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Plant aquaporins: their molecular biology, biophysics and significance for plant water relations

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Abstract

Over the last decade, considerable advances have occurred in understanding the molecular biology and biophysics of water permeation across plant membranes and tissues. Spurred on by the rapid advances in cloning and functional characterization of a superfamily of major intrinsic proteins, some of which function as aquaporins, the biophysics of transport of water and small non-electrolytes across plant membranes is being re-examined based on the proposed function of these membrane-integral proteins in their native membranes. This review focuses on a number of issues that are central to an understanding of aquaporin function: (1) the need to be able to test for water-channel activity in native membranes; (2) the implications of the observed solute/water selectivity of aquaporins; (3) the putative functional roles of aquaporins at the cell, tissue and organ levels in plants; and (4) information that can be obtained from studies of the abundance, diversity and expression patterns of aquaporins. It is clear that to answer many of the critical questions that remain concerning aquaporin function, combined studies using appropriate molecular and biophysical techniques will be required.

Key words: Aquaporin, hydraulic conductivity, major intrinsic protein, osmotic flow, water channel, water permeability.

Introduction

Aquaporins belong to a highly conserved group of membrane proteins called the major intrinsic proteins (MIPs) with molecular masses of between 26 and 30 kDa. The aquaporin polypeptide typically contains six membrane-spanning α helices, with the N- and C-termini both located on the cytoplasmic side of the membrane. The N- and C-terminal halves of the polypeptide show significant sequence similarity to each other and are arranged as a tandem repeat; each half has a smaller hydrophobic loop that includes a highly conserved asparagine-proline-alanine (NPA) motif (Reizer *et al*., 1993; Park and Saier, 1996; Fig. 1A). Structurally, the two halves of the polypeptide show obverse symmetry, with the loops containing the NPA motif overlapping in the middle of the lipid bilayer (in the so-called 'hourglass' model) to form two hemipores that together create a narrow, water-filled channel (Jung *et al*., 1994; Walz *et al*., 1995, 1997; Cheng *et al*., 1997; Fig. 1B). It has been suggested that this distinctive intramolecular two-fold axis of symmetry accounts for the fact that aquaporins can mediate bidirectional water flow, i.e. they do not show rectification, in contrast to many ion channels (Cheng *et al*., 1997).

AQP1 (CHIP28), an abundant aquaporin in erythrocytes and the plasma membrane of various epithelia, was the first protein shown to form a water-selective channel (Preston *et al*., 1992; van Hoek and Verkman, 1992; Agre *et al*., 1998). It has now been crystallized and structurally resolved to 3–6 Å using electron-beam techniques (Jap

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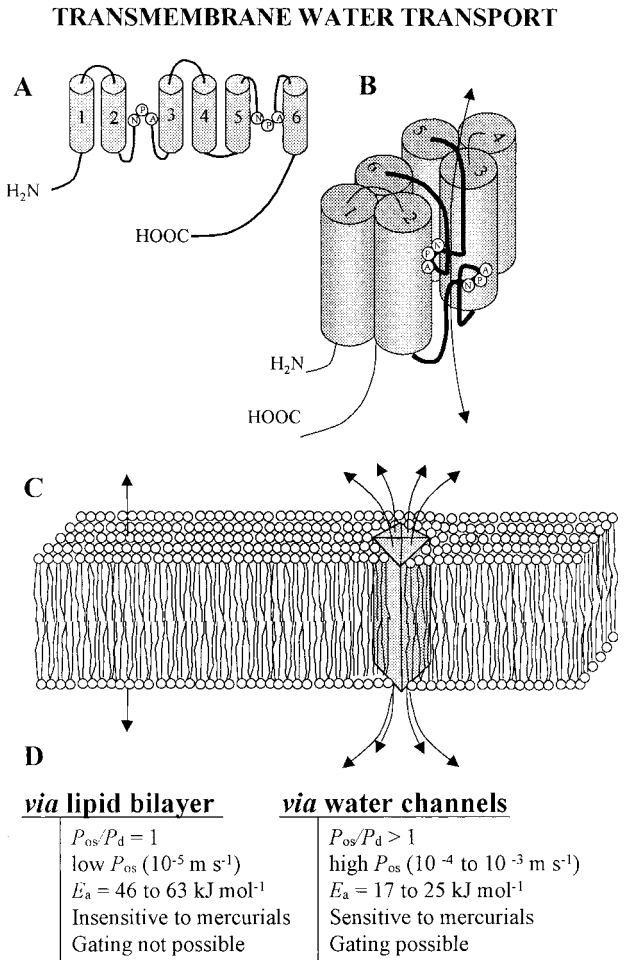


