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The evolutionary aspects of aquaporin family

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Ishibashi K, Kondo S, Hara S, Morishita Y. The evolutionary aspects of aquaporin family. *Am J Physiol Regul Integr Comp Physiol* 300: R566–R576, 2011. First published December 9, 2010; doi:10.1152/ajpregu.90464.2008.—Aquaporins (AQPs) were originally identified as channels facilitating water transport across the plasma membrane. They have a pair of highly conserved signature sequences, asparagine-proline-alanine (NPA) boxes, to form a pore. However, some have little conserved amino acid sequences around the NPA boxes unclassifiable to two previous AQP subfamilies, classical AQPs and aquaglyceroporins. These will be called unorthodox AQPs in this review. Interestingly, these unorthodox AQPs have a highly conserved cysteine residue downstream of the second NPA box. AQPs also have a diversity of functions: some related to water transport such as fluid secretion, fluid absorption, and cell volume regulation, and the others not directly related to water transport such as cell adhesion, cell migration, cell proliferation, and cell differentiation. Some AQPs even permeate nonionic small molecules, ions, metals, and possibly gasses. AQP gene disruption studies have revealed their physiological roles: water transport in the kidney and exocrine glands, glycerol transport in fat metabolism and in skin moisture, and nutrient uptakes in plants. Furthermore, AQPs are also present at intracellular organelles, including tonoplasts, mitochondria, and the endoplasmic reticulum. This review focuses on the evolutionary aspects of AQPs from bacteria to humans in view of the structural and functional diversities of AQPs.

water channel; classical aquaporin; aquaglyceroporin; unorthodox aquaporin; evolution

THE DISCOVERY OF A SPECIALIZED water-permeating channel, aquaporin (AQP), has opened a new field of biological research (60). Over the past 15–20 years, there has been great progress in the understanding of the diverse roles of AQPs in our bodies in health and disease as well as in other organisms. AQPs have evolved mainly to facilitate permeation of small molecules, including water, solutes, and possibly gasses, and regulate several cell functions, including osmolarity, energy metabolism, migration, adhesion, proliferation, and differentiation (1, 9, 30, 31, 43, 95, 114, 116). Intracellular water and solute transports between organelles may also be regulated by AQPs (21, 25, 67, 84, 86). Accordingly, AQPs play an important role in physiology and pathophysiology of our bodies such as osmoregulation, lipid metabolism, organogenesis and regeneration, and vascular and cancer biologies (1, 18, 19, 29, 74, 116). Because no specific AQP inhibitors are yet available, AQP knockout mice have revealed their physiological significance of AQPs. Surprisingly, relatively minor phenotypes have been observed in most AQP null mice (114), suggesting that unidentified compensatory mechanisms may be present (128).

Recent microarray techniques have enabled us to examine gene expression systematically in cells and organs expressing different arrays of genes under some stresses or diseases (4). Unexpected expression of AQPs has been identified in such studies. The following are a list of such studies using microarray technique, in which changes of AQP expression are identified. Expression profiling analyses in detached retina revealed AQP0 expression, which was regarded as lens specific (20), suggesting its possible role in the retina. Gene expression profiles in three different mouse models of experimental colitis revealed a downregulation of AQP8 (109). Vitamin D administration to vitamin D-deficient rats also decreased AQP8 expression in duodenal mucosa (62). AQP10 was downregulated in the small intestine of cholera patients (23). AQP5 was activated soon after the administration of estrogen in mouse uteri (61). The administration of a thiazolidinedione used for the treatment of type 2 diabetes increased AQP9 expression in rat white adipose tissues (100).

Although their importance is unclear in the above studies, all may well be a clue to identify new roles of AQPs. In fact, new substrates for AQPs were identified in plants with such studies. Under a boron-limited condition, an AQP was identified as an upregulated gene in the root, which functions as an influx channel for boron (104). Another plant AQP was found through chromosomal mapping as a gene responsible for an inherited disease in rice with silicon deficiency (78). Such reverse genetic approaches will lead to the discoveries of new and sometimes unexpected functions of AQPs.

Structurally, AQPs have six transmembrane domains with the NH_2- and COOH-termini in the cytoplasm. The pore is

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made of two highly conserved short hydrophobic stretches of amino acid residues named asparagine-proline-alanine (NPA) boxes, which are the signature sequences for AQPs. With the progress of the genome projects, more and more AQP-like sequences have been identified on the basis of amino acid sequence similarities, especially around NPA boxes. They may belong to the AQP family although most of them have not yet been functionally characterized. Some have new primary structures with extra residues at the NH_2- and/or COOH-termini (33, 126) or an alternative splicing (81).

Three-dimensional (3D) structural analyses of AQPs have revealed a uniform structure: a tetramer with a pore in each subunit (19, 22, 117, 122). Some AQPs have a larger pore to permeate glycerol and possibly urea (39). Such structural studies may predict permeating substances and gating mechanisms by phosphorylation or interaction with NH_2-terminal residues (22). These 3D structural studies have also provided an unexpected putative function, cell adhesion by forming a junction between the plasma membrane by interacting with an opposing AQP molecule (99). Such a nontransporting function of AQPs has increasingly been identified (19, 37, 106). The physiological significance of this function, however, awaits further confirmation (129).

This review focuses on the possible evolutionary aspects of the AQP family. To this end, the diversities of AQPs in structure, function, and subcellular localization will first be overviewed.

A Diversity of AQP Structures

The AQP family was divided into two subfamilies based on the distinct primary sequences: classical AQPs and aquaglyceroporins (1, 12, 45). Moreover, both seem to have specific functions: water channels and glycerol channels, respectively. The signature sequence for aquaglyceroporins will be the aspartic acid residue (D) in the second NPA box, which expands the pore to accept larger molecules such as glycerol (39), while its absence indicates a classical AQP (Fig. 1).

Genome projects, however, revealed the presence of AQP-like sequences without apparent NPA boxes highly conserved in most AQPs (14, 42, 46). In plants, such AQPs are named SIPs (short intrinsic basic proteins). Recently, another such subfamily is identified in trees and named XIPs (uncategorized X intrinsic proteins), which are prevalent in fungi and plants, excluding monocots (14). Although their NPA boxes are deviated, their overall sequences are similar to classical AQP, especially around the first NPA box (Fig. 1). Interestingly, they have a highly conserved cysteine residue at the second NPA box, NPARC (Fig. 1). This cysteine residue will be a signature sequence for XIPs. The first NPA is more deviated although its upstream sequence is ISGGH, which is conserved in many AQPs. Thus both SIPs and XIPs will belong to classical AQPs. XIPs are also present in *Dictyostelium discoideum* with highly deviated second NPA boxes, NIA and NMA (Fig. 1). Currently, XIPs seem to be absent in animals.

AQP-like sequences with deviated NPA boxes are also found in multicellular animals. The amino acid sequences upstream of the first NPA box are completely different from other AQPs. A phylogenetic tree in Fig. 2 indicates that they are distantly related members beyond SIP. Despite their lower homology with each other, they have a highly conserved cysteine residue at the downstream of the second NPA box, NPAxxxxxxxxC (Fig. 1). This cysteine residue will be a signature sequence for this subfamily, which will be functionally indispensable as its disruption in AQP11 gives a similar phenotype with AQP11 null mice (107). In fact, this cysteine residue is also found in a fungi, *Ustilago maydis*, and bacteria, *Chlorobium tepidum* and *parvum* (Fig. 1). However, their upstream sequences of the first NPA are highly similar to AQP1 and thus they are phylogenetically included as members of an aquaglyceriporin and classical AQPs, respectively. This third subfamily was previously referred to as "unorthodox AQPs" focusing on deviated NPA itself and unconventional functions, thus AQP6 and AQP8 are also included (95). In this review, however, we use this name to indicate the primary structure only, i.e., highly deviated subgroups of AQP-like sequences with a conserved cysteine residue as indicated in the phylogenetic tree beyond SIP (Fig. 2). The above half will be orthodox AQPs as used in the classification of zebrafish AQPs (112). Although all unorthodox AQPs currently have a conserved cysteine, the identification of any deviated AQPs without this cysteine will make further subdivision of unorthodox AQPs necessary. Because unorthodox AQPs are highly deviated from both classical AQPs and aquaglyceroporins, it is difficult to construct a simulation model based on the known 3D structures of AQPs. Therefore, 3D structure analyses of unorthodox AQPs are necessary to understand the molecular and the pore structures of this family. The distributions of three families of AQPs from bacteria to humans are summarized in Table 1.

A Diversity of AQP Functions

Several critical residues for permeable substrates through AQPs have been identified by mutagenesis and 3D analyses. However, there still are many controversies on the specific function of each AQP. As shown in Table 1, there are only classical AQPs [plasma membrane intrinsic protein (PIP), tonoplast intrinsic protein (TIP), nodulin-26-like intrinsic protein (NIP), short intrinsic basic proteins (SIP), and XIP] in plants; however, the functions of plant AQPs seem to overlap with those of animal AQPs. For example, the absence of aquaglyceroporins in plants is compensated by the extended functions of NIPs. The absence of unorthodox AQPs might be compensated by SIPs or XIPs.

Functions of classical AQPs. The importance of water transport through classical AQPs is well documented in plants and animals. The absence of PIPs in roots, for example, inhibits plant growth by limiting water absorption, leading to a compensatory root growth (77). The absence of AQP2 in mice and humans is a cause of diabetes insipidus (114). The absence of AQP5 decreases salivary gland secretion in mice (114). These results suggest that the role of classical AQPs is mainly water transport. Milder phenotypes in the absence of classical AQPs in mice and humans may indicate the presence of compensatory water transport by solute transporters such as urea transporters (128).

