular network along cytoskeletal elements (119). When AVP-mediated stimulation has diminished, AQPs return to the endosomes via a protein kinase C (PKC)- and clathrin-mediated process (87, 88). In addition to stimulating AQP2 trafficking, vasopressin also stimulates the transcription of AQP2 through a cAMP-responsive element (CRE) present in the 224-bp 5′-flanking lesion of the AQP2 gene (67). The deletion of the core sequence of CRE or introduction of mutation into the CRE abolished the responsiveness to cAMP (67). It was thought that the cAMP and PKA cascade phosphorylates CRE-binding protein, followed by stimulation of transcription; but a recent study in this cell line showed that vasopressin-induced cAMP stimulated the synthesis of AQP2 and that this effect was not mediated by PKA but via other signal cascades (103). Also, actin provides a track guiding the movement of the vesicles to the targeted plasma membrane domain, and actin depolymerization may be required for docking of the vesicle and fusion to the plasma membrane (66, 88, 104). Subapical storage vesicles for AQP2 express myosin Vb and Rab 11 (75), important for actin depolymerization during exocytosis. Furthermore, hypertonicity itself stimulates AQP2 expression through the TonE and its transcription factors (TonEBPs) (103, 104). TonE is present in the AQP2 gene, suggesting that TonE/TonEBP contributes to AQP2 transcription (103).

Two AQPs from H. japonica (AQPh2 and AQPh3) have been identified (37, 116) that have high homology to human (CAG46822.1), mouse (AAD23034.1), and rat (NP113891.1) AQP3 (45). Suzuki and coworkers have suggested that these four genes form an anuran-specific, phylogenetically distinct MIP subclass. AQPh2 and AQPh3 are both expressed in frog ventral skin, whereas AQPh2 is also expressed in the urinary bladder (36). AQPh2 and AQPh3 immunoreactive proteins were translocated to the apical plasma membrane in the principal cells of the first-reacting cell layer in the ventral pelvic skin following challenge with AVT (90). The effect of AVT on AQPh mRNA or protein synthesis, however, has not been measured. AQPh3 during metamorphosis, when the animals are undergoing a transition from an aquatic to a terrestrial environment, suggests a possible role for AVT-regulated AQPs in this process (35).

AQPh3. BIRDS. We recently identified qAQPh3 from quail kidneys (GenBank EU794000). It has 81% homology to human (CAG46822.1), mouse (AAD23034.1), and rat (NP113891.1) AQP3 (45). The qAQPh3 showed six transmembrane domains, two NPA motifs, and a putative N-linked glycosylation site. Xenopus oocytes injected with qAQPh3 cRNA swelled rapidly. The water permeability of oocytes injected with qAQPh3 is lower than in those injected with qAQPh1 or qAQPh2 (Fig. 3) but was inhibited by HgCl2. A digoxigenin-labeled qAQPh3 riboprobe was specifically hybridized in the collecting tubules and ducts of quail kidneys in both superficial (cortical) and deeper (medullary) areas (Fig. 4 and Yang Y, Yamamoto K, Nishimura H; unpublished observations), suggesting that qAQPh3 plays a role in water exit from CDs.

AQPh3 in mammals and other vertebrates. AQPh3 is selective to both water and glycerol/urea (46). AQPh3 and AQPh4 are coexpressed at the basolateral membrane of CD epithelial cells (principal cells), with relatively more AQPh3 in the cortical segments of the CD and more AQPh4 in the inner medullary CD (28). AQP3 is expressed constitutively on the basolateral membrane, and no trafficking occurs from the vesicles. Water deprivation increases AQP3 protein in the inner medulla (28).

