Minireview

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The Aquaporins, Blueprints for Cellular Plumbing Systems*

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Membrane Water Permeability

Plasma membranes provide an effective barrier to the extracellular environment. Water was long believed to move through lipid bilayers by simple diffusion; however, membranes from different tissues vary in their permeability to water. The variability is particularly evident in mammalian kidney where proximal tubules and descending thin limbs of Henle's loop have constitutively high water permeability and are responsible for reabsorption of more than 150 liters per day in adult humans. In contrast, ascending thin limbs have very low water permeability. Renal distal tubules empty into collecting ducts where stimulation with vasopressin causes an increase in water permeability (see Ref. 1 for review). These observations led to the suggestion that specialized water transport molecules must exist in membranes with intrinsically high water permeability. Nevertheless, despite extensive studies, the molecular identity of water transport proteins remained elusive until recently.

The well defined features of membrane water permeability permitted serendipitous identification of the first known water channel. While purifying the 32-kDa subunit of the red cell Rh blood group antigen, a new 28-kDa polypeptide was discovered (2). Detailed biochemical studies of this newly identified tetrameric membrane protein were made easy by its low solubility in N-lauroylsarcosine, which permitted simple purification (3). The abundance of the protein in rat renal proximal tubules and descending thin limbs (2) sparked the idea that the 28-kDa polypeptide may be the long sought water channel, and its unique N-terminal amino acid sequence permitted cloning from an erythroid cDNA library (4).

Functional Analyses of AQP1

The *Xenopus* oocyte expression system has been extremely useful for study of water transport. Oocytes injected with cRNA for the 28-kDa polypeptide exhibit remarkably high osmotic water permeability ($P_f \sim 200 \times 10^{-4}$ cm s^{-1}) and rapidly explode in hypotonic buffer (Fig. 1), whereas control oocytes exhibit minimal permeability (5). First referred to as "CHIP28," this protein is now designated aquaporin-1 (AQP1)[1] by the Human Genome Nomenclature Committee (see http://www.gene.ucl.ac.uk/nomenclature). Despite its large water permeability, AQP1 failed to confer a measurable increase in membrane currents (5). Consistent with these results, highly purified AQP1 protein reconstituted into proteoliposomes exhibits high unit water permeability ($P_f \sim 3 \times 10^9$ water molecules $subunit^{-1}$ s^{-1}), whereas permeation by other small solutes or even protons was undetectable (6).

The prevailing view is that AQP1 is a constitutively active, water-selective pore that permits osmotically driven movement of water. A report that forskolin induces a cation current in AQP1-expressing oocytes (7) was not reproduced by multiple other scientific groups. Permeation by CO_2 has recently been proposed, because rates of pH change are about 40% higher in oocytes expressing AQP1 (8). Permeation by O_2, nitric oxide, and other gases is also likely; however, the high background permeability raises questions about the physiological relevance. Thus, although the evidence that AQP1 functions as a water channel is incontrovertible, existence of still undiscovered transport functions cannot be excluded.

Structural Studies of AQP1

Sequence analysis suggested that AQP1 is a six-transmembrane domain integral protein (4). The N- and C-terminal halves of the protein are related sequences comprised of three bilayer spanning domains and connecting loops B and E, which each contain the signature motif Asn-Pro-Ala (Fig. 2, *top*). Cys-189 in loop E was shown to be the site of mercurial inhibition, and Loop C was localized at an extracellular location demonstrating the obverse symmetry of the N- and C-terminal halves of the molecule (9). In the proposed "hourglass" model (10), loops B and E correspond to two hemipores, which overlap midway between the leaflets of the bilayer, creating a constitutively open, narrow aqueous pathway (Fig. 2, *bottom*).