Similarly, water transport through classical AQPs is important in single-cell organisms for the adaptation to a rapidly changing environment by facilitating water transport (105). Aqy1, a classical AQP in yeast, *Pichia pastoris*, was shown to have a gating mechanism that is important for forming colonies

Sequence alignments of AQPs at the first and the second NPA boxes

	First NPA boxes	Second NPA boxes
Classical AQPs		
AQPZ	-VGHISGGHFNPAVTIGLWAG-	-SIPVTNTSVNPARSTAVAIFQG-
Meth	-FGRISGCHINPAVTIALFAT-	-IGNLTGASLNPARTFGPYLGDW-
Chl.T	-MGTVSGAHLNPAVTLAFAMR-	-AAPVSGASMNPVRSLAPALV**C**G-
Chl.P	-MGTVSGAHLNPAVTIAFAMR-	-AAPISGASMNPVRSLAPALV**C**G-
Cript	-FFRVSGGLFNPAVSLGMVLA-	-GVPYSGGALNPVRSLGPAVVTH-
Tryp1	-FGYISGGHFNPAVTMAVFLV-	-VGRISGGAFNPAAATGLQLALC-
Tryp2	-FGYISGAHFNPAITFATFIN-	-VGGFTGGAFNPAVATGTQLVGC-
Leish	-FGYISSSHFNPAVSIAVFLV-	-AGRISGGAFNPAAASGLQVAMC-
D.disA	-VSGVSGCNLNPAVTLANLLS-	-GFNFSGGALNPVRVLGPSIISG-
D.disB	-ISGISGCQLNPAVTVGCVTT-	-LNLFTGGSLNPARSFGPAVFSD-
D.disC	-FADVSGAHFNPAVTFATCVT-	-GGSVSGGAFNPARVFGTALVGN-
D.disD	-CAPVSGGHLNPSITLATFFA-	-IAPNYIFGFNIAR**C**LSPAIVLS-
D.disE	-CAPVSGGHLNPSITIATFFS-	-ISPNYIFGFNMAR**C**LCPAIVTG-
XIP1.1	-APATSGGHVNPCITWTEMLT-	-FSGYGGAGINPGR**C**IGPAVVLG-
TIP1.1	-GANISGGHVNPAVTFGAFIG-	-GGAFSGASMNPAVAFGPAVVSW-
PIP2.6	-TAGISGGHINPAVTFGLFLA-	-TIPITGTGINPARSFGAAVIYN-
NIP1.2	-LGHISGAHFNPAVTIAFASC-	-AGPVSGASMNPGRSLGPAMVYS-
SIP1.1	-TVIFGSASFNPTGSAAFYVA-	-GSKYTGPAMNPAIAFGWAYMYS-
AQP1	-VGHISGAHLNPAVTLGLLLS-	-AIDYTGCGINPARSFGSAVLTR-
AQP8	-LGNISGGHFNPAVSLAVTVI-	-GGSISGACMNPARAFGPAVMAG-
Aquaglyceroporins		
GlpF	-TAGVSGAHLNPAVTIALWLF-	-MGPLTGFAMNPAR**D**FGPKVFAW-
Entero	-LFVFGGVCINPAMALAQAIL-	-LGGTTGFAMNQAR**D**LGPRIAYQ-
Ustil	-CATTSGTQFHPAFTIAQVVF-	-CFSSSNVVANSAR**D**IGARLV**C**S-
P.viv	-AAKLSGAHLNLAVTVGFATI-	-FGGNTGFALNPSR**D**LGARLLSL-
GIP	-VGHISG-FFNPAVALAAAVV-	-GGGMTGPALNPAR**D**LGPALVSG-
AQP3	-AGQVSGAHLNPAVTFAMCFL-	-MGFNSGYAVNPAR**D**FGPRLFTA-
Unorthodox AQPs		
CeAQP9	-IEFQRDAVAHPCPLVTNCYR-	-GINYTGMYANPIVAWACTFN**C**L-
CeAQP10	-NIFNRGAMTNCAPIFEQFVF-	-LYVVGVPGLNPIVATARLYG**C**R-
CeAQP11	-ALCNRTAFCSPLAPIEQYLF-	-VTFVGDQALDPLVASTLFFG**C**R-
Dros	-GRVWGDASACPYTHMEDVVE-	-AFNFSGGYFNPVLATALKWG**C**R-
Urch1	-LTFDGDSTANTCMIWQSMLK-	-GLEWTGMMFNPALAAGITLN**C**G-
Urch2	-NEELSNAGDAPLGQAVQVQP-	-GLEYTGAPMNPILGFASGWG**C**K-
ZF1	-GFSFRGAICNPTGALELLSR-	-GGRLTGAVFNPALAFSIQFP**C**P-
ZF2	-TAVMQDVSGNPAVTLLRLLQ-	-ANNYTSGYVNPALAYAVTLT**C**P-
Xeno	-GFTFNKASGNSAVSLQDFLL-	-AGSYTGAFFNPTLAAALTFQ**C**S-
Chic11	-GLTLPGSTCNPCGTLQPLWG-	-GGNLTGAIFNPALAFSLHPH**C**F-
Chic12	-AACANGAASNPTVSLQEFLL-	-AAPATGAFFNPALATASTFL**C**A-
AQP11	-GLTLVGTSSNPCGVMMQMML-	-GGSLTGAVFNPALALSLHFM**C**F-
AQP12	-GVTLDGASANPTVSLQEFLM-	-AGPFTSAFFNPALAASVTFA**C**S-

Fig. 1. Sequence alignments of aquaporins (AQPs) at the first and second asparagine-proline-alanine (NPA) boxes. Highly conserved NPAs are underlined. The aspartic acid (D in bold print) in the second NPA box will be a signature residue for aquaglyceroporins. The second NPA box has a conserved cysteine (C in bold print) in unorthodox AQPs: NPA (L/V/A/I)AXXXXXXC. This cysteine will be a signature residue for unorthodox AQPs. AQPZ, GlpF, *Escherichia coli* (NP_415396, NP_418362); Entero, *Enterococcus faecalis* V583 (Gene ID: 1200713); Ustil, *Ustilago maydis* 521 (XP_758316.1); Meth, *Methanoculleus marisnigri* JR1 (Gene ID: 4846532); Chl.T, *Chlorobium tepidum* (NP_662357.1); Chl.P, *Chlorobium parvum* (YP_001999162.1); Cripto, *Cryptococcus neoformans* var. neoformans JEC21 (Gene ID: 4935143); Tryp1/2, *Trypanosoma cruzi* (XP_815990, AF31269.1); Leish, *Leishmania major* (CAJ08765.1); D.disA, B, C, D, E, *Dictyostelium discoideum* (Gene ID: 3398231, 3392160, 3392764, 3395408, 3387173, 3391439); XIP1.1, uncategorized X intrinsic protein: *Physcomitrella patens* (XP_001758094.1); TIP1.1, tonoplast intrinsic protein; PIP2.6, plasma membrane intrinsic protein; NIP1.2, nodulin-26-like intrinsic proteins; SIP1.1, short basic intrinsic protein: *Arabidopsos thaliana* (P25818, Q9ZV07, Q8LFP7, Q9M8W5); GIP, *P. patens* (AY611236); AQP1/3/8: *Mus musculus* (NP_031498, NP_057898, NP_031500); CeAQPs, *Caenorhabditis elegans* (NP_001021552.1, NP_496105.1, NP_499821.2); Dros, *Drosophila melanogaster* (AAF58409.2); Urch1/2 sea urchin, *Strongylocentrotus purpuratus* (XP_780933.1, XP_787329.1); ZF1/2, zebrafish: *Danio rerio* (AAH95775.1, AAH95564.1); Xeno, *Xenopus laevis* (AAH82904.1); Chic11/12, *Gallus gallus* (XP_424343.1, NP_001030011.1); AQP11/12, *Mus musculus* (NP_780314, NP_808255).

following multiple freezing/thaw cycles (22). This hypothesis, however, is controversial in that the absence of AqpZ, a classical AQP in bacteria, decreases the colony size (13) or does not decrease (103), and the two classical AQPs in yeast, *Saccharomyces cerevisiae,* are dysfunctional with deletion or absence at the plasma membrane (63).

Water transport may also regulate cell functions such as cell migration and proliferation through changing the cell volume. For example, AQP1 is expressed in the endothelium of tumor microcapillaries to enhance a rapid vascular proliferation in growing tumors (116). The expression of AQPs was inducible in some tumors (18) and diseases (98), which will be applied to anti-angiogenesis therapy for cancer (116). AQP1 has also been shown to play an important role in chondrocyte migration and adhesion (69, 80). The cell undergoing apoptosis decreases its size due to a rapid water movement out of the cell (51). Accordingly, hepatocellular carcinoma cells with low AQP8 expression have decreased resistance against apoptotic stimuli (50). The result suggests that the induction of AQP expression will stimulate cancer cell death by apoptosis, which also can be applicable to cancer therapy.