AQP3 is a phylogenetically old aquaGLP and has been found in teleost fish (23, 24). AQP3 mRNA and immunoreactivity are apparent throughout the branchial epithelium from both fresh water (FW)- and sea water (SW)-acclimated eels, but especially so within the chloride cells, which also contained specific antisera for the β-subunit of the Na-K-ATPase (24). An AQP3 isoform has been detected in intra-epithelial macropore-like cells within the intestine of FW- and SW-acclimated eels and in the mucous cells of the rectal epithelium of SW-acclimated fish (24). However, AQP3 levels are lower in SW- than in FW-acclimated fish, suggesting that AQP3 is unlikely to have a role in increasing osmotic water permeability. AQP3 is also expressed in the kidney, but its function remains to be elucidated.

To date, four anuran sequences similar to mammalian AQP3 have been reported (56), including AQP3 from X. laevis (106), AQP from X. tropicalis (GenBank Accession number CR855446), AQPh3BL from H. japonica (4), and HC-3 from H. chrysoscelis (139). HC-3 from H. chrysoscelis shows 82% identity and 94% amino acid similarity with mammalian AQP3, and functionally it performs as a GLP, with low water permeability and high glycerol permeability (139). HC-3 mRNA exhibited both tissue-specific and thermoselective patterns of expression. Glycerol concentrations increased in the liver and skeletal muscle in cold-acclimated frogs compared with warmth-acclimated frogs (56). The increase in glycerol concentration in these tissues corresponds well with an increase in HC-3 mRNA abundance in muscle, liver, and bladder in cold-acclimated frogs (139). Amphibians that naturally accumulate glycerol represent a natural model for studying the roles and regulation of glycerol-transporting AQPs.

AQP4. BIRDS. Two distinct qAQP4 cDNAs were isolated from quail medullary cones: long (L, open reading frames) and short (S) cDNA encoding 335 (qAQP4-L) and 301 (qAQP4-S) amino acids with, respectively, 80% and 87% identity to human long- and short-form AQP4 (129). qAQP4-S is identical to qAQP4-L from the second initiation site. Both isoforms have two NPA motifs but lack cysteine at the known mercury-sensitive site. qAQP4-L and qAQP4-S are expressed in membranes of Xenopus laevis oocytes, but both failed to increase the water permeability (Pf) of oocytes exposed to a hypotonic solution (Fig. 3). When glutamate (Q242) was replaced with histidine, a known mammalian structure, qAQP4 did not increase Pf (129). With conventional RT-PCR and real-time PCR, qAQP4-L/S mRNA signals were detected in the brain, lung, heart, intestine, adrenal gland, skeletal muscle, liver, and kidney (higher in the medulla than in the cortical region). qAQP4-L mRNA was detected only in the brain and adrenal gland. Orthogonal arrays of intramembranous particles (OAPs), AQP4-specific membrane organization (39, 109, 121, 128) seen in mammalian AQP4, were not detected in quail CDs. Furthermore, ISH of qAQP4 mRNA by using a digoxigenin-labeled riboprobe failed to show specific hybridization signals along the quail CDs. This suggests that although qAQP4-L and qAQP4-S have high homology to mammalian AQP4 long and short forms, their physiological function may be different.

Chicken qAQP4 (cAQP4) (101) has 99% amino acid homology with qAQP4 (129). cAQP4 mRNA have been detected in the hypothalamus, proventriculus, kidney, muscle, and other organs (101). Immunoreactive AQP4 has been detected in...
circumventricular organs of chicken brain (34). After water deprivation, cAQ4 mRNA in the hypothalamus measured by real-time PCR increased, whereas it decreased in the kidney (101). The physiological significance of this finding remains to be elucidated. The evolution of AQ44 appears phylogenetically old (Fig. 1), and an AQ44 homologue has been reported in cyclostomes (78). Elucidation of the role of AQ44 in birds and other nonmammalian animals may shed light on functions of AQ44 other than a water channel (48).

