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Aquaporins and Aquaglyceroporins:Observing the relationship between structure and function

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AQUAPORINS AND AQUAGLYCEROPORINS: OBSERVING THE RELATIONSHIP BETWEEN STRUCTURE AND FUNCTION

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ABSTRACT

Because water is so critical for life, water transport has been studied extensively. In the last 15 years, molecular research led to the discovery and study of a family of water channel proteins. The aquaporin family of proteins consists of a vast array of integral membrane proteins that transport water in different organisms, organs, and tissues. However, despite having a conserved hourglass structure, aquaporins have slightly different functions. For instance, aquaglyceroporins also transport glycerol. In order to compare the structures of the different aquaporins, homology modeling superimposed different aquaporins on AQP 1. There was overlap in highly conserved regions, such as the NPA motif and the Ar/R region. Oocyte water transport assays verified that AQP 1 enhances oocyte water permeability. Incubation with $HgCl_2$ greatly inhibited AQP 1 function, but incubation with $AgNO_3$ and H_3BO_3 did not. Compared to other aquaporins, NIP 5;1 and NIP 6;1 have extended N-termini. These N-termini were truncated by forty amino acids in order to observe possible roles of the additional residues. Swelling assays showed how NIP 5;1 Δ40N, NIP 6;1, and NIP 6;1 Δ40N are not permeable to water. Glycerol uptake assays showed how these proteins transport glycerol. Although much knowledge has already been gained about aquaporins, further research needs to be continued in order to elucidate more knowledge about aquaporins and related diseases.

INTRODUCTION

Life in an Aqueous Environment

Imagine what life would be like without water. Water plays such a critical role in life that, in its absence, human beings would not be alive long enough to even ponder that scenario. Life on Earth would cease to exist if water were not present since all physiological and biochemical processes of living organisms rely on water.

Water has characteristic chemical properties that allow it to be such a key player in life. Water is a polar molecule that possesses an asymmetric partial charge due to its bent structure[1] (Fig. 1 below).
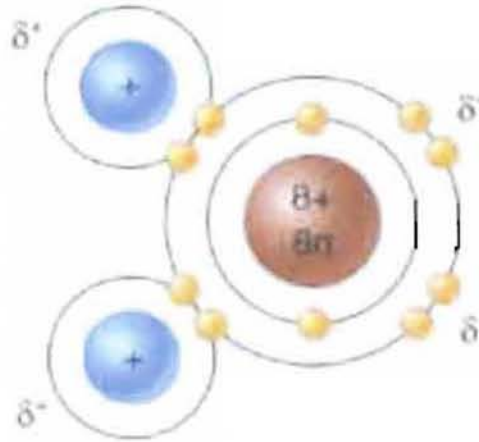
Fig. 1. Molecular geometry of water (blue hydrogens and red oxygen). From www.vrmedialab.dk/pr/students/studentprojects/chemistry.html.

The oxygen atom has a partial negative charge while the two hydrogen atoms have partial positive charges. This polarity allows the formation of several hydrogen bonds between the hydrogen of one water molecule and the oxygen of another[1]. The polarity of water and its ability to make a network of hydrogen bonds contribute to its property as a solvent for hydrophilic biomolecules, allowing for rapid diffusion, interaction, and facilitation of biochemical reactions.

Another outcome of chemistry in an aqueous environment is the formation of lipid bilayer membranes that constitute cellular and subcellular compartments. Membranes are composed principally of lipids and proteins and are fluid and dynamic in nature[1]. Membrane bilayers are amphipathic structures, containing both hydrophobic and hydrophilic components. The nonpolar core of the membrane presents an energetic barrier to the diffusion of polar molecules and is fundamental to the formation of cellular and subcellular compartments. The nonpolar core of the bilayer impedes the natural flow of polar molecules, such as water, across

these membranes. Despite this restriction to diffusion, membranes do exhibit water

permeability, and because of the high concentration of water (55.5 M), diffusion does occur in

response to osmotic or pressure gradients[2] (Fig. 2 below). However, due to the hydrophobicity

of the lipid bilayer core, the rate of simple diffusion is slow with a high energy of activation.
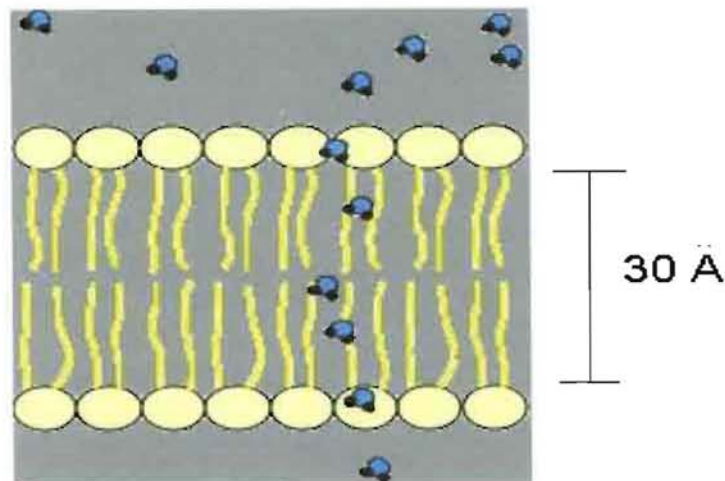
Fig. 2. Simple diffusion of water (blue and black) across bilayer
membrane (yellow). Courtesy of Dr. Dan Roberts.

Water Transport and Membrane Proteins

Although water appears to be in abundant supply, living organisms depend on various

mechanisms to transport and manage the water content of cells and tissues in response to osmotic

and pressure gradients. For example, in humans, because water makes up such a big component

of the body (60% in males and 50-55% in females[4]), the body must have some mechanism to

efficiently and bidirectionally transport water into and out of the cells in the various tissues. This

movement of water is important for a variety of essential physiological processes, such as

production and reabsorption of cerebrospinal fluid, concentration and dilution of urine, and

salivation[16].

Proteins constitute an important functional component of membranes, providing a

facilitated aqueous pathway that allows the flow of water and polar solutes across the

hydrophobic membrane bilayer. For these transport processes to occur, proteins must traverse

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the membrane. Such proteins are referred to as "integral membrane proteins" that span the entire width of the membrane and provide selective conduits for water and metabolite transport (Fig. 3 below). These protein channels, or pores, also serve as "selective gates," allowing the transport of some solutes but not others, and allow for regulation by posttranslational mechanisms, such as protein phosphorylation[7].
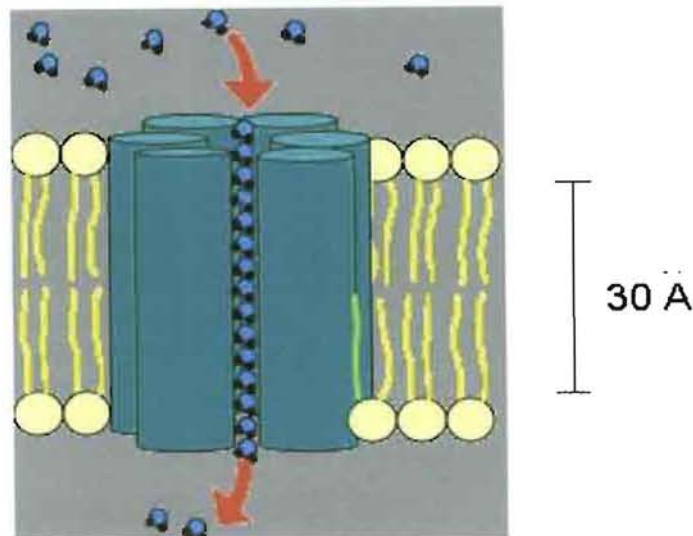
Fig. 3. Facilitated diffusion of water (blue and black) across bilayer membrane (yellow) through transmembrane protein channel (green). Courtesy of Dr. Dan Roberts.

Discovery of Aquaporins

It had been long conjectured that the cell membrane must have openings to allow the rapid transport of water and other particles into and out of the cell. In 1896, Overton concluded that the lipid solubility of the solute was a very important factor of permeability[7]. Because small hydrophilic solutes crossed the membrane in an inverse relation to their sizes, Overton proposed that the plasma membrane must be mainly lipid with aqueous patches[7]. Stein and Danielli further clarified these "aqueous patches" in 1956 when they suggested that the flow of water and other ions was due to hydrophilic pores[7]. Despite these early ideas, it was only in the past fifteen years that molecular research on water channels has truly advanced. Although aquaporins were

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not categorized until the 1990's, their water transport activity has been studied since the nineteenth century[7]. Utilizing simple technologies, Nägeli, Pfeffer, and others studied plant protoplasts' responses to hypertonic solutions[7]. When placed in such media, the protoplasts shrank due to the rapid efflux of cellular water. Eventually, water permeability was observed in other cells, including marine eggs and RBCs[7].

In 1970, Robert Macey discovered that water transport in human red blood cell (RBC) membranes could be inhibited by mercurials[11]. Since these compounds do not inhibit simple diffusion of water through bilayers, it was concluded that the mercurials were exerting their effects on membrane protein components and not lipids[8]. After the observation that reducing agents restored the membrane's water permeability, Macey proposed that a free sulfhydryl (SH) must be present in the water channel protein's pore in the form of a cysteine amino acid[11]. Hg^{+2} binds to the cysteine side chain and blocks the pore[8].