Interestingly, classical AQPs also transport other substrates than water. NIPs in plants transport nutrients such as silicon and boron (78, 104). AQP6 transports anions such as nitrate as well as water at least in in vitro conditions (40). AQP8 also transports ammonia and free radicals such as H_2O_2 (10), as is the case with TIPs in plants (16). Big Brain (bib) of *Drosophila* was reported to be a cation channel (126) or not (106) without

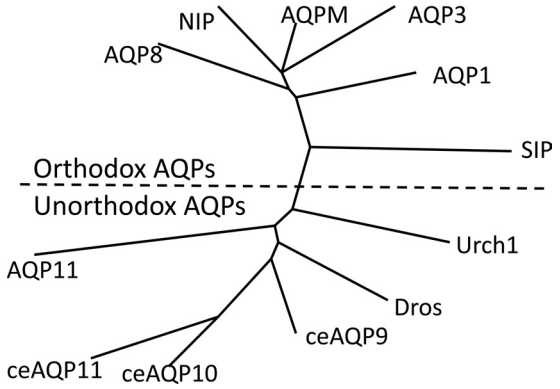

Fig. 2. Phylogenetic tree of AQPs. The tree is based on COBALT multiple alignment with a maximum sequence difference of 0.9 at http://www.ncbi.nlm.nih.gov/blast/treeview/treeView.cgi (Cobalt RID C7EAU3PF212). Classical AQPs: AQP1, AQP8, AQPM, NIP, SIP; Aquaglyceroporin: AQP3; Unorthodox AQPs: AQP11, ceAQP9, ceAQP10, ceAQP11, Dros, Urch1. Abbreviations are as in Fig. 1.

water channel activity, regulating endosomal pH (55) or mediating the cell adhesion (106).

Some biological membranes have low permeabilities to ammonia and CO_2 gasses such as the plasma membrane of gastric parietal cells, cells in kidney thick ascending limbs of Henle's loop, colonic epithelia, and *Xenopus* oocytes (119). Because the presence of unstirred layer may mask such gas transports, a study setting pH microelectrodes at the surface of *Xenopus* oocytes was conducted (85), in which the expression of AQP1, -4, or -5 activated CO_2 and NH_3 transports. Therefore, some specialized pathways for ammonia and CO_2 gasses must be present in the plasma membrane. In fact, CO_2 gas permeation through classical AQPs in plants was demonstrated (108), and tetramer composition studies further indicated that CO_2-related transport processes in tobacco AQPs are different from that of water where monomer is a functional unit (88). The CO_2 permeability of AQP1 in erythrocytes, however, seems to be small, since no significant phenotypes were observed in AQP1 null mice and humans (114) with minimal compensation by other CO_2 transporters such as RhAG (94). Another gas, nitrate oxide at vascular endothelia (34) and germinating seeds (70), and O_2 in the lung (17) may also be transported through classical AQPs. Obviously, further studies are required to prove their physiological importance.

Functions of aquaglyceroporins. Aquaglyceroporins seem to function primarily as a glycerol channel rather than a water channel. In fact, its prototypic glycerol channel, GlpF in *Escherichia coli*, transports little water, and the water permeability of aquaglyceroporins is generally lower than that of classical AQPs. The water transport through aquaglyceroporins is still important as revealed by polyuria in AQP3 null mice (114) and high expression of AQP9 in male reproductive organs (89). Interestingly, aquaglyceroporins also play a crucial role in metalloid homeostasis by transporting antimonite and arsenite (11), which may be transported in ionic forms, although no ions have been shown to permeate aquaglyceroporins. Surprisingly, AQP9 transports much larger substrates such as lactate, purine, and pyrimidine (113), and a 3D structure analysis of AQP9 suggested a larger pore size (117).

Table 1. *The distribution of aquaporins*

Organisms	Classial AQPs	Aquaglyceroporins	Unorthodox AQPs
Bacteria			
Escherichia coli	1	1	
Haemophilus influenzae	1	1	
Pseudomonas aeruginosa		1	
Salmonella typhimurium	1		
Methanothermobacter marburgensis	1		
Fungi			
Saccharomyces cerevisiae	2	2	
Aspergillus nidulans	1	4	
Ustilago mydis	2	3	
Magnaporthe grisea	3	1	
Protozoa			
Leishmania major	4	1	
Trypanosoma cruzi	4		
Trypanosoma brucei		3	
Toxoplasma gondii	1		
Plasmodium falciparum		1	
Dictyostelium discoideum	5		
Nematode			
Caenorhabditis elegans	3	5	3
Plants*			
Arabidopsos thaliana	35 (13, 10, 9, 3, 0)		
Populus trichocarpa	55 (15, 17, 11, 6, 6)		
Insect			
Drosophila melenogaster	7		1
Vertebrates			
Danio rerio	8	7	2
Gallus gallus	5	3	2
Homo sapiens	7	4	2

Nos. represent the no. of genes in each organism. *In plants, classical aquaporine (AQPs) are further divided into five subfamilies: plasma membrane intrinsic protein, tonoplast intrinsic protein, nodulin-26-like intrinsic protein, short intrinsic protein, and uncategorized X intrinsic protein.

Aquaglyceroporins also play a role in osmoregulation through the transport of glycerol. When suddenly exposed to a hypotonic environment, Fps1p in *S. cerevisiae* is important for the excretion of intracellular glycerol accumulated during hypertonic adaptation. It is also important for yeast mating, since its absence disturbed the cytosolic osmotic balance, leading to a decrease in mating efficiency (91), although such was not observed in *Schizosaccharomyces pombe*, which has a single glycerol channel homolog with no facilitated glycerol transport (58). In mammals, the osteoclast differentiation is accompanied by an increase in cell volume by forming multinucleated osteoclasts from mononuclear precursors in which higher AQP9 mRNA levels were observed in the osteoclast differentiation, specifically at the fusion process. Moreover, a nonspecific AQP9 inhibitor, phloretin, dramatically reduced the osteoclast size and the number of nuclei per osteoclast, suggesting the role of AQP9 in the cell fusion (3). A recent report using AQP9 null mice, however, showed an absence of bone phenotypes (72).

Recently, aquaglyceroporins of both malarial parasites and host erythrocytes have been shown to be important for the absorption of nutrients. Glycerol in particular is an important substrate for lipid biosynthesis of malarial parasites in erythrocytes. AQP9 null mice with decreased glycerol transport at the plasma membrane of erythrocytes survived longer after the infection with *Plasmodium berghei* due to unfavorable growth of malaria parasites (71, 92). Similar effect will be expected in humans whose erythrocytes express AQP3 instead of AQP9 (97), although a recent study on AQP3 polymorphisms in the people tolerant to malaria revealed no genetic biases for the AQP3 gene (5).

Because glycerol is produced by fat degradation and used for gluconeogenesis, its transport will also modulate fat and glucose metabolisms. AQP7 may play a major role (36, 121) that is highly expressed in adipose tissues (35) or at the capillary endothelial cell of adipose tissues (101). In fact, AQP7 null mice became profoundly hypoglycemic during prolonged fasting due to impaired glycerol supply to the liver from the adipose tissue. Larger adipocytes were also noted, and the amount of intra-abdominal fat was increased in AQP7 null adult mice, which was only noticed after three months without obvious body weight changes (29, 35), which, however, has not been observed in other AQP7 null mice with smaller pancreatic islet cells and enhanced insulin secretion (76). Because AQP7 is also expressed at the brush-border membrane of the proximal tubule, AQP7 null mice lost glycerol in the urine (102), although there were no changes in blood glycerol levels and growth rates. Single nucleotide polymorphisms in AQP7 have been associated with obesity and type 2 diabetes (74). AQP9 may also function as a glycerol channel to facilitate glycerol uptake in the liver. AQP9 null mice, however, had no apparent abnormalities with a slight increase of blood glycerol (96). Because oral glycerol intake similarly increased blood glucose in normal and AQP9 null mice, the absence of AQP9 may be compensated by other glycerol transporters that are functionally known but not molecularly identified in the liver (118). Because mice depend more on glycerol metabolism than humans, care must be taken in applying mouse data of AQP7 and AQP9 to humans.

Surprisingly, AQP3 has recently been shown to be important for skin regeneration and tumor progression (31). Initially, it was thought that the moisture of the skin was maintained by AQP3, which facilitates water transport across the plasma membrane at the basal layer of keratinocytes (15). Further studies, however, revealed that the glycerol transport seems to be more important than water transport, since oral glycerol feeding reversed the dry skin caused by AQP3 defect (30). The transport of glycerol through AQP3 has been shown to be critical for cell growth in skin repair and tumor growth in the skin presumably because glycerol is a fuel for cell proliferation (116). Thus, overexposure to glycerol or increased AQP3 expression in the skin may stimulate the growth of basal skin cancer cells (115). Because the recovery from colitis in AQP3 null mice was facilitated by oral administration of glycerol (111), the AQP3 expression in colonic epithelia may also be important for the growth. If it is the case, the overexpression of AQP3 in colon cancer cells may stimulate the tumor growth. Obviously, these issues should be critically examined in humans.

Functions of unorthodox AQPs. AQP11 was not regarded as an AQP because of its deviated NPA boxes and originally named AQPX1 (41). Moreover, water and glycerol channel activities were absent in the *Xenopus* oocytes expressing AQP11 (26, 112) or difficult to study for its poor expression at the plasma membrane (47, 83). However, a recent reconstruction vesicle study has clearly shown that AQP11 is indeed a water channel that transports water as efficient as AQP1 (123). Therefore, AQP11 is definitely a member of the AQP family.