**AQ44 in Mammals and Other Vertebrates.** AQ44 has been cloned independently as a mercury-insensitive water channel by two groups (for review, see Ref. 126) from the rat lung and brain cDNA library and is expressed in the basolateral membrane of the principal cells of the inner medulla CD (117), bronchial epithelia, eye epithelia, and other tissues. AQ44 in the CD is constitutively expressed and is not stimulated by AVP/water deprivation. In Brattleboro rats, in which hypothalamic AVP is genetically absent, long-term administration of desmopressin (dDAVP) increased the AQ44 protein abundance in the inner medulla CD1, connecting tubule, and cortical CD (94). It remains to be determined whether AQ44 mRNA and protein are physiologically regulated in intact vertebrate animals. Madrid et al. (65) identified in epithelial Madin-Darby canine kidney (MDCK) cells two independent COOH-terminal signals that regulate a sorting of AQ44 molecules from the Golgi apparatus to the basolateral membrane, a dileucine-like motif (ETEDLIL), and a tyrosine-based motif (GSYMEV). Mutation of these motifs significantly impairs basolateral targeting of AQ44 (65). The two motifs in the equivalent positions in qAQ44 are ETEDDIL and GKYIEV, respectively. As discussed above, recent evidence suggests that AQ44 may play a role in cell migration (93). AQ44 deletion slows migration of astrocytes in transwell cell culture and in vitro wounds assays (93). The OAP structures are not seen in transgenic mice lacking AQ44, suggesting that the OAP protein is likely to be AQ44 (121).

**Other AQPs in Birds and Other Vertebrates.** In chickens, AQ55 (CAH25504), AQ77 (XM_424498), AQ88 (XM_414866), AQ99 (XM_413787), AQP11 (XP_003640646.1), and AQP12 (NP_001103149.1) were identified and reported in GenBank, but functional information about avian AQPs is not available. In ducks given hypertonic saline for drinking water, the nasal gland AQP1 (endothelial cells) and AQP5 (epithelial cells) are down-regulated, possibly to enhance water retention while preventing dilution of secreted salt (72). AQP5 mRNA and protein have been also detected in intestines from chickens (97), although their function remains to be determined. The presence of immunoreactive AQPs 2, 3, and 9 has been reported in gonadal accessory organs of turkeys (136, 137). In chicken cultured hepatocytes, a high glucose level stimulates AQP8 via the cAMP, phosphatidylinositol 3-kinase (PI3-K)/Akt, PKC, and mitogen-activated protein kinases (MAPKs) pathways (114). Blood glucose levels are high in birds (16).

In mammals, AQP5 is a water channel expressed in the salivary gland trachea, nasopharynx, and airway epithelia, alveolar type 1 cells, ear, eye, placenta, and pancreas (56). AQP6 is a unique intracellular water channel (53) expressed in vesicles of the epithelial cells of the proximal tubules and in acid-secreting intercalated cells of the CD (133, 134) that may be important for the regulation of anion permeability and acid-base balance (133, 134). The gene sequence locus of AQP6 is similar to that of AQP2, suggesting that their evolution may be closely related. AQP7 is permeable to urea, glycerol, water, and arsenite (56) and is expressed in the testis, sperm, kidney (proximal straight tubule at the brush border), adipose tissue, and skeletal muscle. AQPs 3, 7, and 9 are expressed in the plasma membrane of adipocytes. AQP7 and AQP3 may facilitate glucose efflux from adipose tissue, whereas AQP9 may reduce the glycerol influx to hepatocytes (99). These aquaGLPs may be metabolic regulators in human diabetes and obesity (122).

Calamita and coworkers (17) found AQP8 and the strikingly high $P_f$ of the rat liver inner mitochondrial membrane. AQP8-mediated water transport across the mitochondrial and its subcompartments may be particularly important for the rapid mitochondrial volume homeostasis that characterizes the mitochondrial plasticity and cell bioenergetics relevant to cell life-and-death (17, 18). More recently, the function of AQP8 in mitochondria has been investigated, leading to interesting results about the role of AQP8 in the diffusional transport of ammonia (111) and the urea cycle (112). AQP8 also permeates urea and NH$_3$ (56) and is expressed in the testis, sperm, gastrointestinal tract, placenta, kidney (proximal tubule and CD), airways, liver, salivary glands, glial and neuronal cells, and pancreas.