In the late 1980's, Gheorghe Benga observed the first water channel protein in situ in the human RBC membrane[7]. Benga examined the binding of ^{203}Hg-PCMBS to the membrane proteins of resealed red blood cell ghosts[7]. In order to avoid having the ^{203}Hg-PCMBS bind to all sulfhydryls, SH groups not involved with water permeability were blocked by N-ethyl-maleimide (NEM)[7]. Because water permeability was inhibited, Benga hypothesized that one of the bands on the SDS-PAGE gel was a water channel protein[7]. Gregory Preston, working in the laboratory of Dr. Peter Agre at Johns Hopkins, eventually cloned the cDNA of a major RBC protein known as CHIP28[8]. Working with Bill Guggino and Agre, Preston studied the water transport properties of CHIP28 in *Xenopus laevis* oocytes by performing oocyte swelling assays[10]. They microinjected oocytes with either 0.05 µL water or with up to 10 ng of in vitro-transcribed CHIP28 RNA[10]. After 24 hours post injection, they demonstrated the expression of the CHIP28 protein by doing an immunoblot using an antibody to the carboxyl-terminal

cytoplasmic domain of CHIP28 (anti-CHIP)[10]. This protein increased in abundance for up to 72 hours[10].

Then they measured the corresponding water permeability of CHIP28-expressing oocytes by assessing changes in cell volume with videomicroscopy upon transfer of both sets of oocytes from a 200 to a 70 mosm solution[10]. Because *Xenopus* oocytes normally have very low water permeability, the control oocytes without aquaporin cDNA swelled at a very low rate in the presence of hypotonic media[10]. On the other hand, when placed in the hypotonic media, the test CHIP28-expressing oocytes swelled to 1.3 to 1.5 times their initial volume and quickly lysed[10]. Based on the changes in size of the oocytes, they calculated the rates of swelling and the coefficients of osmotic water permeability (P_f) [10]. Agre concluded that the presence of the protein resulted in the drastic increase in water permeability so he termed this family of proteins "aquaporins," meaning "water pore," and CHIP28 was renamed "Aquaporin 1" (AQP 1)[3].

Phylogeny of Aquaporins

Since the initial discover of AQP 1, aquaporins (AQPs) have been found in a wide variety of species, ranging from bacteria to mammals[9]. More than 150 aquaporin genes have been sequenced, including at least ten human aquaporins. In addition to the water-selective AQPs, other AQP-related proteins have been found that are multifunctional by transporting other uncharged solutes such as glycerol[9]. Thus, within the aquaporin family of proteins, two subsets of proteins exist- classic aquaporins that transport only water and aquaglyceroporins that allow glycerol to pass through the pore in addition to water[6]. Although aquaporins have the same general structure, differences in a few amino acids play a critical role in distinguishing aquaglyceroporin transport from aquaporin transport. This is discussed in much greater detail below. It has also been observed that the various mammalian aquaporins do not exhibit identical water permeability[6].

The phylogeny of bacterial aquaporins is simple with only one glyceroporin and one classical aquaporin apparent. In *Escherichia coli*, a glycerol facilitator GlpF was discovered that provides a facilitated pathway for glycerol uptake for metabolic purposes[9]. Later an aquaporin gene *aqpZ* was identified in *E. coli* and was proposed to play a role in surviving in hypotonic environments[16].

The *aqpZ* gene encodes for a polypeptide that was 28-38% identical to mammalian aquaporins and which has the conserved hourglass topology of other aquaporins[19]. In *Xenopus* oocytes injected with *aqpZ* cDNA, a 15-fold increase in water permeability occurred[19]. Because of these results, AqpZ is believed to be a prokaryotic water channel[19]. In contrast, GlpF exhibits minimal water permeability while transporting glycerol instead[20].

In contrast to microbes, complex multicellular organisms have a more diverse array of AQP genes. Due to the higher organization of cells, tissues, and organs in mammals, mammalian aquaporins are distributed unevenly. For instance, human aquaporins are found in various quantities and locations in different tissues, such as those in the kidney, eye, and brain[6]. At least ten genes of the human aquaporin family, AQP0- AQP9, have been sequenced[9]. While all ten have water permeability, only AQP3, AQP7, and AQP9 can additionally transport glycerol[9]. A phylogenetic tree of aquaporins visually shows how closely AQP gene sequences are to each other (Fig.4).
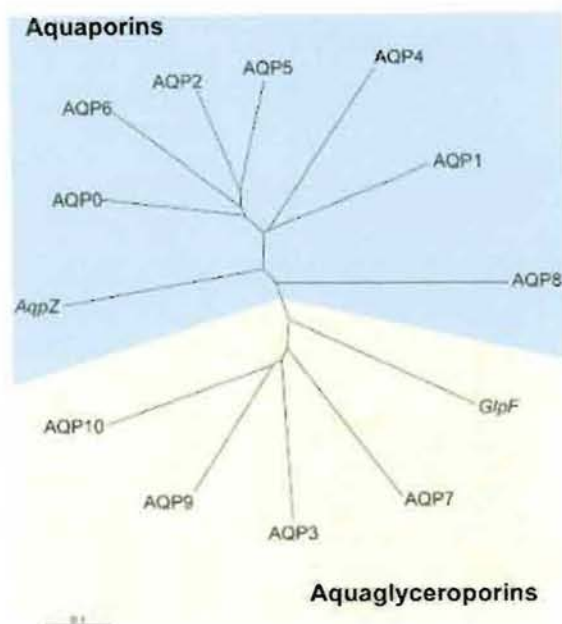
Fig. 4. Human aquaporin gene family and *E. coli* homologs.
Subsets correspond to classic aquaporins and aquaglyceroporins.
From Agre, P. The Aquaporin Water Channels. *Proceedings of the American Thoracic Society* 3 (2006) 5-13.

Because of their fundamental role in water transport, aquaporins have been a hotspot for research because of their association with various medical conditions dealing with water homeostasis[16]. As discussed below, a number of pathological conditions have been linked to defects in aquaporin function.

In addition to mammals and microbes, higher plants also rely on aquaporins for the necessary water uptake for biochemical processes, such as photosynthesis. Since plants need local environmental water in order to synthesize food, to adapt to osmotic stress, and to regulate cell volume homeostasis, plants have been known to express several aquaporins[11]. For example, *Arabidopsis thaliana* has at least 35 aquaporin homologs[13].

Plant aquaporins can be further divided into four subfamilies[21]. These plant aquaporins include plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic membrane proteins (TIPs), nodulin 26-like intrinsic proteins (NIPs), and small basic intrinsic proteins (SIPs)[21]. These subfamilies are also further divided (Fig. 5)[14]. *Arabidopsis* has 13 PIPs, 10 TIPs, 9 NIPs, and 3

SIPs[13].
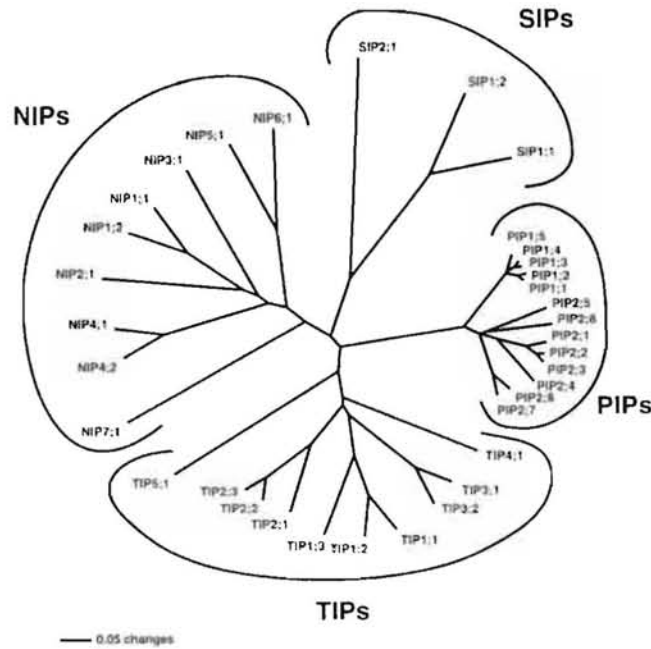
Fig. 5. Plant aquaporin gene family. An unbranched phylogenetic tree of the four subfamilies of plant aquaporins are PIPs, TIPs, NIPs, and SIPs. From Johanson, U. *et al.*, The Complete Set of Genes Encoding Major intrinsic Proteins in Arabidopsis Provides a Framework for a New Nomenclature for Major Intrinsic Proteins in Plants. *Plant Physiology* 126 (2001) 1358-1369.

Like human aquaporins, *Arabidopsis* aquaporins generally show some preferential expression in certain organs. For instance, some are expressed in the roots while others are expressed in the flowers. However, cells heavily involved with large fluxes of water, such as root epidermal cells, xylem parenchyma cells, and phloem companion cells, have a high amount of plant aquaporins[14]. Like mammalian aquaporins, plant aquaporins have varying water permeabilities[13]. In addition to transporting water, some plant aquaporins are also permeable to other solutes, such as glycerol and urea[13]. NIPs have been found to represent the class of aquaglyceroporins in plants. For example, soybean Nodulin-26 functions as a water channel and glycerol permease in *Xenopus laevis* oocytes[37], and the *Arabidopsis* homologue, AtNIP1;1, and AtNIP1;2 show glycerol permeability upon expression in yeast[13].

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To investigate the functional role of aquaporin activity in plants, Ralf Kaldenhoff genetically modified *Arabidopsis thaliana* so that it would have only 20% of the normal level of the PIP1b group of aquaporins[11]. Despite the diminished amount of expressed aquaporin, these mustard plants compensated by increasing root tissue mass to increase surface area to allow adequate uptake of water through the roots[11].

Aquaporin Function and Pathophysiology

In considering their cellular function, it is important to recognize that aquaporins serve as major water transport proteins that increase the rate of water flow dramatically across the membrane. For instance, AQP 1 can enhance the water permeability of a *Xenopus* oocyte's membrane by 100 times[6]. Because of the bidirectional water flow through aquaporins, a cell's water homeostasis can be rapidly modulated in response to osmotic gradients[6]. In humans, aquaporins greatly impact tissues that are heavily involved with the production and secretion of bodily fluids[8]. The various human aquaporins are found in varying amounts in different tissues and organs in the human body. As expected, they contribute to the contrasting functions of those organs.