AQP11 is widely expressed in many cells such as proximal tubular cells, hepatocytes, intestinal epithelial cells, hippocampus, and Purkinje cells in the brain, salivary glands, and testicular spermatozoa (26, 64, 83, 107, 125). However, AQP11 disruption affected only the kidney, leading to uremic death from polycystic kidneys (83). Interestingly, before developing the cysts, proximal tubular cells accumulated huge intracellular vacuoles that have not been observed in other cystic kidney diseases. How can defective water transport inside the cell lead to vacuole formation as AQP11 is expressed intracellularly (26, 83)? Currently, little is known about the movement of intracellular water. In fact, the primary cultured proximal tubular cells from AQP11 null mice had a defective endosomal pH regulation (83) that may be paralleled to the bib defect in fruit flies with cytoplasmic endosome accumulation (55). Endosomal water channel will facilitate vesicular shrinkage (93) and may concentrate intravesicular H ions to lower the endosomal pH, leading to the fusion with late endosomes and lysosomes. The identification of intracellular AQP will be a clue to clarify the water movement in the cell and to study water transport in relation to the compartments of intracellular organelles, which can be linked to a wide range of cellular functions.

Although AQP11 is most abundantly expressed in the testis, the phenotypic analysis of the testis in AQP11 null mice was hampered by the premature death of renal failure due to polycystic kidneys. The accumulated expression of AQP11 in the residual bodies of the elongated spermatids, which are rich in granule and vesicles, was reported (125). It is speculated that AQP11 may be important to eliminate these surplus intracellular organelles and their contents through phagocytosis and degradation by the Sertoli cell, which supports spermatogenesis with the clearance of apoptotic spermatogenic cells (125).

A recent report on AQP12 null mice has shown that they suffer from acute pancreatitis more than wild mice because of a decreased capacity of exocytosis in pancreatic acinar cells (87). Because AQP12 is also expressed inside the cell, vacuole sizes will be regulated by AQP12 water transport to swell and expel the vacuolar contents in pancreatic duct lumens. Similar roles will be expected for AQP11 in the salivary gland (64). Such mechanisms for synaptic and exocrine secretion were previously speculated with classical AQPs (53). In contrast to AQP11 working in endocytosis, AQP12 seems to be important for exocytosis. Further studies on these mechanisms, including other members of unorthodox AQPs, will be necessary.

Nonchannel functions of AQP. A nonchannel function such as cell adhesion has also been reported (19). Some AQPs have been shown to be the target for pathogenic molecules. Neuromyelitis optica (NMO) is a rare inflammatory demyelinating disorder affecting optic nerves and spinal cords; the condition is prevalent in Asia and was previously regarded as a subtype of multiple sclerosis. The target antigen for the serum autoantibody in NMO patients has been identified as AQP4 (32, 52). This autoantibody is not only useful for the diagnosis and monitoring of the disease but also for the selection of the treatment: immunosuppression first for the anti-AQP4 antibody-positive patients rather than interferon administration, since it worsens the symptoms. *Pseudomonas aeruginosa* cytotoxin was reported to bind to AQP1 (73), which may be responsible for hemolysis in bacteremia as erythrocytes have AQP1 at the cell surface (Colton blood group antigen) (2). Recently, AQP1 was also identified as a novel placental target of polychlorinated biphenyls (PCBs), which significantly reduced the uterine AQP1 in interleukin-10 (IL-10)-deficient mice associated with defective spiral artery transformation, leading to increased amniotic fluid, intrauterine growth defect, and smaller fetal size with postnatal neuromuscular abnormalities (110). The results will provide a new treatment for environmental toxicities for recombinant IL-10-reversed PCB-induced defects by increasing AQP1 expression.

A diversity of AQP Subcellular Localizations

Although most AQPs are localized at the plasma membrane, some have been shown to be present inside the cell. In plants, most TIPs are present at intracellular vacuoles, tonoplasts, and SIPs, and some NIPs have been shown to be localized at the endoplasmic reticulum (ER) (46, 75, 79). Therefore, intracellular AQPs are not uncommon in plants. On the other hand, intracellular AQPs are relatively rare and not well characterized in animals. In insects, bib in *Drosophila* has been shown to be localized at the endosome to regulate the Notch signaling by modulating endosome maturation, trafficking, and acidification (55). In animals, AQP8 and AQP9 were reported to be localized at mitochondria (21, 25, 67). AQP1 and AQP6 were also shown to be localized inside the cells such as exocrine vesicles and synaptic vesicles, respectively (53). These results, however, require further confirmation since different results have also been reported (124). AQP10 was shown to be localized at intracellular vesicles of enterochromaffin cells in humans although its role in these cells is unclear (68). AQP11 was reported to be localized inside the cell in the kidney, brain, and spermatids (26, 83, 125), whereas AQP12 was localized intracellularly in the pancreatic acinar cells (47, 87).

What is the role of intracellular AQPs? Their physiological significance in water transport is debated, since intracellular organelles are so small that surface-to-volume ratios may be large enough to facilitate water movement in the absence of AQPs. The intracellular matrix around the organelles may inhibit rapid movement of water, especially in the cells where water is transported massively. In plants, very abundant AQPs (TIPs) are present at vacuoles (tonoplasts) (77) to transport water freely inside the cell. The absence of phenotypes in TIP null plants (8) may indicate that TIP is not essential for plants or that the other members of the TIP family may redundantly transport water across the tonoplast. Alternatively, because TIPs also transport ammonia and H_2O_2, they may be needed for decomposition, a breakdown of dead organisms, or storing substrates in the tonoplast (16).

In animal cells, the roles of intracellular AQPs are not clear except for AQP11 whose disruption in mice produced a fatal kidney disease, polycystic kidney disease, and for AQP12 whose disruption slowed blocked exocytosis of pancreatic zymogen granules when maximally stimulated (83, 87). In both systems, the volume regulation of intracellular vesicle is important under the background of a massive water movement. Possible roles of AQPs in intracellular water movement (86) and in the mitochondrial function, especially ammonia transport (25), were reviewed previously.

Intracellular localization of AQPs may be destined by primary sequences. The deviated NPA boxes may cause intracellular retention. The deletion of one or both NPA boxes resulted in defective plasma membrane targeting in AQP4 (27), suggesting that AQPs with poorly conserved NPA boxes such as unorthodox AQPs may be retained inside the cell and function as intracellular AQPs. Such a double-deletion mutant of both NPA boxes in AQP1 still transported water as efficient as native AQP1 (54). These surprising observations, however, will not fit the current results of 3D analyses of AQPs (24). It is possible that unorthodox AQPs may form a different 3D structure with their deviated NPA boxes.

Evolutionary Aspects of AQPs

Table 1 shows the distributions of classified AQPs from bacteria to humans into three families. From these distributions and functions, putative evolutionary pathways of AQPs will be speculated.

The first AQP may have originated in bacteria. For example, *E. coli* has two AQPs: AqpZ and GlpF, which may correspond to the ancestor forms of a classical AQP and an aquaglyceroporin, respectively. A recent mutational analysis of AQPs suggested that AQPs may have evolved in two steps: the initial formation of optimal NPA boxes preventing passage of inorganic cations and then the subsequent formation of a filter (ar/R) to shut off proton permeability (122). The size of the filter will specify the spectrum of permeating substrates such as water, glycerol, urea, and ammonia. The first AQP may have a larger pore permitting the uptake of nutrients and release of waste products, which was most likely an aquaglyceroporin. Next, the loss of the signature D near the second NPA box and the shortening of the loop 3 may have converted aquaglyceroporins to classical AQPs that are now specialized for water transport. Unorthodox AQPs may be derived from classical AQPs for the purpose of intracellular water transport for

animal cells that do not have TIPs, special to plants. Because unorthodox AQPs are absent in lower organisms and plants, it could have been obtained by horizontal gene transfer from cohabitating ancient bacteria because deviated NPA boxes may not adversely affect single-cell organisms and have been accumulated in their genomes.

Interestingly, not all bacteria have a set of classical AQPs and aquaglyceroporins. Genome projects revealed the absence of AQPs in many archaea like thermophilic archaea who live in the ocean near submarine volcanoes where water channels may not be necessary with higher water diffusion at high temperatures. An exceptional classical AQP, AqpM, from *Methanothermobacter marburgensis* has a wider pore structure to accept larger molecules (65). On the other hand, the loss of aquaglyceroporins may have been caused by a parasitic life style of microorganisms in which nutrients are easily obtained. Interestingly, mycoplasma has a nearly minimal bacterial genome but keeps a classical AQP, which may still be important even in this slim organism. Alternatively, other channels may have compensated for the loss of AQPs. Interestingly, a recent 3D analysis of the formate transporter in bacteria revealed that it has a similar structure to AQPs although the primary structure is quite different (120). It is possible that some transporters or channels other than AQPs may function as water and nutrient channels although the formate channel itself seems not to permeate water.

Because eukaryotes have evolved from symbiosis of prokaryotes, they could have inherited many AQPs from prokaryotes. The AQP gene numbers in protozoa, however, are diverse and may have changed by environmental factors (9). Interestingly, *Cryptosporisium parvum* does not have any AQPs, probably because of its symbiotic life style. Other protozoa have a few AQPs: *D. discoideum* have five AQPs all belonging to classical AQPs while *Trypanosoma brucei* has only three aquaglyceroporins. Three pathogenic Trypanosomatidae (*Leishmania major*, *T. cruzi*, and *T. brucei*) have different families of AQPs that may reflect different nutrient uptakes to adapt to their environments rather than evolutionary restriction. On the other hand, *P. falciparum* has only one AQP, PfAQP, an aquaglyceroporin with little sequence variations (6). In contrast, *Toxoplasma gondii* also has only one AQP, TgAQP, a classical AQP with a wide range of permeability (90). Therefore, protozoa seem to have amended the function of each AQP family to adapt to their environment.