More recently, new members of the AQPs were reported as AQP10, AQP11, and AQP12 (41, 43, 44) that are considered an AQP superfamily. There are two isoforms of human AQP10, a nonfunctional nonsliced variant and authentic AQP10. Authentic AQP10 is an aquaGLP, discovered after AQPs 3, 7, and 9, and expressed intracellularly at the enterochromaffin cells (42). AQP11 has been identified in plants and in nonvertebrate and vertebrate animals (42). AQP11 is poorly permeable to water, and its authentic substrate is not known. AQP11-knockout mice were born normally, but revealed vacuolated proximal tubules at birth and eventually developed polycystic kidneys (70), suggesting a possible role of AQP11 in intravesicular homeostasis (42). These data demonstrate that AQP11 is essential for proximal tubular function. AQP12 is also expressed inside the cell, selectively in acinar cells of the pancreas, and AQP12-mediated water transport regulates vacuoles to swell and expel the vacuolar contents into the pancreatic duct lumens (43).

**Function and Regulation of AQPs in Birds and Other Vertebrates**

**Gene mutation.** Deletion of AQP genes has been used for studying their function in mammals, but there is no such data available in birds. AQP knockout mice are grossly normal in survival and appearance. Given free access to food and water, AQP1 and AQP3 null mice were remarkably polyuric, consuming 2–3 (AQP1) to 7–10 (AQP3) times more fluid compared with wild-type mice (61, 63), whereas AQP4 null mice were not polyuric but manifested only a mild reduction in maximal urine-concentrating ability (62). AQP1 null mice show low plasma osmolality ($P_{osm}$) (50%), low proximal fluid absorption in free-flow microperfusion, and low water flux in isolated perfused tubules (105). It has been speculated that loss of water permeability in the DL and vasa recta reduces the osmotic gradient along the medulla and impairs urine concentration (21, 92). AQP2 mutation, usually hereditary (familial) in humans, causes nephrogenic diabetes insipidus (NDI) (107, 108). NDI patients cannot concentrate urine because of impaired water transport in the CD and thus excrete a large amount of water.
amount of hypotonic urine, leading to thirst, dehydration, and low plasma volume. Most congenital (hereditary) NDI is due to type 2 vasopressin (V2) receptor mutation, and only about 10% is ascribed to mutations of the AQP2 gene (premature termination of translation) (8, 60, 108). While the former is an X-linked disease, the NDI due to AQP2 mutation appears to be autosomal recessive (27) or dominant (71). In contrast, acquired NDI is more common and has multiple causes, including impaired urine-concentrating capacity due to acute or chronic nephron damage or low-protein diet, lithium-related polyuria, urinary tract obstruction, or hypothyroidism (107, 108). Genetic ablation of AQP2 in the connecting tubules (CNTs) of mice suggests that the CNTs play a role in regulating basal body water balance but not in the pathology of lithium-induced nephrogenic DI (55). There are many descriptive studies on up- and downregulation of AQPs in response to changes in environmental conditions (122). Other than in the case of AQP2, however, the biological significance of transcriptional regulation of AQP expression is uncertain (122).

Water homeostasis in changing internal and external environments. It appears that vertebrates have evolved from SW environments, followed by invasion to FW and terrestrial environments. The role of the kidney can be classified in three categories: 1) excretion of excess water as a dilute urine, as exemplified in hyperosmoregulation by FW fish and aquatic amphibians; 2) water conservation in hyperosmotic media by marine fish or in arid environments by certain species of amphibians and reptiles; and 3) conservation of water by urine concentration, as seen in birds and mammals (for review, see Refs. 14, 25, 29, 79, 81). The roles of AQPs in each of the osmoregulation patterns and changing environments are important but not clearly elucidated. AQP3 (64–75% homology to mammalian AQP3) is expressed in the gill chloride cells, intestine, and kidney of FW-adapted and SW-adapted tilapia (124). European eels (silver stage) acclimated to SW for 3 wk, however, showed a 76% reduction in AQP3 in gill extract (23).