Kidney

Due to its important role in water reabsorption and urine concentration, the kidney contains at least seven types of aquaporins[18]. Because of specialized functions, renal aquaporins are localized in different parts of the nephron and collecting duct[18] (Fig. 6). The capillaries in the glomeruli of the kidney must filter upwards of 180 liters of filtrate each day so that the final daily urine output is only one liter[6].
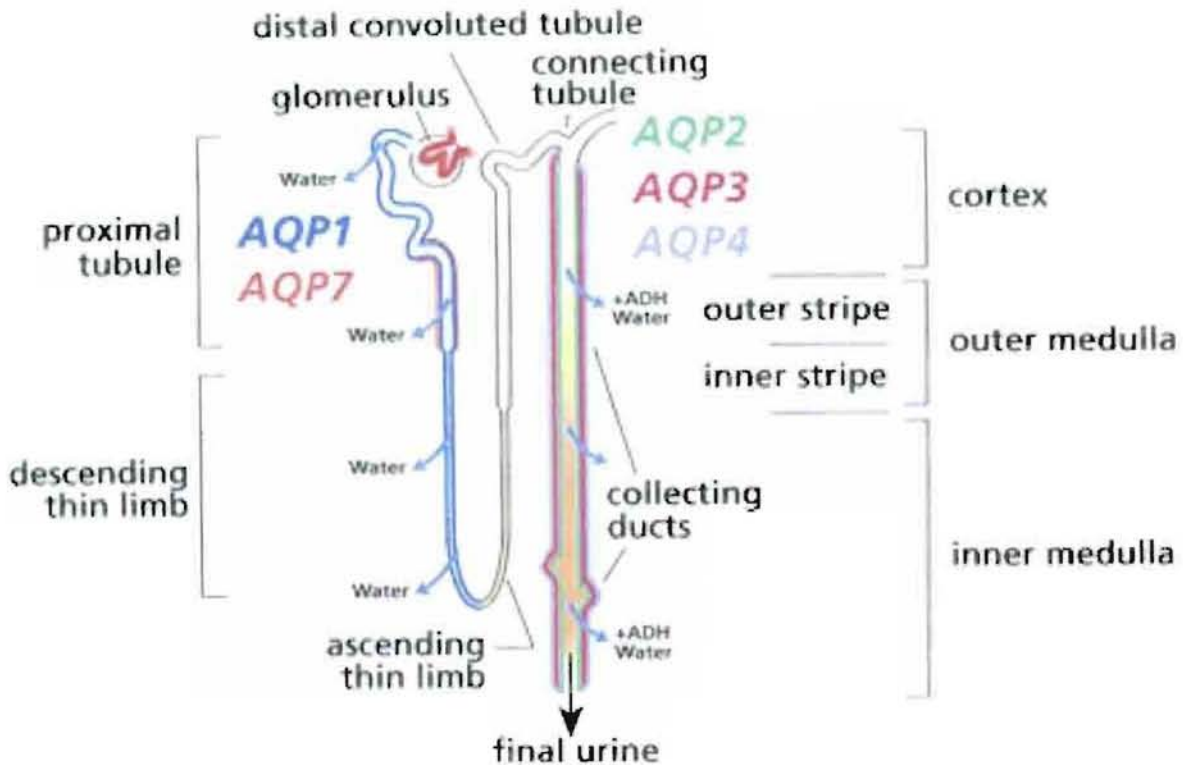
Fig. 6. Aquaporins in a nephron in a kidney. The colors of the figure correspond to the colors of the AQPs.

Located abundantly in the apical and basolateral sides of the membrane of the proximal convoluted tubules and descending limbs of the loop of Henle, AQP 1 comprises about 1% of all membrane proteins in the kidney cortex[18]. Due to differences in osmotic permeability, AQP 1 guides the path of water molecules from inside the hypotonic proximal tubule to the hypertonic interstitial fluid surrounding the nephron[18]. It is responsible for approximately 70% of the kidney's water reabsorption[3]. As a result, urine becomes more concentrated[18]. Thus, AQP 1 plays an important role in handling water and concentrating urine in the kidney.

The physiological importance of AQP 1 has been examined in mice and humans who completely lack AQP 1[18]. Six individuals have mutated AQP 1 proteins, in which alanine-45 was converted to valine[18]. These individuals lack the Colton blood-group antigen that is normally found on AQP 1[16]. Although the Colton-null individuals do not normally display clinical symptoms, they demonstrated a moderate urinary concentrating defect when they were

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stressed with water deprivation[15]. In contrast to AQP 1-null humans, AQP 1-null mice cannot concentrate their urine at any time[16]. Therefore, it is speculated that their bodies must compensate for the lack of AQP 1 during unstressed times[16]. At this time though, the mechanism of compensation is not concrete.

In the principal cells of the collecting duct, AQP 2 is necessary for kidney to reabsorb more water from the kidney's filtrate and to concentrate urine[7]. Unlike AQP 1, AQP 2 requires the presence of vasopressin in order to localize to the apical membrane[17]. Vasopressin, an antidiuretic hormone (ADH), promotes water reabsorption[32]. By binding to receptors in the basolateral plasma membrane, vasopressin activates adenylate cyclase, which raises cAMP levels leading to an activation of protein kinase A[14]. A cAMP-dependent protein kinase A phosphorylates AQP 2 in intracellular vesicles[14]. This phosphorylation stimulates AQP 2 to fuse with the apical surface of the plasma membrane, increasing the plasma membrane water permeability[14].

Plasma osmolarity and blood pressure/ volume regulate vasopressin secretion[32]. In times when blood osmolarity falls below a certain threshold value, vasopressin is not released from the posterior pituitary[32]. Without vasopressin, AQP 2 is restricted to intracellular vesicles[17]. Thus, water is not as actively reabsorbed. On the other hand, when either blood osmolarity rises above the threshold value or blood volume decreases, vasopressin is actively released in order to preserve water[32]. With vasopressin, AQP 2 increases the water permeability of the collecting duct apical membrane by five times[17].

Unlike AQP 1-null individuals who can lead lives without any symptoms, humans who lack functional AQP 2 suffer from a disease called nephrogenic diabetes insipidus (NDI)[17]. Mutations in the transmembrane and pore-forming domains of the AQP 2 gene prevent proper folding and cellular trafficking of the protein[17]. As a result, these individuals with NDI produce

high amounts of dilute urine and have a daily urine output of twenty liters[6]. These people are chronically thirsty and consistently in danger of dehydration[8].

Although its functional importance in the kidney is less emphasized, the aquaglyceroporin AQP 3 contributes to the reabsorption of water into the blood[5]. AQP 3 is different from AQP 2 because it is located in the basolateral membrane of principal cells. As such, AQP 3 allows reabsorbed fluid to exit the collecting duct[16]. The physiological function of AQP 3's glycerol permeability in the kidney has not been identified[16].

Despite being genetically similar in sequence to AQP 2, AQP 6 has extremely low water permeability[5]. While most aquaporins are located in the cell membrane, AQP 6 can be found in intracellular vesicles in the intercalated cells of the collecting duct[34, 15]. Also, unlike AQP 1 and many other aquaporins, it is not inhibited by mercurials[34, 15]. Rather, oocytes expressing rat AQP 6 show increased water permeability in the presence of Hg^{+2}. Because AQP 6 conducts anions well, it may play a role in acid-base regulation[16]. AQP 6 colocalizes with H+-ATPase in acid-secreting α-intercalated cells of renal collecting duct[34]. At pH levels below 5.5, AQP 6 rapidly conducted anions[34].

Eye

AQP 0 is primarily found in the fiber cells of the lens and was the first member of the Major Intrinsic Protein/ Aquaporin family to be identified[32]. In the lens, AQP 0 helps fiber cells to maintain transparency[6]. Point mutations in the AQP 0 gene result in congenital cataracts because the AQP 0 proteins were either not properly folded or not properly trafficked to the plasma membrane[32, 17]. AQP 0 has a low water permeability that is 40 times less efficient than that of AQP 1[32]. Because there is extensive intercellular coupling in the lens, Andreas Engel proposed that AQP 0 might play a part in cell-to-cell adhesion, which is unique to AQP 0[32]. Similar to membrane junctions, AQP 0 tetramers tightly associate with each other head-to-

head[32].

Brain

It is critical to regulate the volume of fluid inside the cranium because of the physical restraints from the skull[16]. In the brain, aquaporins allow water to flow reversely from blood or cerebrospinal fluid (CSF) into the brain, which can lead to hydrostatic pressure during edema[8]. Serious damage can arise if there is too much pressure on the brain. For example, hydrocephalus, a condition in which there is excess fluid on the brain, occurs if excess CSF accumulates.

The choroid plexus, capillaries in the ventricles of the brain, produces CSF, which circulates through the subarachnoid space in the brain. CSF, which mainly protects the brain, passes through AQP 1 located in the apical membrane microvilli of the choroid plexus[16]. After circulating through the lateral, third, and fourth ventricles, CSF is then reabsorbed. Production and reabsorption of CSF should be equal in order to avoid problems with the buildup of pressure on the brain.

Because AQP 4 is abundant in astroglial cells, which maintain the blood-brain barrier, AQP 4 may play a role in water crossing that barrier[17]. Nagelhus speculated that AQP 4 might also allow fluid to cross cell membranes in response to changes in potassium concentrations because of its association with potassium channels[17].