Caenorhabditis elegans is the first to have unorthodox AQPs with abundant aquaglyceroporins, 5 out of 11 AQPs (45%), suggesting that glycerol may be important for its osmoregulation and nutrion. Their functional studies, however, showed that not all aquaglyceroporins transport glycerol and, surprisingly, one is even water selective, while all three classical AQPs do not transport glycerol (38). Two AQPs seem to have no transport activities, and the functions of three unorthodox AQPs were not examined. Moreover, the physiological roles of nematode AQPs are still unclear, since even multiple AQP knockouts up to quadruple AQP mutants revealed minimum abnormalities (38). However, a recent report indicates that aqp-1, an aquaglyceroporin, is important for the longevity in a low-sugar diet, suggesting that AQPs may play a role in metabolism rather than water transport (66). Because unorthodox AQPs have first appeared in nematodes and not in protozoa, unorthodox AQPs may have a role in cellular activities specific to multicellular animals such as cellular differentiation, apoptosis, organogenesis, mating, and intercellular communication.

The complexity of AQPs seems to decrease in insects. A fruit fly, *D. melanogaster*, has eight AQPs: seven classical AQPs and one unorthodox AQP that was not initially included as a member of the AQP family, since the first NPA is CPY (57) (Fig. 1). The reason for fewer AQPs in insects may lie in the fact that insects are at a constant risk for dehydration as a result of their high surface area-to-volume ratio, which necessitates minimum water loss by limiting the number of AQPs as AQPs facilitate water efflux from the cell. Alternatively, the absence of aquaglyceroporins in sects simply should have decreased the number of AQPs. In fact, facilitated water loss from cells will be necessary for survival in extreme dryness: larvae of the sleeping chironomid can survive complete dehydration by entering anhydrobiosis in which water rapidly flows out of the larval body as accumulated trehalose prevents cell damage caused by dehydration. Rapid dehydration will be necessary to increase trehalose concentration to a steady state where water content is $<3\%$, which is conducted by classical AQPs during the induction of anhydrobiosis (59). Interestingly, one classical AQP identified from the silkworm larva, *Bombyx mori*, has been shown to increase glycerol and urea uptake similar to protozoan AQPs (56). Because insects use trehalose for the osmolyte, glycerol may be necessary for protecting them from freezing. Overwintering freeze-tolerant larvae of *Chilo suppressalis* can survive in freezing temperature by accumulating glycerol and losing water through activation of AQPs to prevent freezing injury (49). Recently, an AQP named RsAQP1 with a NPARD sequence at the second NPA box has been identified in a mite, *Rhipicephalus sanguineus* (7), which will be an aquaglyceroporin with the signature D although its function was reported to be water selective. Because mites are evolutionally earlier than insects, the ancestors of insects may have had aquaglyceroporins but lost them through the evolution with the acquisition of the glycerol transport function by classical AQPs. Conversely, because glycerol transporter may not be necessary in insects and mites, a remnant aquaglyceroporin in mites has been functionally converted to a water-selective AQP. Further search for aquaglyceroporins in insects and mites may reveal their possible horizontal gene transfers from bacterial aquaglyceroporins.

In contrast to animals, plants have developed unique sets of classical AQPs without aquaglyceroporins and unorthodox AQPs. *Arabidopsis thaliana* has 35 AQPs, which are further subdivided into four groups: PIPs, TIPs, NIPs, and SIPs (75, 77). The absence of aquaglyceroporins in plants can be explained by functional conversion of classical AQPs to aquaglyceroporins as is the case with protozoa and insects. NIPs can transport small molecules other than water, such as glycerol, silicon, and boron (78, 104), which are thought to be derived from bacteria by horizontal gene transfer (127): symbiotic bacteria in the root may have had ancestors of NIPs for the uptake of nutrients and possibly for the efflux of metabolites and wastes. Some NIPs are expressed at the ER membrane, which may be reminiscent of previous intracellular symbiotic states (75, 79). The absence of unorthodox AQPs in plants is intriguing and may be related to the presence of intracellular TIPs and SIPs, which may have made intracellular unorthodox AQPs redundant. Another subfamily of classical

AQPs is identified in poplar trees, which is absent in *A. thaliana*. It is called XIP (14). Similar to SIPs, XIPs have less conserved NPA boxes. Currently, no functional studies have yet been reported with XIPs. An aquaglyceroporin, GIP, found in a moss, *Physcomitrella patens,* functions as a glycerol channel that may have come from bacteria by a horizontal gene transfer (28).

In vertebrates, all three families of AQPs are present. In humans, there are 13 AQPs in total: classical AQPs, AQP0, -1, -2, -4, -5, -6, and -8; aquaglyceroporins, AQP3, -7, -9, and -10; and unorthodox AQPs, AQP11 and -12. Although vertebrates have undergone two rounds of whole genome duplications, the total number of AQPs is relatively small compared with nematodes, which have 11 AQPs. Many might have been lost through the evolution as exemplified by AQP10, which has turned to a nonfunctional pseudogene in mice (82). In fact, the numbers of aquaglyceroporins and unorthodox AQPs have been decreased in humans from nematodes. Although the zebrafish underwent another round of whole genome duplication, its AQP number is not twice as many as that of humans (112). Interestingly, the orthologs of AQP2, AQP6, and AQP10 are missing in the chicken (48). Further analyses of tissue distribution and hormonal regulation of each AQP ortholog in vertebrates will be useful to obtain an insight into the functions and roles of mammalian AQPs (44).

Perspectives and Significance

Accumulating evidence indicates that pore selectivity of AQPs is more diversified than previously thought. Most results have been derived from the studies on classical AQPs and aquaglyceroporins. Because unorthodox AQPs have highly variable NPA boxes, they may have unusual pore structures. Little is known about the 3D structure and the function of this subfamily. With much more knowledge, the further grouping of unorthodox AQPs will be necessary as in the case with plant classical AQPs, which are now composed of five subfamilies.

Among diversified functions of AQPs, the structural basis of gas permeation through some classical AQPs will be intriguing, with potential application for construction of artificial gas channels. Possible stimulations of cell migration and proliferation by AQPs should also be substantiated to be applicable to developmental and cancer biologies.

Because AQPs play an important role in the adaptation of organisms to the environmental challenges, knowledge in lower lives will be applicable to plants and animals, and vice versa. Such interdisciplinary works as comparative physiology and endocrinology in particular should be encouraged and will be rewarding. For example, an aquaglyceroporin in nematode has been shown to be important for the longevity in a low-sugar diet (66), which may be applicable to agriculture and even to medicine.

Although many studies have substantiated the importance of plasma membrane AQPs for the regulation of water and solute homeostasis, the significance of intracellular AQPs has been little appreciated with poor knowledge on water and solute transport between intracellular organelles. Intracellular AQPs will be expected to open a new research field.

Finally, still, no specific inhibitors against AQPs are available yet. The accumulating knowledge on 3D structures of AQPs will facilitate the search for these inhibitors. Obviously, the development of therapeutic drugs to modulate AQP function and expression is also needed, which will be applicable to agriculture and medicine.

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES

1. **Agre P, Bonhivers M, Borgnia MJ.** The aquaporins, blueprints for cellular plumbing systems. *J Biol Chem* 273: 14659–14662, 1998.
2. **Agre P, Smith BL, Preston GM.** ABH and Colton blood group antigens on aquaporin-1, the human red cell water channel protein. *Transfus Clin Biol* 2: 303–308, 1995.
3. **Aharon R, Bar-Shavit Z.** Involvement of aquaporin 9 in osteoclast differentiation. *J Biol Chem* 281: 19305–19309, 2006.
4. **Alexandersson E, Fraysse L, Sjövall-Larsen S, Gustavsson S, Fellert M, Karlsson M, Johanson U, Kjellbom P.** Whole gene family expression and drought stress regulation of aquaporins. *Plant Mol Biol* 59: 469–484, 2005.
5. **Bahamontes-Rosa N, Tena-Tomás C, Wolkow J, Kremsner PG, Kun JF.** Genetic conservation of the GIL blood group determining aquaporin 3 gene in African and Caucasian populations. *Transfusion* 48: 1164–1168, 2008.
6. **Bahamontes-Rosa N, Wu B, Beitz E, Kremsner PG, Kun JF.** Limited genetic diversity of the Plasmodium falciparum aquaglyceroporin gene. *Mol Biochem Parasitol* 156: 255–257, 2007.
7. **Ball A, Campbell EM, Jacob J, Hoppler S, Bowman AS.** Identification, functional characterization and expression patterns of a water-specific aquaporin in the brown dog tick, Rhipicephalus sanguineus. *Insect Biochem Mol Biol* 39: 105–112, 2009.
8. **Beebo A, Thomas D, Der C, Sanchez L, Leborgne-Castel N, Marty F, Schoefs B, Bouhidel K.** Life with and without AtTIP1;1, an Arabidopsis aquaporin preferentially localized in the apposing tonoplasts of adjacent vacuoles. *Plant Mol Biol* 70: 193–209, 2009.
9. **Beitz E.** Aquaporin water and solute channels from malaria parasites and other pathogenic protozoa. *ChemMedChem* 1: 587–592, 2006.
10. **Bienert GP, Møller AL, Kristiansen KA, Schulz A, Møller IM, Schjoerring JK, Jahn TP.** Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *J Biol Chem* 282: 1183–1192, 2007.
11. **Bienert GP, Schüssler MD, Jahn TP.** Metalloids: essential, beneficial or toxic? Major intrinsic proteins sort it out. *Trends Biochem Sci* 33: 20–26, 2008.
12. **Calamita G, Bishai WR, Preston GM, Guggino WB, Agre P.** Molecular cloning and characterization of AqpZ, a water channel from Escherichia coli. *J Biol Chem* 270: 29063–29066, 1995.
13. **Calamita G, Kempf B, Bonhivers M, Bishai WR, Bremer E, Agre P.** Regulation of the Escherichia coli water channel gene aqpZ. *Proc Natl Acad Sci USA* 95: 3627–3631, 1998.
14. **Danielson JA, Johanson U.** Unexpected complexity of the aquaporin gene family in the moss Physcomitrella patens (Abstract). *BMC Plant Biol* 8: 45, 2008.
15. **Dumas M, Sadick NS, Noblesse E, Juan M, Lachmann-Weber N, Boury-Jamot M, Sougrat R, Verbavatz JM, Schnebert S, Bonté F.** Hydrating skin by stimulating biosynthesis of aquaporins. *J Drugs Dermatol* 6: s20–s24, 2007.
16. **Dynowski M, Schaaf G, Loque D, Moran O, Ludewig U.** Plant plasma membrane water channels conduct the signaling molecule H2O2. *Biochem J* 414: 53–61, 2008.
17. **Echevarría M, Muñoz-Cabello AM, Sánchez-Silva R, Toledo-Aral JJ, López-Barneo J.** Development of cytosolic hypoxia and hypoxia-inducible factor stabilization are facilitated by aquaporin-1 expression. *J Biol Chem* 282: 30207–30215, 2007.
18. **Endo M, Jain RK, Witwer B, Brown D.** Water channel (aquaporin 1) expression and distribution in mammary carcinomas and glioblastomas. *Microvasc Res* 58: 89–98, 1999.