Avian kidneys possess looped nephrons that operate countercurrent urine concentration by utilizing Na recycling and AVT-regulated AQP2 (Fig. 5) (84, 85). They lack, however, an efficient vasa recta mechanism to effectively remove the water from the tip of the medullary cone and transport it to the cortex and systemic circulation. Moreover, as the DL of Henle’s loop lacks water permeability (84) and AQP1 (132 and Yang Y, Yamamoto K, Nishimura H; unpublished observations), reaching osmotic equilibrium across the DL epithelium is likely to take longer. In most birds examined, the maximum osmolar urine-to-plasma (U/P) ratio was below 2.5 after dehydration (12). During dehydration or salt loading, the inulin U/P ratio or creatinine U/P ratio increases in reptiles and birds, indicating that renal tubules effectively absorb water (110). In contrast, significant tubular water absorption continues during water loading in desert quail; only 79% of loaded water is excreted, despite the increase in the single nephron glomerular filtration rate and the number of filtering nephrons (13). It is not known whether this slow adaptation occurs because avian kidneys cannot efficiently turn on and off AVT secretion or AVT binding to the receptor, or because AQP2 trafficking and the turnover pathway are not effectively operating for controlling renal tubule water permeability. As mentioned above, increases in cAMP production and in water permeability evoked by AVT in the CD epithelial cells are also smaller in adult quail (85) (also in newborn mammals, 59) than in mature mammals. Thus osmotic control via the ADH-CD AQP2-water permeability axis exists in birds, but the efficiency of the system may be lower than in mature mammals. Furthermore, although AVT stimulation of AQP2 isoforms exists in amphibians, the evidence is not as clear as in birds or mammals. In avian kidneys, the hypsomotic or isosmotic urine that drains from the cortical loopless nephrons to the medullary CDs increases medullary urine volume flow (26). Considering the smaller number of looped nephrons, usually 10–30% (25), and the fact that only a limited number of nephrons in Henle’s loop reach the tip of the medullary cone (15), the looped nephrons of avian kidneys must work efficiently to double the urine osmolality. Furthermore, in birds, the P osm increases considerably during water deprivation; the higher P osm apparently reduces the osmolar U/P ratio (12).

AQP homologues play important roles in amphibians for osmoregulation and during cold acclimation (139) (for review, see Ref. 56). HC-1 (AQP1 equivalent) from Hyla japonica (37) and HC-2 (AQP2 equivalent) show significant water permeability. In contrast, HC-3 has high permeability to glycerol but shows low water permeability, and tissue-specific levels of expression change depending on environmental temperature (139). In addition, GLP serves important roles in glycerol-facilitated cryopreservation as a cryoprotectant for cell preservation without the loss of viability.

AQP2s during development and their roles. In quail metanephric kidneys, the PAS-positive condensation area where nephrogenesis takes place spreads throughout the whole surface area at embryonic day 10–15 (E10–E15) (86). Condensate area remains considerably widespread after birth and gradu-
Coturnix japonica developed a may be important for neonatal PCT (96). may suggest that tubule properties other than AQP1 expression of the PCT is approximately the same as in adults (96). This is lower than in the adult PCT, whereas osmotic permeability luted tubules (PCT) from neonatal rabbits, expression of AQP1 may have a role (52). In the juxtamedullary proximal convo-
lungs in preparation for alveolar gas exchange in which AQP1 regulation (85). At birth, water is rapidly absorbed from the abdominal cavity (99), suggesting that AQP2 may be in part operating without AVT function of the AVT-cAMP axis may be lower than in rats (85). In contrast, diffusional and osmotic water fluxes are considerable in quail, suggesting that AQP2 may be in part operating without AVT regulation (85). At birth, water is rapidly absorbed from the lungs in preparation for alveolar gas exchange in which AQP1 may have a role (52). In the juxtamedullary proximal convoluted tubules (PCT) from neonatal rabbits, expression of AQP1 is lower than in the adult PCT, whereas osmotic permeability of the PCT is approximately the same as in adults (96). This may suggest that tubule properties other than AQP1 expression may be important for neonatal PCT (96).