Since AQP 4 is the predominant water channel in the brain, it could possibly provide a mechanism to relieve brain edema, which follows water intoxication and ischemic stroke[35]. Cerebral edema increases intracranial pressure and has a high mortality due to the skull's unyielding structure[27]. In AQP 4- knockout mice, the absence of AQP 4 protected them from cytotoxic edema because water could not enter the brain parenchyma as efficiently[35]. As a result of this, AQP 4 inhibitors have been sought since they may reduce cytotoxic brain swelling during

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trauma[35].

Lung

Although less is known about the role of aquaporins in the lung, it is very important to maintain water homeostasis in the lung[15]. If fluid balance is altered, gas exchange may be impaired[15]. Four aquaporins have been found in the respiratory tract of humans, rats, and mice[15]. These include AQP 1, AQP 3, AQP 4, and AQP 5[15].

In humans, the apical and basolateral membranes around the airways contain AQP 1 while the apical membrane of bronchiolar epithelium has AQP 3[15]. AQP 4 is found in high levels in the basolateral membrane of ciliated columnar cells in bronchial, tracheal, and nasopharyngeal epithelium[15]. In contrast, AQP 5 is plentiful in the epithelium of the bronchial tubes and nasopharynx[15].

As in cases of deficient aquaporins in other organs, symptoms, such as acute pulmonary edema, appear when AQP 1, AQP 4, or AQP 5 deletions occur[15]. Also, because aquaporins might be involved with regulating the airway surface liquid layer, aquaporin dysfunction might be linked to cystic fibrosis[16]. Furthermore, asthma might be partially related to AQP 5 distribution in the lung because of the proximal location of some forms of human asthma to the site of AQP 5 on chromosome 12q[17]. Because the functions of the different lung aquaporins are not well known, these speculations are not concrete, and more work, particularly with AQP-knockout animal models, is needed.

Secretory Glands

AQP 5 permits water flow from the apical membrane of acinar cells in the ducts of salivary, sweat, and lacrimal glands, which are involved in fluid secretion[15]. A lack of AQP 5 in mice resulted in decreased salivation[15]. Because of the distribution of AQP 5 in secretory glands, AQP 5 may contribute to symptoms of Sjögren's syndrome, such as dry eyes and dry

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mouth[15]. Tsubota et al. reported that defective AQP 5 trafficking is responsible for those symptoms of Sjögren's syndrome[28]. Hope for treatment of dry eye exists if AQP 5 can be properly distributed to the apical membrane[28].

Aquaglyceroporins: functions of solute transport in mammalian physiology

Unlike the aquaporins previously discussed, AQP 7 and AQP 9 are aquaglyceroporins[8]. Due to its presence in adipocytes and its glycerol permeability, AQP 7 plays a key role in the cell's ability to utilize energy when triglycerides are metabolized into fatty acids and glycerol[8]. AQP 9 then comes into factor by providing a route through which glycerol can pass in order to enter hepatocytes so it can be used during gluconeogenesis to synthesis glucose[8]. An increase in AQP 9 after a period of fasting, which increases blood glycerol levels, supports this speculated function[22]. AQP 9 may also transport urea from the liver after amino acid deamination[22]. While AQP 9 has a broad solute permeability, it does not transport water as well as other aquaporins[22].

As one can see, without proper transport of water, many tissues and organs would develop problems and lead to different diseases. By allowing water molecules to rapidly traverse the membrane bilayer, aquaporins prevent these defects from occurring frequently. Furthermore, multifunctional aquaglyceroporins serve additional important metabolic functions. Because of their ubiquitous existence, aquaporins are essential in many organisms, their tissues, and their organs.

Aquaporin Structure

Despite differences in transport rates and selectivity, aquaporins generally have a conserved structure and membrane topology referred to as the "hourglass fold." The hourglass fold consists of six tilted transmembrane α-helices, organized into two tandem repeats of three helices. The helices are separated by five loops, two of which contain conserved "NPA bases" and insert into the bilayer[2] (Fig. 7). Loop C connects the molecule's two repeated halves, which

are oriented at 180° to each other[12, 11]. The hydrophilic amino and carboxyl termini face the

cytoplasm[25]. The 30°-tilt of the six transmembrane helices and the two membrane-inserting but

non-membrane-spanning loops (loops B and E) are responsible for its hourglass fold[36] (Fig. 8).

Loops B and E each have a conserved asparagine-proline-alanine (NPA) motif[17].
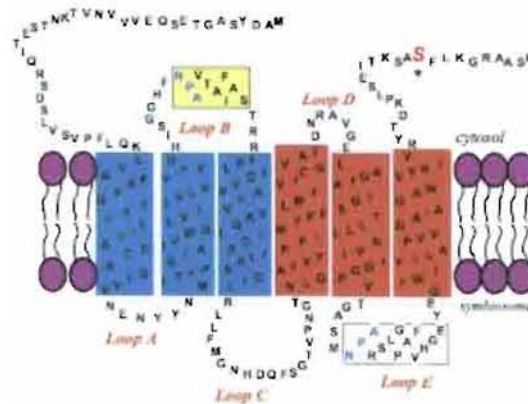
Fig. 7. The conserved topology and position of conserved
bases of Nodulin 26. Courtesy of Dr. Dan Roberts.
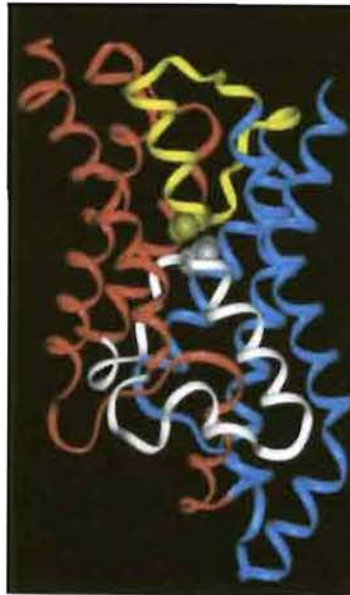
Fig. 8. Hourglass fold of GlpF, using the same color
coordination scheme as in Fig. 7. Courtesy of Dr. Dan
Roberts.

In the hourglass model of aquaporin, loops B and E enter the bilayer opposite to each

other, loop B from the intracellular side and loop E from the extracellular side[8]. Overlap of

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loops B and E creates an aqueous pore[8]. This aqueous pore is shaped like a dumbbell when viewed from the side and consists of three components, an extracellular vestibule, a selectivity filter, and an intracellular vestibule[25].

The extracellular vestibule, shaped like a cone, is widest at the mouth with a diameter of approximately 15 Å[25]. Then over a distance of about 20 Å, the extracellular vestibule tapers until it reaches its narrowest point of 2.8 Å, which is just big enough for a single water molecule to pass[25, 11]. While most aquaporins also have a phenylalanine-leucine pair at this narrowing, almost all glyceroporins have a leucine-leucine pair at this location[12]. This difference could be due to glyceroporins' need for a larger pore to accommodate small solutes[12].

At the pore's narrowest point, the selectivity filter begins[25]. Short α-helices of Loops B and E create a partial positive charge in the center of the membrane[17]. Contributing to this positive charge is Arg-195, an amino acid that is highly conserved among all aquaporins[17]. Also, His-180 provides a second positive charge when it is protonated at an acidic pH[17]. Together, these three positive areas block the passage of protons, a critical event that will be discussed shortly[17]. Beyond the constriction site, the pore begins to widen to approximately 4 Å for the next 15 Å[25]. While the walls of transmembrane helices 1, 2, 4, and 5 are hydrophobic, the two highly conserved polar asparagines of the two NPA motifs come together about 8 Å past the constriction point[17]. Hydrophilic carbonyl oxygens, extending away from the NPA motifs, line the pore[25]. Also near this pore is Cys-189, providing the sulfhydryl that binds with mercurials[8]. The proximity of Cys-189 near the pore explains why water permeability is inhibited when mercurials are present[8]. The narrow water channel is blocked by the large mercurial[8]. At the end of the selectivity filter is His-76, which is another highly conserved residue[25]. The cone-shaped intracellular vestibule then widens to a diameter of 15 Å[25].

In addition to the hourglass fold, all aquaporins have the signature NPA motif in loops B and E[21]. When water is in bulk solution, it is liquid because of hydrogen bonds between adjacent water molecules[8]. The close vicinity of water molecules facilitates the movement of protons along them in what is known as the Grotthus effect[11, 8]. Fortunately, when water forms hydrogen bonds with the side chains of the asparagines, the NPA region breaks the water chain by flipping the water molecules and, consequently, reorienting the water's dipole[21]. As a result, protons cannot pass through aquaporins, despite the hydrogen-bonded network of water.

This is crucial for the maintenance of proton gradients[8]. Proton gradients are necessary to energetically drive biochemical processes of energy transformation, such as oxidative phosphorylation and photosynthesis[1]. They are also essential for maintenance of membrane potentials and intracellular pH[12].

In addition to the necessity of proton gradients, it is important that protons are repelled so that organisms do not become acidotic[11]. Acidosis would become a problem if the kidneys reabsorbed acid along with water. Because aquaporins are not permeable to protons, the kidneys can reabsorb hundreds of liters of water from the glomerular filtrate while excreting acid[17].

Besides the NPA motifs, aquaporins have another conserved region referred to as the aromatic arginine region (Ar/R)[21]. The Ar/R region is located on the extracellular side of the pore[21]. It is composed of arginine and cysteine residues from Loop E, phenylalanine from Helix 2, and histidine of helix 5[21]. This conserved region sterically excludes large molecules from entering the pore and provides a size selectivity filter (2.8 Å diameter)[21]. It also allows water to pass through the pore in a single-file manner by shedding other water molecules[21]. Water molecules are kept in an energetically favorable condition by forming critical hydrogen bonds within the pore[21].