The availability of highly purified red cell AQP1 permitted physical studies of the protein (see Ref. 11 for review). Freeze fracture electron microscopy of reconstituted red cell AQP1 confirmed the proposed tetrameric assembly of AQP1. Reconstitution of purified AQP1 at high concentrations produced highly uniform lattices (membrane crystals) with full retention of the water permeability. Fourier transform infrared analyses of AQP1 membrane crystals demonstrated the lack of β structure and the existence of α-helices oriented at 21–27° from the perpendicular. Electron diffraction analysis of cryopreserved specimens at tilts of up to 60° revealed AQP1 tetramers at a resolution of 3–6 Å with individual subunits containing six bilayer-spanning α helices (12) (Fig. 3, *left*). These physical studies revealed that the intrasubunit structure strongly resembles the proposed hourglass (Fig. 3, *right*). The current challenge is to extend this information to the atomic level of resolution.

Expression of AQP1

Distribution of AQP1 protein in proximal convoluted tubules and descending thin limbs have been defined in rat and human kidney (13, 14). AQP1 is constitutively present in the apical plasma membranes (brush borders) and in basolateral membranes in quantities sufficient to account for the huge volumes of water reabsorbed in the proximal nephron. AQP1 is also present in many capillary endothelia and in multiple water-permeable epithelia including cerebrospinal fluid secretory epithelia of choroid plexus, aqueous

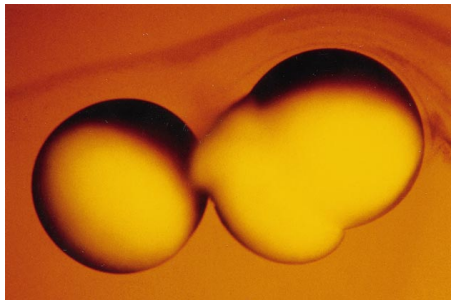

FIG. 1. **Water permeability of AQP1 expressed in *Xenopus* oocytes.** Control oocytes exhibit negligible water permeability in hypo-osmotic buffer (*left*) whereas oocytes expressing AQP1 rapidly swell and explode (*right*).

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[1] The abbreviations used are: AQP, aquaporin; TIP, tonoplast intrinsic protein.

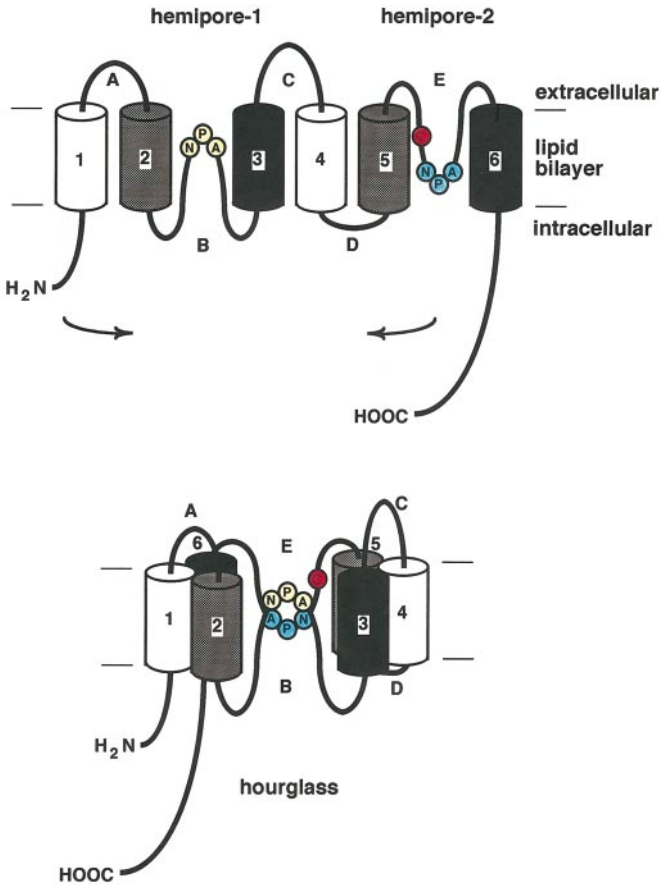

FIG. 2. **Hourglass model for AQP1 membrane topology.** *Top,* each AQP1 subunit contains six bilayer-spanning domains comprised of two obversely symmetrical hemipores (TM1–3 and TM4–6). *Bottom,* when juxtaposed, Asn-Pro-Ala motifs in loops B and E form a single aqueous channel spanning the bilayer (the "hourglass") flanked by the mercury-sensitive residue (Cys-189). Modified with permission from Jung *et al.* (10).

humor secretory, absorptive epithelia in eye, and bile secretory epithelia (15).