19. **Engel A, Fujiyoshi Y, Gonen T, Walz T.** Junction-forming aquaporins. *Curr Opin Struct Biol* 18: 229–235, 2008.
20. **Farjo R, Peterson WM, Naash MI.** Expression profiling after retinal detachment and reattachment: a possible role for aquaporin-0. *Invest Ophthalmol Vis Sci* 49: 511–521, 2008.
21. **Ferri D, Mazzone A, Liquori GE, Cassano G, Svelto M, Calamita G.** Ontogeny, distribution, and possible functional implications of an unusual aquaporin, AQP8, in mouse liver. *Hepatology* 38: 947–957, 2003.
22. **Fischer G, Kosinska-Eriksson U, Aponte-Santamaría C, Palmgren M, Geijer C, Hedfalk K, Hohmann S, de Groot BL, Neutze R, Lindkvist-Petersson K.** Crystal structure of a yeast aquaporin at 1.*15 angstrom reveals a novel gating mechanism PLoS Biol* 7: e1000130, 2009.
23. **Flach CF, Qadri F, Bhuiyan TR, Alam NH, Jennische E, Holmgren J, Lönnroth I.** Differential expression of intestinal membrane transporters in cholera patients. *FEBS Lett* 581: 3183–3188, 2007.
24. **Fu D, Lu M.** The structural basis of water permeation and proton exclusion in aquaporins. *Mol Membr Biol* 24: 366–374, 2007.
25. **Gena P, Fanelli E, Brenner C, Svelto M, Calamita G.** News and views on mitochondrial water transport. *Front Biosci* 14: 4189–4198, 2009.
26. **Gorelick DA, Praetorius J, Tsunenari T, Nielsen S, Agre P.** Aquaporin-11: a channel protein lacking apparent transport function expressed in brain (Abstract). *BMC Biochem* 7: 14, 2006.
27. **Guan XG, Su WH, Yi F, Zhang D, Hao F, Zhang HG, Liu YJ, Feng XC, Ma TH.** NPA motifs play a key role in plasma membrane targeting of aquaporin-4. *IUBMB Life* 62: 222–226, 2010.
28. **Gustavsson S, Lebrun AS, Nordén K, Chaumont F, Johanson U.** A novel plant major intrinsic protein in Physcomitrella patens most similar to bacterial glycerol channels. *Plant Physiol* 139: 287–295, 2005.
29. **Hara-Chikuma M, Sohara E, Rai T, Ikawa M, Okabe M, Sasaki S, Uchida S, Verkman AS.** Progressive adipocyte hypertrophy in aquaporin-7-deficient mice: adipocyte glycerol permeability as a novel regulator of fat accumulation. *J Biol Chem* 280: 15493–154936, 2005.
30. **Hara-Chikuma M, Verkman AS.** Physiological roles of glycerol-transporting aquaporins: the aquaglyceroporins. *Cell Mol Life Sci* 63: 1386–1392, 2006.
31. **Hara-Chikuma M, Verkman AS.** Prevention of skin tumorigenesis and impairment of epidermal cell proliferation by targeted aquaporin-3 gene disruption. *Mol Cell Biol* 28: 326–332, 2008.
32. **Hayakawa S, Mori M, Okuta A, Kamegawa A, Fujiyoshi Y, Yoshiyama Y, Mitsuoka K, Ishibashi K, Sasaki S, Hattori T, Kuwabara S.** Neuromyelitis optica and anti-aquaporin-4 antibodies measured by an enzyme-linked immunosorbent assay. *J Neuroimmunol* 196: 181–187, 2008.
33. **Hedfalk K, Bill RM, Mullins JG, Karlgren S, Filipsson C, Bergstrom J, Tamás MJ, Rydström J, Hohmann S.** A regulatory domain in the C-terminal extension of the yeast glycerol channel Fps1p. *J Biol Chem* 279: 14954–14960, 2004.
34. **Herrera M, Garvin JL.** Novel role of AQP-1 in NO-dependent vasorelaxation. *Am J Physiol Renal Physiol* 292: F1443–F1451, 2007.
35. **Hibuse T, Maeda N, Funahashi T, Yamamoto K, Nagasawa A, Mizunoya W, Kishida K, Inoue K, Kuriyama H, Nakamura T, Fushiki T, Kihara S, Shimomura I.** Aquaporin 7 deficiency is associated with development of obesity through activation of adipose glycerol kinase. *Proc Natl Acad Sci USA* 102: 10993–10998, 2005.
36. **Hibuse T, Maeda N, Nagasawa A, Funahashi T.** Aquaporins and glycerol metabolism. *Biochim Biophys Acta* 1758: 1004–1001, 2006.
37. **Hiroaki Y, Tani K, Kamegawa A, Gyobu N, Nishikawa K, Suzuki H, Walz T, Sasaki S, Mitsuoka K, Kimura K, Mizoguchi A, Fujiyoshi Y.** Implications of the aquaporin-4 structure on array formation and cell adhesion. *J Mol Biol* 355: 628–639, 2006.
38. **Huang CG, Lamitina T, Agre P, Strange K.** Functional analysis of the aquaporin gene family in Caenorhabditis elegans. *Am J Physiol Cell Physiol* 292: C1867–C1873, 2007.
39. **Hub JS, de Groot BL.** Mechanism of selectivity in aquaporins and aquaglyceroporins. *Proc Natl Acad Sci USA* 105: 1198–1203, 2008.
40. **Ikeda M, Beitz E, Kozono D, Guggino WB, Agre P, Yasui M.** Characterization of aquaporin-6 as a nitrate channel in mammalian cells. Requirement of pore-lining residue threonine 63. *J Biol Chem* 277: 39873–39879, 2002.
41. **Ishibashi K.** New members of mammalian aquaporins: AQP10-AQP12. *Handb Exp Pharmacol* 190: 251–262, 2009.
42. **Ishibashi K.** Aquaporin subfamily with unusual NPA boxes. *Biochim Biophys Acta* 1758: 989–993, 2006.
43. **Ishibashi K, Hara S, Kondo S.** Aquaporin water channels in mammals. *Clin Exp Nephrol* 13: 107–117, 2009.
44. **Ishibashi K, Kuwahara M, Sasaki S.** Molecular biology of aquaporins. *Rev Physiol Biochem Pharmacol* 141: 1–32, 2000.
45. **Ishibashi K, Sasaki S.** The dichotomy of MIP family suggests two separate origins of water channels. *News Physiol Sci* 13: 137–142, 1998.
46. **Ishikawa F, Suga S, Uemura T, Sato MH, Maeshima M.** Novel type aquaporin SIPs are mainly localized to the ER membrane and show cell-specific expression in Arabidopsis thaliana. *FEBS Lett* 579: 5814–5820, 2005.
47. **Itoh T, Rai T, Kuwahara M, Ko SB, Uchida S, Sasaki S, Ishibashi K.** Identification of a novel aquaporin, AQP12, expressed in pancreatic acinar cells. *Biochem Biophys Res Commun* 330: 832–838, 2005.
48. **Isokpehi RD, Rajnarayanan RV, Jeffries CD, Oyeleye TO, Cohly HH.** Integrative sequence and tissue expression profiling of chicken and mammalian aquaporins. *BMC Genomics Suppl* 2: S7, 2009.
49. **Izumi Y, Sonoda S, Yoshida H, Danks HV, Tsumuki H.** Role of membrane transport of water and glycerol in the freeze tolerance of the rice stem borer, Chilo suppressalis Walker (Lepidoptera: Pyralidae). *J Insect Physiol* 52: 215–220, 2006.
50. **Jablonski EM, Mattocks MA, Sokolov E, Koniaris LG, Hughes FM Jr, Fausto N, Pierce RH, McKillop IH.** Decreased aquaporin expression leads to increased resistance to apoptosis in hepatocellular carcinoma. *Cancer Lett* 250: 36–46, 2007.
51. **Jablonski EM, Webb AN, McConnell NA, Riley MC, Hughes FM Jr.** Plasma membrane aquaporin activity can affect the rate of apoptosis but is inhibited after apoptotic volume decrease. *Am J Physiol Cell Physiol* 286: C975–C985, 2004.
52. **Jarius S, Paul F, Franciotta D, Waters P, Zipp F, Hohlfeld R, Vincent A, Wildemann B, Vanderbilt.** Mechanisms of Disease: aquaporin-4 antibodies in neuromyelitis optica. *Nat Clin Pract Neurol* 4: 202–214, 2008.
53. **Jeremic A, Cho WJ, Jena BP.** Involvement of water channels in synaptic vesicle swelling. *Exp Biol Med (Maywood)* 230: 674–680, 2005.
54. **Jiang Y.** Expression and functional characterization of NPA motif-null aquaporin-1 mutations. *IUBMB Life* 61: 651–657, 2009.
55. **Kanwar R, Fortini ME.** The big brain aquaporin is required for endosome maturation and notch receptor trafficking. *Cell* 133: 852–863, 2008.
56. **Kataoka N, Miyake S, Azuma M.** Aquaporin and aquaglyceroporin in silkworms, differently expressed in the hindgut and midgut of Bombyx mori. *Insect Mol Biol* 18: 303–314, 2009.
57. **Kaufmann N, Mathai JC, Hill WG, Dow JA, Zeidel ML, Brodsky JL.** Developmental expression and biophysical characterization of a Drosophila melanogaster aquaporin. *Am J Physiol Cell Physiol* 289: C397–C407, 2005.
58. **Kayingo G, Sirotkin V, Hohmann S, Prior BA.** Accumulation and release of the osmolyte glycerol is independent of the putative MIP channel Spac977.17p in Schizosaccharomyces pombe. *Antonie Van Leeuwenhoek* 85: 85–92, 2004.
59. **Kikawada T, Saito A, Kanamori Y, Fujita M, Snigórska K, Watanabe M, Okuda T.** Dehydration-inducible changes in expression of two aquaporins in the sleeping chironomid, Polypedilum vanderplanki. *Biochim Biophys Acta* 1778: 514–520, 2008.
60. **Knepper MA, Nielsen S.** Peter Agre, 2003 Nobel Prize winner in chemistry. *J Am Soc Nephrol* 15: 1093–1095, 2004.
61. **Kobayashi M, Takahashi E, Miyagawa S, Watanabe H, Iguchi T.** Chromatin immunoprecipitation-mediated target identification proved aquaporin 5 is regulated directly by estrogen in the uterus. *Genes Cells* 11: 1133–1143, 2006.
62. **Kutuzova GD, Deluca HF.** Gene expression profiles in rat intestine identify pathways for 1,25-dihydroxyvitamin D(3) stimulated calcium absorption and clarify its immunomodulatory properties. *Arch Biochem Biophys* 432: 152–166, 2004.
63. **Laizé V, Tacnet F, Ripoche P, Hohmann S.** Polymorphism of Saccharomyces cerevisiae aquaporins. *Yeast* 16: 897–903, 2000.
64. **Larsen HS, Ruus AK, Schreurs O, Galtung HK.** Aquaporin 11 in the developing mouse submandibular gland. *Eur J Oral Sci* 118: 9–13, 2010.
65. **Lee JK, Kozono D, Remis J, Kitagawa Y, Agre P, Stroud RM.** Structural basis for conductance by the archaeal aquaporin AqpM at 1.68 A. *Proc Natl Acad Sci USA* 102: 18932–18937, 2005.
66. **Lee SJ, Murphy CT, Kenyon C.** Glucose shortens the life span of C. *elegans* by downregulating DAF-16/FOXO activity and aquaporin gene expression. *Cell Metab* 10: 379–391, 2009.