PURPOSE

After literary research on the phylogeny, function, and structure of various aquaporins, lab experiments were performed in order to further study aquaporins directly. The first purpose of lab experimentation was to investigate differences in the structural and functional properties of robust (AQP 1 and AQP 2), low activity (AQP 0) aquaporins, and aquaglyceroporins (AQP 3). Water transport assays were done so that the ability of AQP 1 to increase water permeability could be observed. The second goal was to examine the ability of various compounds to inhibit AQP 1 in oocytes. The third aim was to study the functional importance of an unusual N-terminal extension in AtNIP6;1, a plant glyceroporin.

MATERIALS AND METHODS

Homology Modeling

A computational program called Molecular Operating Environment (MOE, Chemical Computing Group, Quebec, Canada) allows the three-dimensional structures of various aquaporins to be investigated. MOE relied on the fact that, because two proteins in the same family usually have similar amino acid sequences, enough of the secondary structural elements should also be similar.

For homology modeling purposes, AQP 1 was used as the structural template since its three-dimensional structure has been solved[25]. The AQP 1 amino acid sequence was aligned with those of other aquaporins. Using the best intermediate algorithm, MOE made ten individual approximations as to what the other aquaporin's three-dimensional structure was, evaluated them separately, and selected the most energetically favorable one[21]. Then in order to hypothesize how various aquaporins differ structurally, the structures of AQP 1 and the other aquaporin were superimposed in order to show structural similarities and differences.

<u>cRNA Synthesis</u>

In order to use *Xenopus laevis* oocytes as expression vectors, cRNA of AQP 1, Nodulin 26, NIP5;1 Δ40N, NIP6;1, and NIP6;1 Δ40N had to first be synthesized (Δ40N stands for the deletion of forty amino acids in the N-terminus). Through reverse transcription, cDNA was cloned from total RNA samples. AQP 1 was cloned in Dr. Peter Agre's lab[10]. After being cloned, amplified by PCR, and gel-purified, the cDNAs were individually inserted in pXβG-FLAG plasmid vectors, downstream of the T3 promoter which drives transcription by the binding of T3 RNA polymerase[21, 37]. 5' and 3' untranslated regions of the *Xenopus* β-globin mRNA surrounded the genes in order to facilitate the expression of the cRNAs in the *Xenopus* oocytes[37]. An N-terminal FLAG epitope was ligated into the vectors so that gene products could be identified in a western blot using anti-FLAG antibodies[21].

The pXβG-FLAG plasmid vectors, each containing a gene of interest, were linearized at a restriction site located on the 3' end of the cDNA open reading frame[21]. cRNA was produced by *in vitro* transcription using T3 RNA polymerase to synthesize the cRNA of AQP 1, Nodulin 26, NIP5;1 Δ40N, NIP6;1, and NIP6;1 Δ40N. The synthesized cRNAs were analyzed by gel electrophoresis on 1% agarose (Fig. 9 & 10), and the cRNA was stored at -80°C until oocyte injection.

Fig. 9. 1% agarose gel electrophoresis . of cRNAs. Lane 1, Nod26; lane 2, NIP 6;1; lane 3, AQP 1; lane 4, GM Nod 70
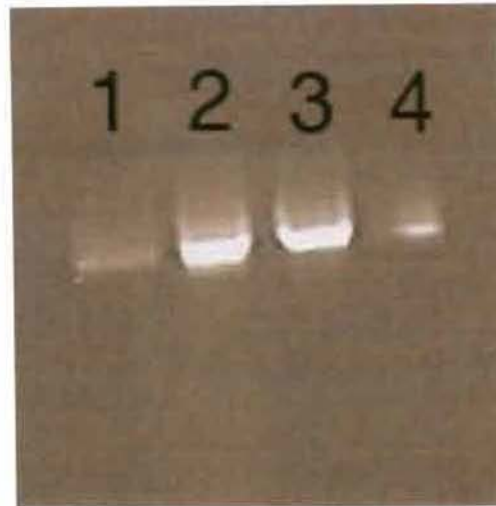
Fig. 10. 1% agarose gel electrophoresis of cRNAs. Lane 1, NodS-FLAG; lane 2, NIP 5;1 Δ40N; lane 3, NIP 6;1-FLAG; lane 4, NIP 6;1 Δ40N

Oocyte Harvesting and Preparation

In the animal facility at the University of Tennessee (Knoxville, TN, USA), surgery was performed on an adult *Xenopus laevis* female. The frog was anesthetized in tricaine solution (MS222, 20μM/ kg) at room temperature for 5-10 minutes. The frog was placed on a sterile surface, ventral side up, and a 1.5 cm incision was made through the skin and muscle layers of the lower abdomen. 5 mL of oocyte sacs were harvested with a sterile scissor and placed in 5 mL Frog Ringers solution without calcium (96 mM NaCl, 2 mM KCl, 5 mM $MgCl_2$, 5 mM HEPES at pH 7.6).

The oocyte lobules were cut up into smaller pieces, and the follicular layer was digested in 10 mL collagenase solution (2 mg collagenase/ mL Frog Ringers without Ca^{+2}). The oocytes were incubated at room temperature for two hours while they were defolliculated. Once 70-80% of the oocytes were without collagen, the oocytes were thoroughly washed with Frog Ringers with Ca^{+2} 4-5 times until the supernatant solution was clear. The oocytes were then incubated in Frog Ringers with Ca^{+2} at 16°C for 2 hours. Under a dissection microscope, 100 stage V and VI

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oocytes that had a distinct light-colored vegetal pole and a dark-colored animal pole were collected and stored at 15°C in Frog Ringers solution until microinjection was performed.

cRNA Microinjection

In order to observe effects on the permeability of various aquaporin constructs, the oocytes had to first be injected with cRNA, as done previously[21]. Oocytes were injected with either cRNA or RNase-free water. Injection pipettes were prepared by breaking the tips of 3.5" RNase-free glass capillaries. The micropipets were backfilled with a small amount of mineral oil and was secured onto a "nanoject" automatic injector (Drummond Scientific Company). Each cRNA sample (1 ng/ mL) was loaded into the tip of the pipette.

The oocytes were placed on a specialized platform that consisted of a small grid in a small Petri dish. There was one oocyte per square of the grid. Oocytes were injected with 46 nL of cRNA or RNase-free water. The validity of injection was assessed by observing the swelling of the oocytes using a dissecting microscope or if the interface of oil-RNA solution moved within the pipette. Uninjected oocytes that were cultured under the same conditions for the same time period served as an additional negative control.

After microinjection, the oocytes were incubated at 16°C for 72 hours in a 96-well plate (single oocyte per well) in oocyte media (96 mM NaCl, 2 mM KCl, 5 mM $MgCl_2$, 5 mM HEPES, 0.6 mM $CaCl_2$ at pH 7.6, and 1000 units of penicillin-streptomycin, osmolarity = 195 mOsm/ kg for AQP 1 oocytes, 194 mOsm/ kg for Nodulin 26, NIP5;1 Δ40N, NIP6;1, and NIP6;1 Δ40N oocytes). Every 24 hours, the media was refreshed.

Osmotic Water Transport Assay

Water assays of oocytes were performed by transfer from 100% isoosmotic Frog Ringers (195 mOsm/kg) to hypoosmotic Frog Ringers (60%, 116 mOsm/ kg for AQP 1; 25%, 48 mOsm/ kg for Nodulin 26, NIP5;1 Δ40N, NIP6;1, and NIP6;1 Δ40N oocytes). Video microscopy was

performed, and oocyte images were collected, digitized, and analyzed using the Scion Image software [38]. A camera measured the change in cross-sectional area of the oocyte, which appeared as a circle on the computer screen, every five seconds for a time period of sixty seconds. Assuming the oocyte to be a perfect sphere, the computer used the increase in cross-sectional area to determine the corresponding increase in volume (Eq. 1).

$$\left(\frac{A}{A_0}\right)^{\frac{3}{2}} = \frac{V}{V_0}$$

(Eq. 1)

A is the cross-sectional area of the oocyte at a given time, A_0 is the initial cross-sectional area, V is the oocyte volume at a given time, and V_0 is the initial volume. The calculated initial swelling rate $[d(V/V_0)/dt]$ allowed the osmotic water permeability (P_f) to be determined (Eq. 2).

$$P_f = \frac{(V_0/S_0)(dV/V_0)/dt}{(S_{real}/S_{sphere})V_w(osm_{in} - osm_{out})}$$

(Eq. 2)

V_0 is the initial oocyte volume, S_0 is the initial oocyte surface area, osm_{in} is the osmolarity in the oocyte, osm_{out} is the osmolarity of the media, V_w is the partial molar volume of water, S_{real} is the actual area of the oolemma, and S_{sphere} is the area calculated by assuming a sphere. V_w was 18. In order to correct for the increase in surface area due to the presence of folds and microvilli in the oolemma, an S_{real}/S_{sphere} value of 9 was used for all P_f calculations[37]. Oocyte water permeability data was analyzed using Graphpad Prism.

The effects of metal ions on oocyte AQP 1 water permeability were observed as follows. Fifteen oocytes (five in each media) were incubated in 1 mM $AgNO_3$, 1 mM $HgCl_2$, or 1 mM H_3BO_3 for fifteen minutes at room temperature. The water transport assay was repeated as described earlier.

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<u>Standard Glycerol Uptake Assay</u>

The glycerol permeabilities of oocytes injected with Nodulin 26, NIP5;1 Δ40N, NIP6;1, and NIP6;1 Δ40N cRNA were assayed in standard glycerol uptake assays, as previously described, under isoosmotic conditions (200 mM glycerol, 60 μCi/ mL [^{3}H] glycerol, 5 mM $MgCl_2$, 5 mM HEPES, pH 7.6)[39]. After the oocytes were lysed, liquid scintillation counting measured the uptake of [^{3}H] glycerol.