A fortuitous observation led to the identification of humans who totally lack AQP1. The Co blood group antigen is a structural polymorphism at the extracellular surface of red cell AQP1. The exceedingly rare Co null individuals were found to have mutations in the *AQP1* gene but surprisingly exhibited no obvious clinical phenotype (16). Nevertheless, targeted gene disruption has revealed that Aqp1 null mice become hyperosmolar after fluid restriction (17).

Mammalian Homologs

Vasopressin was long known to regulate water permeability of the apical membrane in renal collecting duct epithelium, but lack of AQP1 at this site predicted the existence of additional aquaporins (5). Homology cloning by polymerase chain amplification using degenerate oligonucleotide primers has been employed by multiple investigators to clone aquaporin homologs. Efforts have rapidly led to the recognition of at least 10 mammalian aquaporins (Fig. 4). All members of the family contain structural motifs similar to AQP1, but each probably has special features needed for function or regulation.

Correlation of sequences and functional properties showed that this protein family is comprised of two subgroups (18): water-selective homologs (aquaporins) and other homologs that are permeated by water, glycerol, and other small molecules (aquaglyceroporins) (Fig. 4).

Aquaporins—Multiple groups are investigating AQP2, a homolog that is only expressed in the principal cells of the renal collecting duct (see Refs. 1, 19, and 20 for reviews). Vasopressin has long been known to cause the redistribution of intracellular vesicles to the apical surface of principal cells. In a classic study of short-term vasopressin regulation of isolated collecting ducts, trafficking of

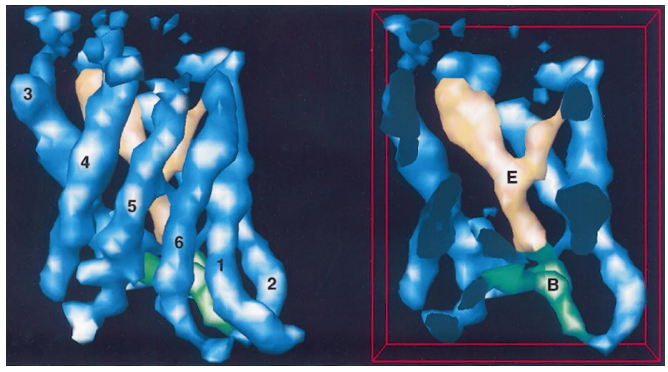

FIG. 3. **Three-dimensional structure of AQP1 at 6-Å resolution established by cryoelectron microscopy.** *Left,* six tilted, bilayer-spanning α-helices in a single subunit. *Right,* cutaway view exposing internal structure reminiscent of the hourglass. Modified with permission from Walz *et al.* (12). Original figure provided by Bernard Heymann, Maurice-Müller Institute, University of Basel, Switzerland.

AQP2 in intracellular vesicles was visualized by immunogold electron microscopy and correlated with measurements of transcellular water permeability (21) (Fig. 5). Thus, multiple studies together indicate that vasopressin binds to a basolateral V2 receptor coupled to adenylyl cyclase; protein kinase A then leads to phosphorylation of sites in the C terminus of the AQP2 protein; AQP2 vesicles are then targeted to the cell surface via the vesicle-targeting proteins causing increased water permeability of the apical membrane. The long term vasopressin regulation of biosynthesis and degradation of AQP2 are also recognized but are not well understood.