67. **Lee WK, Thévenod F.** A role for mitochondrial aquaporins in cellular life-and-death decisions? *Am J Physiol Cell Physiol* 291: C195–C202, 2006.
68. **Li H, Kamiie J, Morishita Y, Yoshida Y, Yaoita E, Ishibashi K, Yamamoto T.** Expression and localization of two isoforms of AQP10 in human small intestine. *Biol Cell* 97: 823–829, 2005.
69. **Liang HT, Feng XC, Ma TH.** Water channel activity of plasma membrane affects chondrocyte migration and adhesion. *Clin Exp Pharmacol Physiol* 35: 7–10, 2008.
70. **Liu HY, Yu X, Cui DY, Sun MH, Sun WN, Tang ZC, Kwak SS, Su WA.** The role of water channel proteins and nitric oxide signaling in rice seed germination. *Cell Res* 17: 638–649, 2007.
71. **Liu Y, Promeneur D, Rojek A, Kumar N, Frøkiaer J, Nielsen S, King LS, Agre P, Carbrey JM.** Aquaporin 9 is the major pathway for glycerol uptake by mouse erythrocytes, with implications for malarial virulence. *Proc Natl Acad Sci USA* 104: 12560–12564, 2007.
72. **Liu Y, Song L, Wang Y, Rojek A, Nielsen S, Agre P, Carbrey JM.** Osteoclast differentiation and function in aquaglyceroporin AQP9 null mice. *Biol Cell* 101: 133–140, 2009.
73. **Lutz F, Mohr M, Grimmig M, Leidolf R, Linder D.** Pseudomonas aeruginosa cytotoxin-binding protein in rabbit erythrocyte membranes. An oligomer of 28 kDa with similarity to transmembrane channel proteins. *Eur J Biochem* 217: 1123–1128, 1993.
74. **Maeda N, Funahashi T, Shimomura I.** Metabolic impact of adipose and hepatic glycerol channels aquaporin 7 and aquaporin 9. *Nat Clin Pract Endocrinol Metab* 4: 627–634, 2008.
75. **Maeshima M, Ishikawa F.** ER membrane aquaporins in plants. *Pflugers Arch* 456: 709–716, 2008.
76. **Matsumura K, Chang BH, Fujimiya M, Chen W, Kulkarni RN, Eguchi Y, Kimura H, Kojima H, Chan L.** Aquaporin 7 is a beta-cell protein and regulator of intraislet glycerol content and glycerol kinase activity, beta-cell mass, and insulin production and secretion. *Mol Cell Biol* 27: 6026–6037, 2007.
77. **Maurel C, Verdoucq L, Luu DT, Santoni V.** Plant aquaporins: membrane channels with multiple integrated functions. *Annu Rev Plant Biol* 59: 595–624, 2008.
78. **Mitani N, Yamaji N, Ma JF.** Characterization of substrate specificity of a rice transporter, Lsi1. *Pflugers Arch* 456: 679–686, 2008.
79. **Mizutani M, Watanabe S, Nakagawa T, Maeshima M.** Aquaporin NIP2;1 is mainly localized to the ER membrane and shows root-specific accumulation in Arabidopsis thaliana. *Plant Cell Physiol* 47: 1420–1426, 2006.
80. **Mobasheri A, Trujillo E, Bell S, Carter SD, Clegg PD, Martín-Vasallo P, Marples D.** Aquaporin water channels AQP1 and AQP3, are expressed in equine articular chondrocytes. *Vet J* 168: 143–150, 2004.
81. **Moe SE, Sorbo JG, Sogaard R, Zeuthen T, Petter Ottersen O, Holen T.** New isoforms of rat Aquaporin-4. *Genomics* 91: 367–377, 2008.
82. **Morinaga T, Nakakoshi M, Hirao A, Imai M, Ishibashi K.** Mouse aquaporin 10 gene (AQP10) is a pseudogene. *Biochem Biophys Res Commun* 294: 630–634, 2002.
83. **Morishita Y, Matsuzaki T, Hara-chikuma M, Andoo A, Shimono M, Matsuki A, Kobayashi K, Ikeda M, Yamamoto T, Verkman AS, Kusano E, Ookawara S, Takata K, Sasaki S, Ishibashi K.** Disruption of aquaporin-11 produces polycystic kidneys following vacuolization of the proximal tubule. *Mol Cell Biol* 25: 7770–7779, 2005.
84. **Morishita Y, Sakube Y, Sasaki S, Ishibashi K.** Aquaporin superfamily (superaquaporins): Expansion of aquaporins restricted to multicellular organisms. *J Pharmacol Sci* 96: 276–279, 2004.
85. **Musa-Aziz R, Chen LM, Pelletier MF, Boron WF.** Relative CO2/NH3 selectivities of AQP1, AQP4, AQP5, AmtB, and RhAG. *Proc Natl Acad Sci USA* 106: 5406–5411, 2009.
86. **Nozaki K, Ishii D, Ishibashi K.** Intracellular aquaporins: clues for intracellular water transport? *Pflugers Arch* 456: 701–707, 2008.
87. **Ohta E, Itoh T, Nemoto T, Kumagai J, Ko SB, Ishibashi K, Ohno M, Uchida K, Ohta A, Sohara E, Uchida S, Sasaki S, Rai T.** Pancreas-specific aquaporin 12 null mice showed increased susceptibility to caerulein-induced acute pancreatitis. *Am J Physiol Cell Physiol* 297: C1368–C1378, 2009.
88. **Otto B, Uehlein N, Sdorra S, Fischer M, Ayaz M, Belastegui-Macadam X, Heckwolf M, Lachnit M, Pede N, Priem N, Reinhard A, Siegfart S, Urban M, Kaldenhoff R.** Aquaporin tetramer composition modifies the function of tobacco aquaporins. *J Biol Chem* 285: 31253–31260, 2010.
89. **Pastor-Soler N, Bagnis C, Sabolic I, Tyszkowski R, McKee M, Van Hoek A, Breton S, Brown D.** Aquaporin 9 expression along the male reproductive tract. *Biol Reprod* 65: 384–393, 2001.
90. **Pavlovic-Djuranovic S, Schultz JE, Beitz E.** A single aquaporin gene encodes a water/glycerol/urea facilitator in Toxoplasma gondii with similarity to plant tonoplast intrinsic proteins. *FEBS Lett* 555: 500–504, 2003.
91. **Philips J, Herskowitz I.** Osmotic balance regulates cell fusion during mating in Saccharomyces cerevisiae. *J Cell Biol* 138: 961–974, 1997.
92. **Promeneur D, Liu Y, Maciel J, Agre P, King LS, Kumar N.** Aquaglyceroporin PbAQP during intraerythrocytic development of the malaria parasite Plasmodium berghei. *Proc Natl Acad Sci USA* 104: 2211–2216, 2007.
93. **Rauch C, Pluen A, Foster N, Loughna P, Mobasheri A, Lagadic-Gossmann D, Counillon L.** On some aspects of the thermodynamic of membrane recycling mediated by fluid phase endocytosis: evaluation of published data and perspectives. *Cell Biochem Biophys* 56: 73–90, 2010.
94. **Ripoche P, Goossens D, Devuyst O, Gane P, Colin Y, Verkman AS, Cartron JP.** Role of RhAG and AQP1 in NH3 and CO2 gas transport in red cell ghosts: a stopped-flow analysis. *Transfus Clin Biol* 13: 117–122, 2006.
95. **Rojek A, Praetorius J, Frøkiaer J, Nielsen S, Fenton RA.** A current view of the mammalian aquaglyceroporins. *Ann Rev Physiol* 70: 301–327, 2008.
96. **Rojek AM, Skowronski MT, Füchtbauer EM, Füchtbauer AC, Fenton RA, Agre P, Frøkiaer J, Nielsen S.** Defective glycerol metabolism in aquaporin 9 (AQP9) knockout mice. *Proc Natl Acad Sci USA* 104: 3609–3614, 2007.
97. **Roudier N, Bailly P, Gane P, Lucien N, Gobin R, Cartron JP, Ripoche P.** Erythroid expression and oligomeric state of the AQP3 protein. *J Biol Chem* 277: 7664–7669, 2002.
98. **Ruiz-Ederra J, Verkman AS.** Aquaporin-1 independent microvessel proliferation in a neonatal mouse model of oxygen-induced retinopathy. *Invest Ophthalmol Vis Sci* 48: 4802–4810, 2007.
99. **Scheuring S, Buzhynskyy N, Jaroslawski S, Gonçalves RP, Hite RK, Walz T.** Structural models of the supramolecular organization of AQP0 and connexons in junctional microdomains. *J Struct Biol* 160: 385–394, 2007.
100. **Seda O, Sedová L, Oliyarnyk O, Kazdová L, Krenová D, Corbeil G, Hamet P, Tremblay J, Kren V.** Pharmacogenomics of metabolic effects of rosiglitazone. *Pharmacogenomics* 9: 141–155, 2008.
101. **Skowronski MT, Lebeck J, Rojek A, Praetorius J, Füchtbauer EM, Frøkiaer J, Nielsen S.** AQP7 is localized in capillaries of adipose tissue, cardiac and striated muscle: implications in glycerol metabolism. *Am J Physiol Renal Physiol* 292: F956–F965, 2007.
102. **Sohara E, Rai T, Miyazaki J, Verkman AS, Sasaki S, Uchida S.** Defective water and glycerol transport in the proximal tubules of AQP7 knockout mice. *Am J Physiol Renal Physiol* 289: F1195–F1200, 2005.
103. **Soupene E, King N, Lee H, Kustu S.** Aquaporin Z of Escherichia coli: reassessment of its regulation and physiological role. *J Bacteriol* 84: 4304–4307, 2002.
104. **Takano J, Wada M, Ludewig U, Schaaf G, von Wirén N, Fujiwara T.** The Arabidopsis major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation. *Plant Cell* 18: 1498–1509, 2006.
105. **Tanghe A, Van Dijck P, Thevelein JM.** Why do microorganisms have aquaporins? *Trends Microbiol* 14: 78–85, 2006.
106. **Tatsumi K, Tsuji S, Miwa H, Morisaku T, Nuriya M, Orihara M, Kaneko K, Okano H, Yasui M.** Drosophila big brain does not act as a water channel, but mediates cell adhesion. *FEBS Lett* 583: 2077–2082, 2009.
107. **Tchekneva EE, Khuchua Z, Davis LS, Kadkina V, Dunn SR, Bachman S, Ishibashi K, Rinchik EM, Harris RC, Dikov MM, Breyer MD.** A newly identified ENU-induced single amino acid mutation in aquaporin-11 resulting in perinatal kidney failure in mice. *J Am Nephrol Soc* 19: 1955–1964, 2008.
108. **Terashima I, Ono K.** Effects of HgCl(2) on CO(2) dependence of leaf photosynthesis: evidence indicating involvement of aquaporins in CO(2) diffusion across the plasma membrane. *Plant Cell Physiol* 43: 70–78, 2002.
109. **Te Velde AA, Pronk I, de Kort F, Stokkers PC.** Glutathione peroxidase 2 and aquaporin 8 as new markers for colonic inflammation in experimental colitis and inflammatory bowel diseases: an important role for H2O2? *Eur J Gastroenterol Hepatol* 20: 555–560, 2008.