<u>Western Blot</u>

To verify whether the Nodulin 26, NIP5;1 Δ40N, NIP6;1, and NIP6;1 Δ40N were properly expressed, an immunoblot was performed[21]. Five oocyte lysates of each were homogenized in 100 μL of oocyte homogenization solution (20 mM Tris-HCl at pH 7.4, 5 mM $MgCl_2$, 5 mM NaH_2PO_4, 80 mM sucrose, 1 mM EDTA, 1 mM dithiothreitol, 1 mM PMSF, 5 μg/ mL leupeptin, and 5 μg/ mL pepstatin A at 4°C) with a plastic mortar and pestle. As a control, five uninjected oocyte lysates were also homogenized in the same fashion. The lysates were spun down at 250 g for 10 minutes at room temperature in order to remove the yolk proteins. The supernatants were saved while the pellets were discarded.

Lysate proteins were resolved by SDS-polyacrylamide gel electrophoresis on 12.5% polyacrylamide gels. The proteins were electrotransferred onto polyvinylidene fluoride membranes overnight at 4°C at 100 mA in 0.2 M glycine, 25 mM Tris, and 20% methanol. The membrane was blocked with 100 mL 10% nonfat dry milk and 1% goat serum in phosphate buffered saline (PBS) at 37°C (shaking) for 1 hour. Excess milk was removed, and the membrane was washed with PBS containing 0.01% tween (PBSt) three times for 5 minutes each. The membrane was incubated with a 1:2000 μL dilution of mouse anti-FLAG antibody in 0.01% nonfat dry milk and 0.1% goat serum in PBS for 1 hour at 37°C. The membrane was washed with PBSt as above and was incubated with a 1:2000 dilution of peroxidase-labeled anti-mouse

IgG antibody for 1 hour at 37°C. Excess antibody solution was removed, and the membrane was washed as before. After PBSt was removed, the blot was incubated for 2-5 minutes at room temperature in a 1:1 mixture of solution 1 (2 mL 1M Tris, 200 μL luminol, 88 μL p-coumaric acid, and 20 mL water) and solution II (2 mL 1 M Tris, 20 mL water, and 12 μL H_2O_2). The blot was wrapped in seran wrap, taped to chromatography paper, and exposed to film. X-ray film detected the chemiluminescent signal[21].

RESULTS

Homology Modeling

In spite of conserved regions among all aquaporins, slight structural distinctions apparently cause functional differences to exist. Using AQP 1 as a template, MOE constructed three-dimensional structures of other aquaporins. After superpositions of these other aquaporins and AQP 1, structural similarities and differences were studied.

The NPA motif was the first region observed using MOE. Overlap of all six amino acids involved demonstrated that the NPA region is structurally highly conserved among all aquaporins (Fig. 11).


Fig. 11. Overlap of NPA regions of AQP 1 (green) and AQP 3 (red). The asn, pro, & ala of each NPA region are indicated on the helical ribbon structure.

Therefore, given the high conservation of the NPA regions in these functionally different aquaporins, one can conclude the NPA region is not responsible for the selectivity of different aquaporins. For example, AQP 3 is an aquaglyceroporin whereas AQP 1 is a classic aquaporin. Aquaglyceroporins and aquaporins would be expected to have differences within their pores. However, this is not the case. Thus, it is significant that two molecules with such different permeabilities still have much overlap of the asparagine, proline, and alanine amino acids.

In addition to the NPA region, the aromatic arginine (Ar/R) region was modeled using MOE. Similar steps were taken in order to superimpose the three-dimensional structure of AQP 1 and AQP 0 (Fig. 12), AQP 2 (Fig. 13), and AQP 3 (Fig 14).
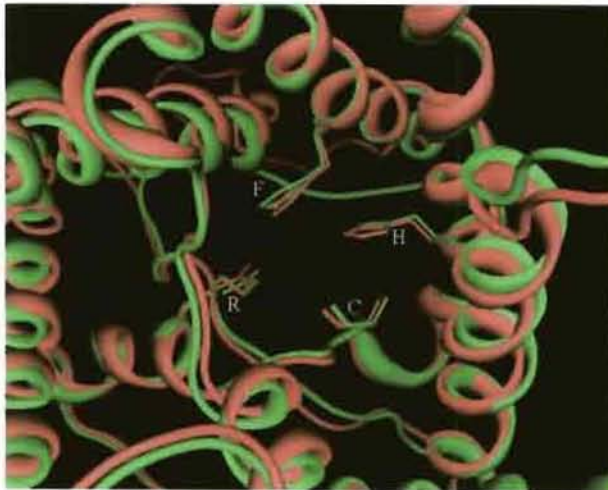
Fig. 12. AQP 0 (green) and AQP 1 (red) Ar/R superposition. Phe from Helix 2, His from Helix 5, Arg and Cys from Loop E.. View looking down the pore axis.
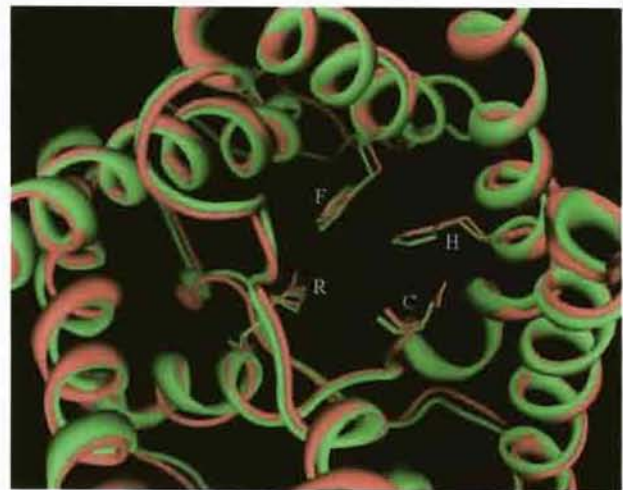
Fig. 13. AQP 2 (green) and AQP 1 (red) Ar/R superposition. Phe from Helix 2, His from Helix 5, Arg and Cys from Loop E. View looking down the pore axis.
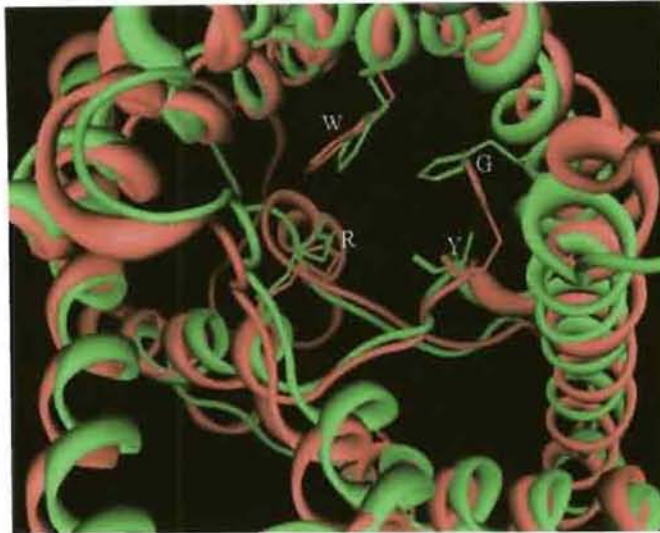
Fig. 14. AQP 3 (red) and AQP 1 (green) Ar/R superposition.
Shown are the letters of the residues of AQP 3. View looking
down the pore axis.

Unlike with the NPA region, some variability between the Ar/R regions of AQP 1 and AQP 3

exists. The classic aquaporins AQP 0 and AQP 2 both showed more overlap when superimposed

with AQP 1. This could be due to the fact that AQP 3 is an aquaglyceroporin because this is not

the case with superpositions of AQP 1 and aquaporins AQP 0 and AQP 2. It can be concluded

that the Ar/R region of AQP 3 differs from that of AQP 1 because it serves as a selectivity filter.

The presence of these different amino acids allows the transport of glycerol to occur.

Tryptophan and tyrosine are more hydrophobic so they facilitate the bonding of glycerol to the

pore. Glycine opens the pore more so that larger molecules, such as glycerol, can pass through

the pore.

As stated earlier, AQP 0 has low water permeability. However, the "selectivity filter"

does not significantly differ from that of AQP 1. In order to decipher a possible explanation,

MOE compared other regions, particularly three specific histidine residues- His-40, His-66, and

His-172 (Fig. 15).
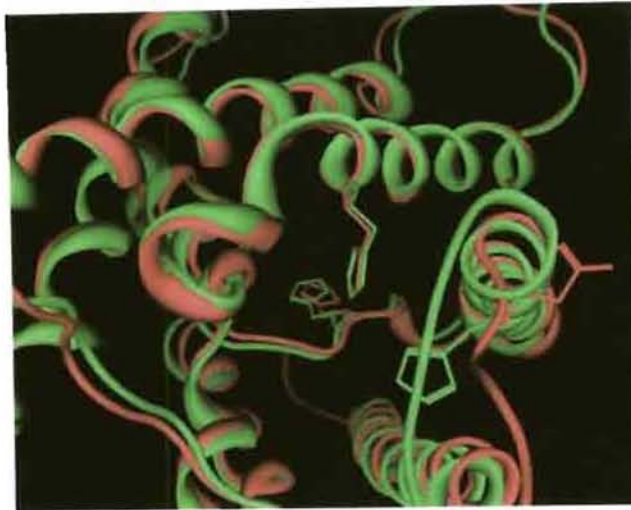
Fig. 15. Superposition of His-40, His-66, and His-172
of AQP 1 (red) and AQP 0 (green)

While the back two histidines are conserved, the front one is not. In fact, it is a completely

different amino acid in a different conformation. This blocks the pore. As a result, water cannot

pass through the pore of AQP 0 as well. Based on this superposition, one can conclude that this

histidine region serves as a gate that regulates what can pass through the pore. Also, the titration

of these histidines at pH 6 may account for the ability of pH to regulate AQP 0[42].