Diabetes insipidus strain of quail. Minvielle et al. (68) developed a Coturnix japonica strain with clinical signs of diabetes insipidus (DI) by screening for polydipsia and polyuria over 20 generations (manifestation in 100%). This DI quail strain appears to represent homozygous autosomal recessive mutation (clearer polyuria in females) with normal levels of vasotocin in the blood and hypothalamus (68). DI quail kidneys have poorly developed medullary cones with a significantly smaller area per kidney, but no significant difference in the number of glomeruli between control and DI quail in either superficial or deeper zones (131 and Nishimura H, Minvielle F, Yang Y, Yamamoto T; unpublished observations). Glomeruli in the deeper area are significantly smaller in DI quail than in normal quail. The DI and CT gene and amino acid sequences are nearly identical, including two NPA motifs, a mercury-sensitive structure, and a putative phosphorylation site. Using the same qAQP fragment riboprobe, we examined ISH of mRNA in the kidneys of DI and control quail. In the normally hydrated state, DI quail show much lower levels of qAQP2 mRNA signals. qAQP mRNA levels were enhanced after water deprivation. Interestingly, the plasma osmolality (measured by freezing-point depression) in normally hydrated DI quail is significantly lower than in normally hydrated normal quail, suggesting hemodilution. Hematocrit in the normally hydrated DI quail was significantly lower than in control quail, supporting the Posm results. Thus it appears possible that in this strain of quail, the mechanism of DI is neither a shortage of AVT in the hypothalamus nor defective renal tubules. Rather, they may have genetically decreased sensitivity to AVT release as a function of Posm, resulting in volume expansion. Accordingly, the role of the renal medulla in reabsorbing water is less important, leading to underdevelopment of the renal medulla and to decreased qAQP2 mRNA and qAQP2 protein.

Perspectives and Significance

AQPs are phylogenetically old molecules. As of today, 13 distinct AQPs/aquaGLPs and numerous isoforms have been found in vertebrates. It is not clear whether they have evolved from the same ancestral molecule or whether a variety of molecules are based on divergent evolution. AQP/aquaGLP molecules and their
functions must have been selected for survival during the phylogenetic advancement of vertebrates and their adaptation to changing environments. The AQP1 isoform is a prototype of the AQP molecule and is widely found in nonmammalian and mammalian tissues. In birds, AQP1 is presumably located along the proximal tubules, but evidence for mRNA (by ISH) remains unclear. AQP2 and isoforms are present in lower vertebrates, but ADH-induced stimulation of AQP2 mRNA and protein has been clearly shown only in mammals and birds. Furthermore, the role of AQP2 in urine concentration only becomes evident with the architectural structure development of the renal medulla. In mammals, AQP3 and AQP4 are renal AQPs constitutively expressed in the basolateral membrane of CD epithelial cells and are responsible for transport of water taken via AQP2 expressed on the luminal membrane. In avian kidneys, AQP3 is expressed along cortical and medullary CDs, whereas the presence of AQP4 appears unlikely in CDs. Thus the role of AQP3 in water transport in renal tubules appears to be evolutionarily older than that of AQP4.