AQP2 may be involved in all imbalances of water metabolism (see Refs. 1, 19, and 20 for reviews). Impaired renal water reabsorption is associated with quantitatively reduced AQP2 in diabetes insipidus, a disorder resulting from inadequate levels of vasopressin leading to secretion of large volumes of dilute urine. Nephrogenic diabetes insipidus occurs when the kidney fails to respond to vasopressin, and mutations have been identified in the gene encoding AQP2 (22). Lithium is widely prescribed for treatment of manic depressive disorder. Polyuria is a problematic side effect of lithium therapy, and marked reduction in AQP2 expression occurs in lithium-treated rats (23). Reduced AQP2 expression has also been documented in several other settings including after reversal of urinary obstruction, in hypokalemia-induced polyuria, and in nocturnal enuresis. In contrast, clinical problems with excessive renal water reabsorption are associated with increased AQP2. Congestive heart failure is a life-threatening complication of coronary vascular disease caused by excessive retention of water. Increased expression of AQP2 has been found in rat models of congestive heart failure and is also believed to explain fluid retention in pregnancy (24, 25).

AQP4 is the predominant aquaporin in brain and resides at the perivascular membrane of astroglial cells where it may function as an exit port for excess brain water. AQP4 is also present in glial lamellae surrounding vasopressin secretory neurons where it has been suggested to function as an osmoreceptor (26), in ependymal cells lining the cerebrospinal fluid-filled cavities (27), and in retina where the protein is particularly abundant in Müller cell end-feet adjacent to the vitreous body and vascular endothelium (28). It has been proposed that AQP4 is the molecular basis of square arrays within astroglial membranes (29). Although it was previously reported that protein kinase A and C do not modulate the water permeability of aquaporins, this is now questioned because phorbol diesters produced ~90% reduction in water permeability by oocytes expressing AQP4 (30). Disruption of the mouse *Aqp4* gene resulted in a minor defect in renal concentration but no detectable neurological abnormality (31).

The fifth aquaporin was cloned from a salivary gland cDNA library, and AQP5 resides in apical membranes of type I alveolar pneumocytes as well as in a subset of salivary and lacrimal glands where it may participate in airway humidification and generation of saliva and tears (32). The gene encoding rat AQP5 is being evaluated as a potential gene transfer to damaged secretory glands (33).

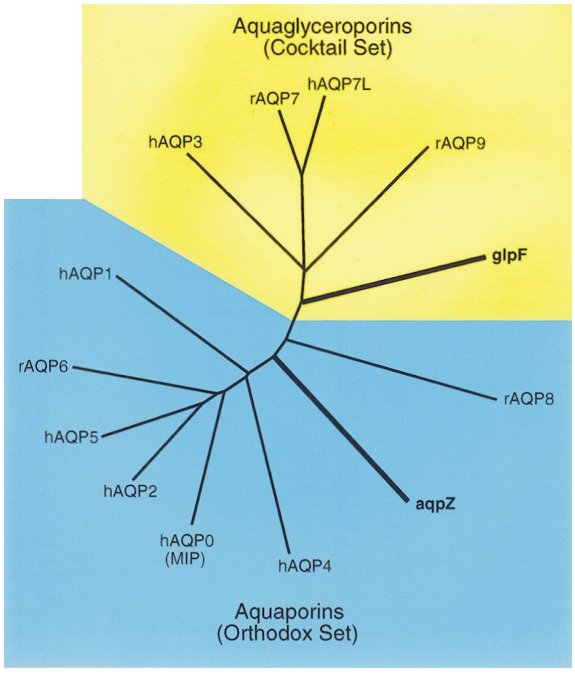

FIG. 4. **Phylogenetic tree of mammalian aquaporins and *E. coli* homologs.** Note the genetic and functional groupings into aquaporins, selective for water (*bottom*) or aquaglyceroporins, permeated by water and glycerol (*top*). *MIP* denotes major intrinsic protein of lens. Generated using ClustalW software (53).