<u>AQP 1 Expression</u>

In order to measure facilitated water diffusion, AQP 1 was expressed in *Xenopus laevis*

oocytes via cRNA microinjection. They were then assayed by the *Xenopus* oocyte swelling

assay developed by Preston *et al*[10]. As discussed earlier, video microscopy captured images and

cross-sectional areas of the oocytes in order to calculate the oocytes' rate of swelling in

hypoosmotic media. Fig. 16A shows the change in volume versus time of AQP 1 oocytes and

control oocytes injected with sterile water. Upon hypoosmotic challenge, AQP 1 oocytes rapidly

underwent an increase in volume whereas the water-injected control oocytes did not. This

showed that control *Xenopus* plasma membranes have extremely low water permeabilities and

that AQP 1 confers a high transport rate similar to previous findings[10].

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After the rate of swelling, $[d(V/V_0)/dt]$, was determined, the osmotic water permeability (P_f) was calculated. As shown in Fig. 16B, the P_f of AQP 1 oocytes was significantly higher than that of the oocytes injected with DEPC-treated water. Furthermore, inhibition of water permeability is shown by the lower P_f of oocytes incubated in HgCl$_2$. Although not as significant as the effects of mercury, AgNO$_3$ slightly inhibited the water permeability of oocytes. In contrast, H$_3$BO$_3$ did not appear to inhibit the water transport property of AQP 1.

A.
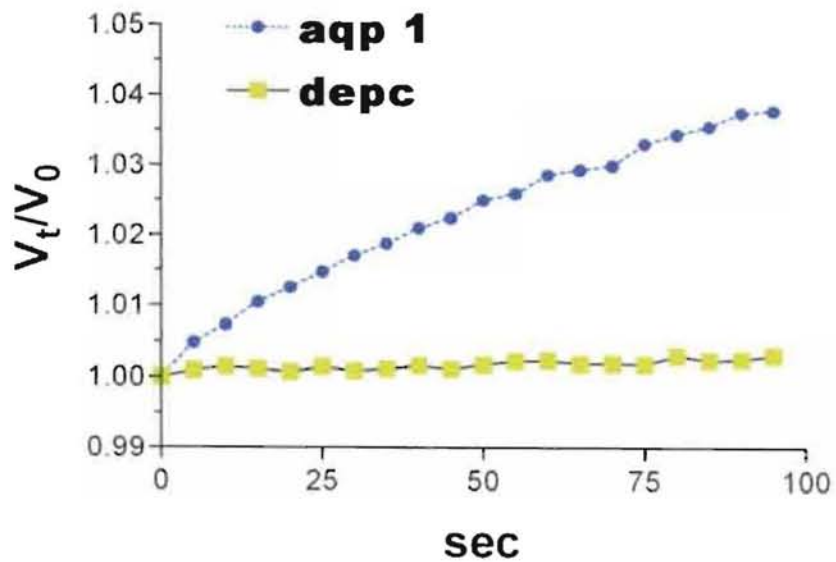
B.
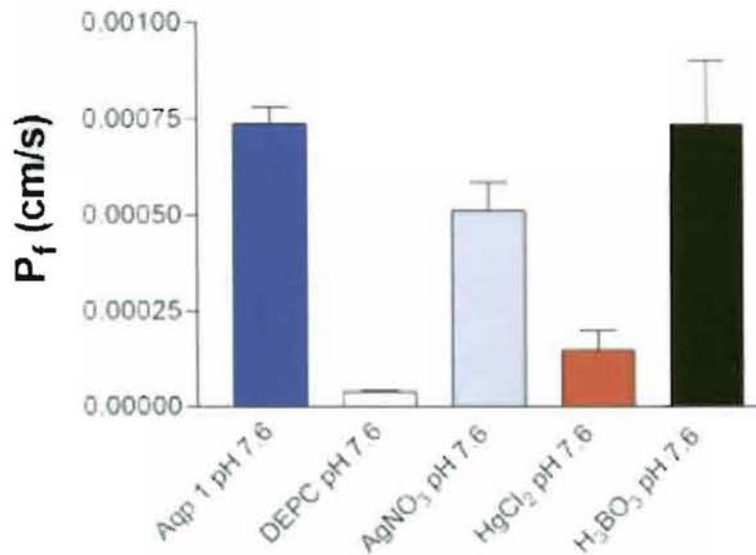
Fig. 16. Water permeabilities of AQP 1 in *Xenopus laevis* oocytes. 46 nL
AQP 1 cRNA or DEPC-treated water was injected into *Xenopus laevis*
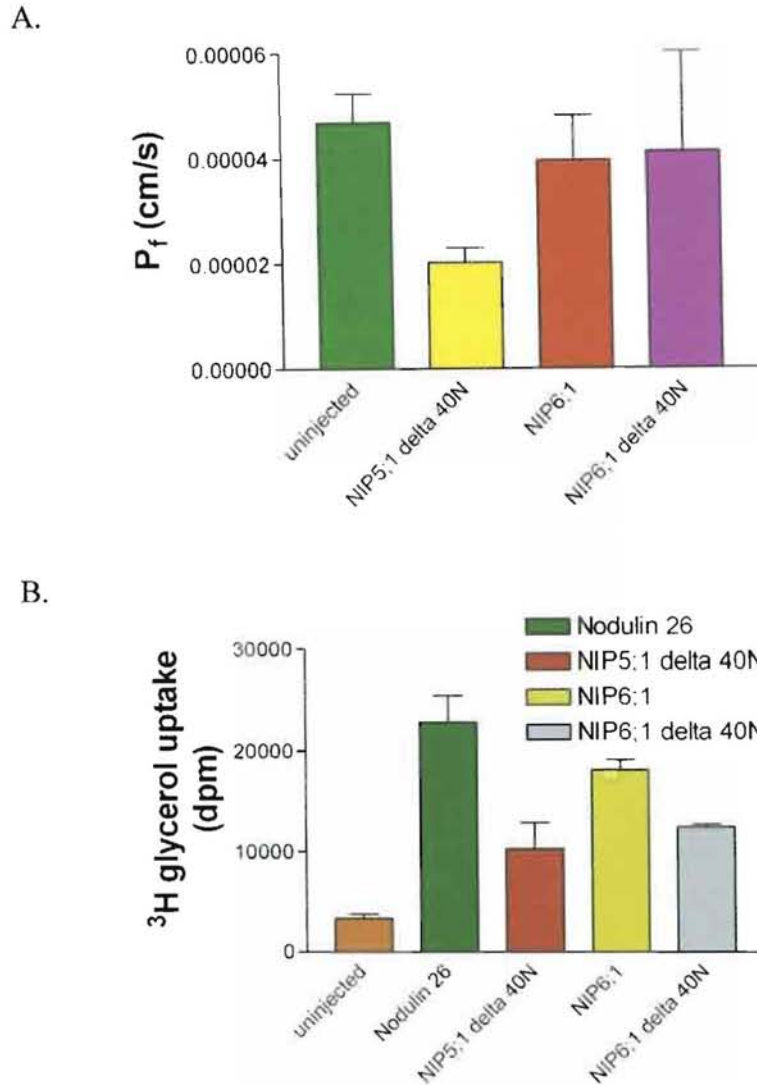oocytes and was assayed for water transport as described in the Materials and
Methods. (A) The increase of the relative oocyte swelling (V_t/V_0) of
individual oocytes was determined and measured as a function of time by
playing the oocytes in 60% hypoosmotic Frog Ringers solution. The relative
volume was assessed at 5 second intervals for a total of 60 seconds. (B)
Osmotic water permeability (P_f) of oocytes was determined for uninjected
control oocytes, oocytes injected with DEPC-treated water, or oocytes
expressing AQP 1, which contain an N-terminal FLAG epitope tag. Error
bars represent the standard error of the mean. There were five replicates.

NIP Subgroup II Deletions

Within the plant subfamily of aquaporins, NIP 5;1 and NIP6;1 have displayed very low

water permeability despite having conserved hydrogen bond donors and acceptors in the

selectivity filter known as the Ar/ R region. NIP 5;1 and NIP 6;1 have extended N-termini,

which may have putative MAP kinase phosphorylation sites. Because Fps1, a fungal

aquaglyceroporin, also has long N- and C-termini that serve to gate the pore[40], forty amino acids

in the N-termini of NIP 5;1 and NIP 6;1 were deleted in order to observe the significance of the

extended termini. Then the water and glycerol permeabilities of the oocytes expressing the

truncated glyceroporin gene were determined by water and glycerol uptake assays.

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In the first repetition, all four groups of oocytes demonstrated extremely low water

permeability (Fig. 17A). In the standard glycerol uptake assay, the glycerol permeability of the

oocytes with the deletions was decreased, as shown in Fig. 17B.

A.

B.