AQP3 is a phylogenetically old molecule and is expressed in gills, gastrointestinal organs, and kidneys of teleost fish (24), suggesting its role in osmoregulatory organs. Although vertebrate AQP3 amino acid sequences are on average reasonably well conserved, the level of conservation shows considerable heterogeneity (11, 23). In chickens, sequences of AQP3s 5, 7, 8, 9, 11, and 12 have been identified, but their physiological roles remain to be determined. AQP8 and isoforms were identified in teleost medullary CDs, whereas the presence of AQP4 appears unlikely in CDs. Thus the role of AQP3 in water transport in renal tubules appears to be evolutionarily older than that of AQP4.

Table 1. Amino acid conservation at sites determinative of quail AQPs versus rat APQs

<table>
<thead>
<tr>
<th>AQP</th>
<th>Position and Location</th>
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<tbody>
<tr>
<td>qAQP1</td>
<td>P1, Transmembrane Helix 3: Thr (nonaromatic); P2, Extracellular Loop E: Ser (small and uncharged); P3, Extracellular Loop E: Ala (small and uncharged); P4, Transmembrane Helix 6: Phe (aromatic); P5, Transmembrane Helix 6: Trp (aromatic)</td>
</tr>
<tr>
<td>ckAQP1</td>
<td>P1, Transmembrane Helix 3: Thr; P2, Extracellular Loop E: Ser; P3, Extracellular Loop E: Ala; P4, Transmembrane Helix 6: Phe; P5, Transmembrane Helix 6: Trp</td>
</tr>
<tr>
<td>rAQP1</td>
<td>P1, Transmembrane Helix 3: Thr; P2, Extracellular Loop E: Ser; P3, Extracellular Loop E: Ala; P4, Transmembrane Helix 6: Phe; P5, Transmembrane Helix 6: Trp</td>
</tr>
<tr>
<td>qAQP2</td>
<td>P1, Transmembrane Helix 3: Thr; P2, Extracellular Loop E: Ser; P3, Extracellular Loop E: Ala; P4, Transmembrane Helix 6: Phe; P5, Transmembrane Helix 6: Trp</td>
</tr>
<tr>
<td>ckAQP2</td>
<td>P1, Transmembrane Helix 3: Thr; P2, Extracellular Loop E: Ser; P3, Extracellular Loop E: Ala; P4, Transmembrane Helix 6: Phe; P5, Transmembrane Helix 6: Trp</td>
</tr>
<tr>
<td>rAQP2</td>
<td>P1, Transmembrane Helix 3: Thr; P2, Extracellular Loop E: Ser; P3, Extracellular Loop E: Ala; P4, Transmembrane Helix 6: Phe; P5, Transmembrane Helix 6: Trp</td>
</tr>
<tr>
<td>qAQP4</td>
<td>P1, Transmembrane Helix 3: Thr; P2, Extracellular Loop E: Ser; P3, Extracellular Loop E: Ala; P4, Transmembrane Helix 6: Phe; P5, Transmembrane Helix 6: Trp</td>
</tr>
<tr>
<td>ckAQP4</td>
<td>P1, Transmembrane Helix 3: Thr; P2, Extracellular Loop E: Ser; P3, Extracellular Loop E: Ala; P4, Transmembrane Helix 6: Phe; P5, Transmembrane Helix 6: Trp</td>
</tr>
<tr>
<td>rAQP4</td>
<td>P1, Transmembrane Helix 3: Thr; P2, Extracellular Loop E: Ser; P3, Extracellular Loop E: Ala; P4, Transmembrane Helix 6: Phe; P5, Transmembrane Helix 6: Trp</td>
</tr>
<tr>
<td>qAQP3</td>
<td>P1, Transmembrane Helix 3: Thr; P2, Extracellular Loop E: Ser; P3, Extracellular Loop E: Ala; P4, Transmembrane Helix 6: Phe; P5, Transmembrane Helix 6: Trp</td>
</tr>
<tr>
<td>ckAQP3</td>
<td>P1, Transmembrane Helix 3: Thr; P2, Extracellular Loop E: Ser; P3, Extracellular Loop E: Ala; P4, Transmembrane Helix 6: Phe; P5, Transmembrane Helix 6: Trp</td>
</tr>
<tr>
<td>rAQP3</td>
<td>P1, Transmembrane Helix 3: Thr; P2, Extracellular Loop E: Ser; P3, Extracellular Loop E: Ala; P4, Transmembrane Helix 6: Phe; P5, Transmembrane Helix 6: Trp</td>
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Five amino acid positions (P1–P5) located in transmembrane helix 3 (P1), extracellular loop E (P2, P3), and transmembrane helix 6 (P4, P5) are conserved in both mammals and birds; however, they differ between the aquaporin (AQP) and glyceroporin (GLP) subfamilies. The five positions were determined by multiple alignment (Clustal X2 program), matching the position with Froger’s method (32).