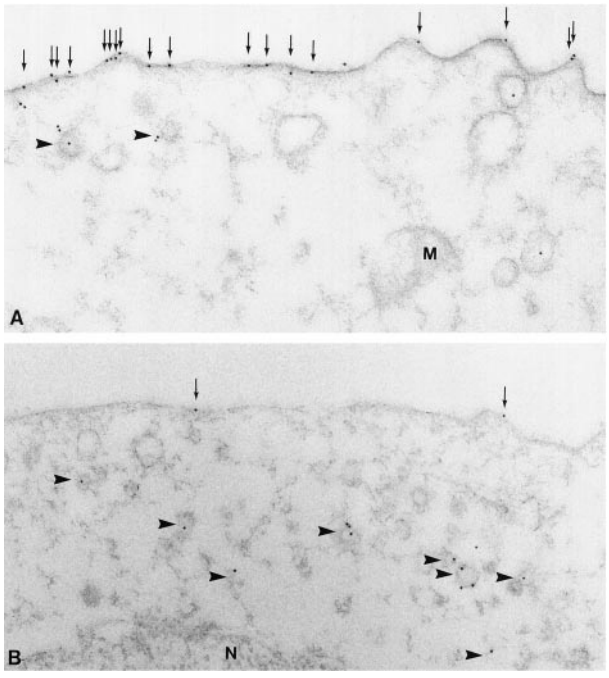

FIG. 5. **Immunogold electron microscopy of rat collecting duct stained with anti-AQP2.** *A,* ultrathin cryosection of isolated rat collecting duct after *in vitro* stimulation with vasopressin. Gold particles are predominantly at the lumenal surface. *B,* similar section from unstimulated collecting duct. Gold particles are predominantly intracellular. × 32,000. *M,* mitochondrion; *N,* nucleus. Figure provided by Søren Nielsen, Department of Cell Biology, University of Aarhus, Denmark.

Other water-selective homologs have been identified and partially characterized. The major intrinsic protein of lens (AQP0) has a low water permeability, but mice with naturally occurring mutations in the gene encoding AQP0 suffer congenital cataracts (34). AQP6 is expressed in kidney but exhibits extremely low water permeability, and its distribution has not been defined (35). The cDNA encoding AQP8 was recently isolated from testis, pancreas, liver, and other tissues (36).

Aquaglyceroporins—Whereas previously cloned aquaporins are only permeable to water, some homologs are permeated by water, glycerol, and other small solutes. AQP3 was noted to be genetically closer to the *Escherichia coli* glycerol transport protein GlpF (see Ref. 20 for review). The structural explanation for how AQP3 may permit transport of water and glycerol is not agreed upon. The existence of independent water and glycerol transporting domains was suggested by one group of scientists; the existence of a single pore was suggested by a second group; a third group incorrectly reported that the molecule was impermeable to water. The abundant expression of AQP3 at the basolateral membranes of principal cells in the collecting duct suggests a role in renal water reabsorption (37). AQP3 is also expressed in multiple other tissues including airways and is particularly abundant in nasopharyngeal epithelium, where roles in mucosal secretions and allergic rhinitis are suspected (32).

Multiple other aquaglyceroporins are now being identified by cDNA cloning and computer search of expressed sequence tagged (EST) cDNA libraries (Fig. 4). A cDNA encoding AQP7 was isolated from rat testis (38). AQP7 may provide a port for water and glycerol as a carbon source and may permit replacement of water by glycerol during cryopreservation of sperm. At the same time, AQP7L, a highly related cDNA, was isolated from human adipose tissue (39), which may facilitate glycerol export during lipolysis. Preliminary evidence supports the existence of another homolog designated AQP9 (Fig. 4).

Multiple Aquaporins in Complex Tissues

Kidney, airways, and eye have been found to contain multiple different aquaporins at specific cellular locations. Several aquaporins have been documented in mammalian kidney (see Refs. 1, 19, and 20 for reviews). These aquaporins presumably function together to provide transcellular water flow. In principal cells of the collecting duct, AQP2 is shuttled to the apical membrane permitting water entry from the lumen, whereas AQP3 and AQP4 reside at the basolateral membranes providing exit ports to the interstitium (37, 40). Pulmonary tissues develop a large capacity for fluid absorption in the perinatal state and provide humidification of the airways and airway secretions (see Ref. 41 for review). To accomplish this, complex developmental expression patterns have evolved (42) with highly specific distribution patterns (32): AQP1 in peribronchiolar capillary endothelium; AQP3 in basal cells of airway epithelium; AQP4 in tall columnar cells; AQP5 in the apical membrane of type I alveolar pneumocytes and salivary acini. The distribution of aquaporins in eye is also complex (43): AQP0 in lens; AQP1 in anterior chamber; AQP3 in bulbar conjunctivae; AQP5 in lacrimal gland and cornea; AQP4 in retina and optic nerve. Regional aquaporin expressions are also found in brain, suggesting integrated functions; AQP1 may mediate cerebrospinal fluid secretion by the choroid plexus, and AQP4 may facilitate reabsorption by ependyma (15, 26, 27).