Fig. 17. Water and glycerol permeabilities of Nodulin 26, NIP 5;1 Δ40N, NIP
6;1, and NIP 6;1 Δ40N in *Xenopus laevis* oocytes. 46 nL cRNA was injected
into *Xenopus laevis* oocytes and was assayed for water and glycerol transport as
described in the Materials and Methods. (A) Osmotic water permeability (P_f) of
oocytes was determined for uninjected control oocytes or oocytes expressing
Nod26, NIP 5;1 Δ40N, NIP 6;1, or NIP 6;1 Δ40N, which contain an N-terminal
FLAG epitope tag. Error bars represent the standard error of the mean. There
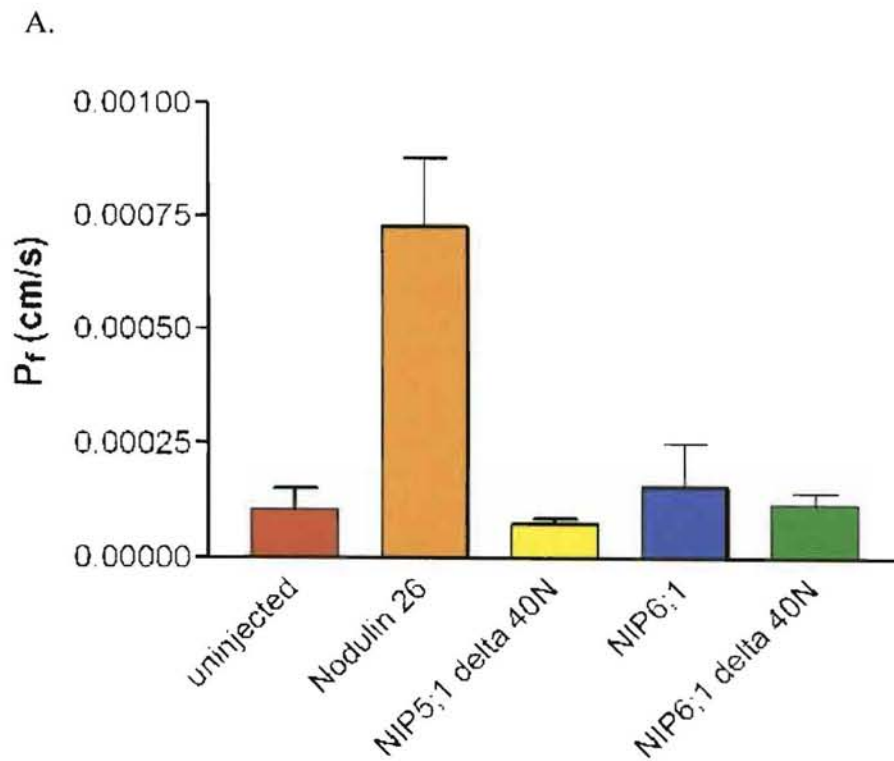were five replicates. (B) Glycerol permeability was assessed by the uptake of
[^{3}H] glycerol of uninjected oocytes or those injected with Nod26, NIP 5;1 Δ40N,
NIP 6;1, or NIP 6;1 Δ40N. Error bars represent the standard error of the mean.
There were two replicates.

In the second repetition, oocytes expressing NIP 5;1 Δ40N, NIP 6;1, and NIP 6;1 Δ40N had P_f values very similar to that of the uninjected control oocytes (Fig. 18A). As in the first repetition, the oocytes with the deletions had lower glycerol permeability (Fig. 18B). The main difference in the results of the two repetitions was that Nodulin 26 initially had a higher P_f but lower glycerol permeability than in the second repetition.
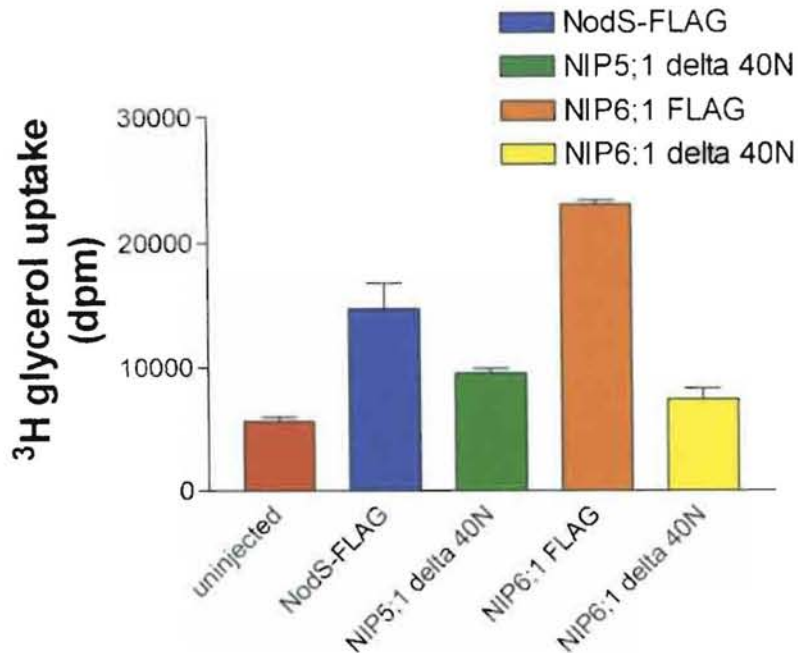
A.

B.

Fig. 18. Water and glycerol permeabilities of Nodulin 26, NIP 5;1 Δ40N, NIP 6;1, and NIP 6;1 Δ40N in *Xenopus laevis* oocytes. 46 nL cRNA was injected into *Xenopus laevis* oocytes and was assayed for water and glycerol transport as described in the Materials and Methods. (A) Osmotic water permeability (P_f) of oocytes was determined for uninjected control oocytes or oocytes expressing Nod26, NIP 5;1 Δ40N, NIP 6;1, or NIP 6;1 Δ40N, which contain an N-terminal FLAG epitope tag. Error bars represent the standard error of the mean. There were five replicates. (B) Glycerol permeability was assessed by the uptake of [³H] glycerol of uninjected oocytes or those injected with Nod26, NIP 5;1 Δ40N, NIP 6;1, or NIP 6;1 Δ40N. Error bars represent the standard error of the mean. There were three replicates.

An immunoblot was performed in order to see whether or not the channel proteins were properly expressed in the oocytes' plasma membranes. However, for some reason, this did not work so there were no conclusive results.

DISCUSSION

Since their discovery in the early 1990s, aquaporins have opened several doors for research regarding water transport in various organisms, organs, and tissues because water is a very important molecule for life. Water transport assays were carried out as described in Preston

et al. in order to observe the role of AQP 1 in water permeability. As mentioned in the Results section, AQP 1 oocytes experienced an increase in volume whereas the water-injected control oocytes did not. This increase in oocyte volume also demonstrated that *Xenopus* oocytes injected with AQP 1 cRNA had higher osmotic water permeabilities (P_f= 7.384 x 10^{-4}) compared to control oocytes (P_f= 3.94 x 10^{-5}). Because *Xenopus* oocytes have been known to have very low water permeability[8], it can be concluded that the increase in water permeability can be attributed to the presence of AQP 1 proteins in the plasma membranes.

In order to see if water transport could be altered by the presence of certain compounds, AQP 1 oocytes were incubated with metal ions, such as Hg^{+2} and Ag^{+1}. In accordance with the research of Robert Macey[11], *Xenopus* oocytes expressing AQP 1 had diminished water permeability after being incubated with mercurials. This is due to Hg^{+2} modification of the sulfhydryl of Cys-189 in loop E[8]. Because of Cys-189's location within the pore near the second NPA motif, the bound mercury atom occluded the pore so water molecules could not pass through the narrow pore of AQP 1. A lower P_f value of 1.478 x 10^{-4} highlights the inhibitory effects of mercurials. Although Ag^{+1} exerted some negative effect on AQP 1 (P_f= 5.11 x 10^{-4}), as seen in Fig. 14B, $AgNO_3$ did not inhibit AQP 1 as much as expected. Although Ag^{+1} does not modify cysteine, it has been reported to affect aquaporins[43]. On the other hand, because the $AgNO_3$ P_f value of 7.35 x 10^{-4} is very close to that of AQP 1 oocytes, H_3BO_3 did not appear to inhibit AQP 1's water transport function at all. These studies on AQP inhibition are important because many researchers are looking for drugs and compounds to block AQP function for therapeutic purposes.

As mentioned before, different classes of aquaporins exist. While AQP 1 is a classic aquaporin that is impermeable to solutes such as glycerol, Nodulin 26 is an aquaglyceroporin permeable to water and glycerol, and NIP 5;1 and NIP 6;1 are glyceroporins that are permeable

to glycerol[21]. Although NIP 5;1 and NIP 6;1 have the same residues in the Ar/ R region as other aquaporins permeable to water, it is unknown why they do not transport water.

Because NIP 5;1 and NIP 6;1 have extended N-termini, it is thought that the N-termini may play a role in gating the pore, as they do in the fungal aquaglyceroporin Fps1[40]. Fps1 regulates intracellular glycerol levels[40]. In order to test this hypothesis, forty amino acids of the extended N-termini of NIP 5;1 and NIP 6;1 were deleted. The truncated NIP 5;1 and NIP 6;1 oocytes had negligible water permeabilities and decreased glycerol permeabilities. The decreased glycerol permeabilities indicate that the N-termini could possibly have a role involved with either transporting glycerol or directing proper protein folding. However, at this time, the exact biochemical cause for these results cannot be deciphered.

SUMMARY

Extensive research on aquaporins has revealed the importance of water transport in all living organisms. Because of the necessity of water for life, the aquaporin family is ubiquitous in all organisms. However, despite having a conserved hourglass structure, aquaporins have slight variances in their function. Homology modeling showed overlap in highly conserved regions, such as the NPA motif and the Ar/ R region. Oocyte water transport assays verified that AQP 1 enhances oocyte water permeability. Incubation with $HgCl_2$ greatly inhibited AQP 1 function, but incubation with $AgNO_3$ and H_3BO_3 did not. Compared to other aquaporins, NIP 5;1 and NIP 6;1 have extended N-termini. These N-termini were truncated in order to observe possible roles of the additional amino acids. Swelling assays showed how NIP 5;1 $\Delta40N$, NIP 6;1, and NIP 6;1 $\Delta40N$ are not permeable to water. Glycerol uptake assays showed how these proteins transport glycerol. Further research needs to be done in order to elucidate more knowledge about aquaporins.

ACKNOWLEDGMENTS

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