Water handling by the kidney is essential for both aquatic and terrestrial life for maintaining body fluid osmolality and volume homeostasis; but the role of the kidney differs, depending on habitats and the phylogenetic stage of the vertebrates. The factors that make kidney functions diverse are the following: 1) architectural organization, such as increases in complexity in nephron segments, development of the vasa recta, and the interaction of vasa recta with tubules, such as those in the countercurrent exchange mechanism, thereby increasing the efficiency of ion and water circulation; 2) intrarenal and systemic regulatory systems involving hemodynamic, humoral, and neural mechanisms; and 3) communication between preglomerular/glomerular structures and tubules, such as glomerulotubular balance or tubuloglomerular feedback and the integration of systemic and intrarenal structures by autoregulation make the entire fluid-ion balance more complex. The roles of AQP2 and AVT in urine concentration depend on architectural development of the renal medulla. It is therefore very important to investigate the role of the kidney in body fluid homeostasis from both aspects: elucidation of cellular/molecular mechanisms and integration at the in vivo level as to how the various systems work in concert.

Furthermore, many structural and functional similarities of the renal medulla exist between birds and neonatal mammals (54, 59, 80). First, both lack the thin ascending limb (AL) of Henle, and the entire AL shows characteristics of diluting segments. During maturation of rat kidneys, apoptosis occurs in the lower segment of the AL, where the Na⁺-K⁺-2Cl⁻ cotransporter is replaced by a thin AL-specific Cl channel. Second, the DLs from quail and neonatal rats show low water permeability; AQP1 expression is absent in DLs from neonatal rat and quail kidneys. In contrast, both show high Na and Cl permeability, suggesting that an increase in the osmotic concentration of tubular urine in the DL of Henle is likely to occur by addition of solute, not by subtraction of water. Third, AQP2...
is present in the CDs of both neonatal rat and quail kidneys, but the AQP2 responses to rat vasopressin and quail AVT are only modest (59, 85). Fourth, the CDs of neonatal rat (and presumably quail) kidneys lack urea permeability and urea transporters (UT), whereas UTA1 mRNA is expressed in mature rat kidneys (59). Thus the contribution of urea to the urine-concentrating mechanism is unlikely in neonatal rats, supporting the fact that urine-concentrating abilities are low in neonatal mammals, including humans. It is thus possible that the ancestral vertebrates from which the mammalian and reptile-bird line evolved may have had primitive urine-concentrating mechanisms and AQP function.

Furthermore, recent evidence indicates that AQPs show functions other than water channel or glycerol transport. Comparative analysis of AQPs in primitive animals may provide useful information as to the fundamental function. The importance of AQP2 analysis of AQPs in primitive animals may provide useful information to understand possible mechanisms of developmental programming in adults.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: H.N. conception and design of research; H.N. and Y.Y. performed experiments; H.N. and Y.Y. analyzed data; H.N. interpreted results of experiments; H.N. and Y.Y. prepared figures; H.N. drafted manuscript; H.N. edited and revised manuscript; H.N. approved final version of manuscript.

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