Non-mammalian Aquaporins

Because entry and release of water from cells and tissues is a fundamental process of life, it is not surprising that aquaporins have been found throughout nature including diverse vertebrate and invertebrate species. Early studies of toad bladder provided some of the first insights that water channels may exist, and homology cloning identified a constitutively active aquaporin in toad bladder and another in frog skin, which is believed to alter skin water permeability during adaptation to salt environments (44). The *Drosophila* mutation *big brain* interferes with development of neurogenic or epidermogenic cells, and AQP_{cic} has been identified in the filter chamber of the sap-sucking insect *Cicadella viridis* (45).

Because plants are rooted, they are entirely dependent upon their local environments for water, and numerous plant aquaporins are being identified. The *Arabidopsis thaliana* EST library was recently found to contain at least 23 different aquaporin homologs (46). Plant homologs appear to be water-selective aquaporins and are comprised of two basic subgroups, which are tightly regulated during development. Plasma membrane intrinsic proteins reside in the plasma membrane and mediate cellular water uptake and release; TIPs reside in the intracellular vacuole (tonoplast) and mediate cellular turgor. A fascinating array of physiological pro-

cesses is now being ascribed to plant aquaporins (see Ref. 47 for review). γ-TIP has been functionally defined and is expressed in the stem. Another aquaporin homolog is involved in the inhibition of self-pollination. Rehydration alters vacuole integrity through α-TIP during seed germination. Aquaporins are involved in other plant processes such as closure of leaf guard cells and root water uptake. Expression of certain plant aquaporins is regulated by light, gibberellic acid, water deprivation, or nematodal infestation. The homolog Nod26 is encoded by a legume gene and is expressed in the symbiosome surrounding nitrogen-fixing bacteria.

The genomes of multiple unicellular organisms are being entirely sequenced at The Institute for Genome Research and elsewhere. Not surprisingly, genes distributed among plant and animal species are present in bacteria. The two major branches of the aquaporin family are each represented by a single homolog in *E. coli*. GlpF, like the mammalian aquaglyceroporins, has been shown to transport glycerol by a porelike mechanism. AqpZ, like mammalian and plant aquaporins, is a selective water channel, and AqpZ is operative during rapid growth (48). The importance of the aquaporin-aquaglyceroporin dualism is being explored in *E. coli* and may provide insight into their presence in higher organisms (Fig. 4). As microbial genomes become sequenced, multiple aquaporin homologs are becoming recognized. The *Saccharomyces* genome contains two open reading frames related to aquaglyceroporins and two others related to aquaporins (49). The slime mold *Dictyostelium discoideum* expresses an aquaporin-like protein in prespore cells (50). The functions of these microbial proteins are currently being evaluated in wild-type organisms and null mutants.

Future Prospects

The molecular studies into the membrane transport of water point to several new challenges. The biophysical roles of aquaporins are also not fully defined, and the possibility of other functions or molecular regulatory mechanisms awaits further study. Likewise, it cannot be assumed that all water transport molecules will be members of the aquaporin family, because some cotransporter proteins carry water along with their major substrate (51). The molecular structure of AQP1 is becoming well understood but is still not at the atomic level of resolution, and development of heterologous expression systems will be needed. Numerous animal and plant homologs remain to be identified, and completion of the human genome project will provide sequences of additional aquaporins but will not identify the sites of expression or physiological functions. It is likely that involvement of aquaporins in clinical disorders will continue to be interesting (52). For these reasons, it seems certain that the aquaporin story is far from complete.

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