110. **Tewari N, Kalkunte S, Murray DW, Sharma S.** The water channel aquaporin 1 is a novel molecular target of polychlorinated biphenyls for in utero anomalies. *J Biol Chem* 284: 15224–15232, 2009.
111. **Thiagarajah JR, Zhao D, Verkman AS.** Impaired enterocyte proliferation in aquaporin-3 deficiency in mouse models of colitis. *Gut* 56: 1529–1535, 2007.
112. **Tingaud-Sequeira A, Calusinska M, Finn RN, Chauvigné F, Lozano J, Cerdà J.** The zebrafish genome encodes the largest vertebrate repertoire of functional aquaporins with dual paralogy and substrate specificities similar to mammals (Abstract). *BMC Evol Biol* 10: 38, 2010.
113. **Tsukaguchi H, Shayakul C, Berger UV, Mackenzie B, Devidas S, Guggino WB, van Hoek AN, Hediger MA.** Molecular characterization of a broad selectivity neutral solute channel. *J Biol Chem* 273: 24737–24743, 1998.
114. **Verkman AS.** Knock-out models reveal new aquaporin functions. *Handb Exp Pharmacol* 190: 359–381, 2009.
115. **Verkman AS.** A cautionary note on cosmetics containing ingredients that increase aquaporin-3 expression. *Exp Dermatol* 17: 871–872, 2008.
116. **Verkman AS, Hara-Chikuma M, Papadopoulos MC.** Aquaporins-new players in cancer biology. *J Mol Med* 86: 523–529, 2008.
117. **Viadiu H, Gonen T, Walz T.** Projection map of aquaporin-9 at 7 A resolution. *J Mol Biol* 367: 80–88, 2007.
118. **vom Dahl S, Häussinger D.** Evidence for a phloretin-sensitive glycerol transport mechanism in the perfused rat liver. *Am J Physiol Gastrointest Liver Physiol* 272: G563–G574, 1997.
119. **Waisbren SJ, Geibel JP, Modlin IM, Boron WF.** Unusual permeability properties of gastric gland cells. *Nature* 368: 332–335, 1994.
120. **Wang Y, Huang Y, Wang J, Cheng C, Huang W, Lu P, Xu YN, Wang P, Yan N, Shi Y.** Structure of the formate transporter FocA reveals a pentameric aquaporin-like channel. *Nature* 462: 467–472, 2009.
121. **Wintour EM, Henry BA.** Glycerol transport: an additional target for obesity therapy? *Trends Endocrinol Metab* 17: 77–78, 2006.
122. **Wu B, Steinbronn C, Alsterfjord M, Zeuthen T, Beitz E.** Concerted action of two cation filters in the aquaporin water channel. *EMBO J* 28: 2188–2194, 2009.
123. **Yakata K, Hiroaki Y, Ishibashi K, Sohara E, Sasaki S, Mitsuoka K, Fujiyoshi Y.** Aquaporin-11 containing a divergent NPA motif has normal water channel activity. *Biochem Biophys Acta* 1768: 688–693, 2007.
124. **Yang B, Zhao D, Verkman AS.** Evidence against functionally significant aquaporin expression in mitochondria. *J Biol Chem* 281: 16202–16206, 2006.
125. **Yeung CH, Cooper TG.** Aquaporin AQP11 in the testis: molecular identity and association with the processing of residual cytoplasm of elongated spermatids. *Reproduction* 139: 209–216, 2010.
126. **Yool AJ.** Dominant-negative suppression of big brain ion channel activity by mutation of a conserved glutamate in the first transmembrane domain. *Gene Expr* 13: 329–337, 2007.
127. **Zardoya R, Ding X, Kitagawa Y, Chrispeels MJ.** Origin of plant glycerol transporters by horizontal gene transfer and functional recruitment. *Proc Natl Acad Sci USA* 99: 14893–14896, 2002.
128. **Zeuthen T.** Water-transporting proteins. *J Membrane Biol* 234: 57–73, 2010.
129. **Zhang H, Verkman AS.** Evidence against involvement of aquaporin-4 in cell-cell adhesion. *J Mol Biol* 382: 1136–1143, 2008.