Fig. 1. Summary of the properties of water permeation through the lipid phase of cell membranes and through water channels embedded in the lipid bilayer. (A) The predicted secondary structure of the AQP1 monomer is shown, with the cylinders denoting membrane-spanning α-helices (N, P and A are single-letter amino acid codes). (B) Proposal for the manner in which the two NPA motifs overlap in the membrane to form a water-filled pore, indicated by the vertical arrow (adapted from Jung et al., 1994). In reality the helices are tilted at an angle of approximately 25° from vertical and the water pore is more centrally located in the monomer (Cheng *et al.*, 1997). (C) In the membrane, four polypeptides form a tetrameric array, with water flow occurring through each monomer. (D) Summary of the features associated with water flow across the lipid bilayer only compared with water flow through a biological membrane studded with aquaporins. Note that P_{os} is given for the entire membrane and not for the individual aquaporin monomers.

and Li, 1995; Cheng *et al.*, 1997; Walz *et al.*, 1997). AQP1 forms tetramers in the membrane (Verbavatz *et al.*, 1993; Walz *et al.*, 1997; Eskandari *et al.*, 1998), but each monomer is thought to facilitate water flow (Shi *et al.*, 1994; Verkman *et al.*, 1996; Fig. 1C). The existence of such an aquaporin was anticipated in the erythrocyte membrane and in many secretory epithelia in animals based on decades of biophysical research that indicated water flow through proteinaceous channels (Verkman, 1992). Since 1993, when the first aquaporin from plants (γ-TIP) was cloned and functionally expressed (Maurel *et al.*, 1993), there has been a growing interest in the molecular biology of MIPs and their bearing on the biophysics of water flow across plant membranes (Chrispeels and Maurel, 1994; Steudle and Henzler, 1995; Maurel, 1997; Schäffner, 1998).

One puzzling aspect of MIPs in plants is their abundance and complexity. In *Arabidopsis thaliana*, 23 MIPS have been identified at the DNA-sequence level (Weig *et al.*, 1997; Schäffner, 1998). Although not all the gene products have been identified at the protein level, eleven of these are proposed to be TIPs (tonoplast intrinsic proteins) and eleven to be PIPs (plasma-membrane intrinsic proteins), with one (NLM1) being most closely related to soybean NOD26 (Weig *et al.*, 1997). Some of the *Arabidopsis* MIPs have been shown to increase the water permeability of *Xenopus* oocytes when expressed in these cells (e.g. γ-TIP, Maurel *et al.*, 1993; δ-TIP, Daniels *et al.*, 1996; RD28, Daniels *et al.*, 1994; PIP1a,b,c and PIP2a,b, Kammerloher *et al.*, 1994; PIP3 and NLM1, Weig *et al.*, 1997). MIPs from other plants have also been functionally tested for aquaporin function, e.g. maize TIP1 (Chaumont *et al.*, 1998), spinach PM28A (Johansson *et al.*, 1998), *Phaseolus vulgaris* seed α-TIP (Maurel *et al.*, 1997*a*), and *Mesembryanthemum crystallinum* MIPA and MIPB (Yamada *et al.*, 1995).

In this article, some of the key issues emerging from recent work on aquaporins in plants will be addressed. In particular, an attempt will be made to assess the extent to which molecular and biophysical techniques have combined to give a more unified picture of water transport in plants and also to highlight the most important questions that remain to be resolved in future work.

Establishing water channel function in native membranes

PIPs and TIPs have been shown by immunolocalization techniques to be located at the plasma membrane and tonoplast, respectively (Johnson *et al.*, 1990; Höfte *et al.*, 1992; Daniels *et al.*, 1994; Kammerloher *et al.*, 1994; Johansson *et al.*, 1996; Daniels *et al.*, 1996; Robinson *et al.*, 1996; Chaumont *et al.*, 1998). Other endomembranes besides the tonoplast in plants have MIPs that may function as aquaporins. One example is the NOD26 protein (Weaver *et al.*, 1994; Rivers *et al.*, 1997) on the symbiosome membrane in soybean nodules (Fortin *et al.*, 1987). However, it is not firmly established that all MIPs function as aquaporins in the native membrane. It has only recently been shown for higher-plant membranes using standard biophysical techniques that water-channel activity can be detected in native membranes, notably the tonoplast (Maurel *et al.*, 1997*b*; Niemietz and Tyerman, 1997). The plasma membrane is interesting in that despite having numerous PIPs, some of which have been shown to increase water permeability when expressed in *Xenopus*

oocytes, the biophysical evidence for water channel activity in isolated plasma membrane is not clear-cut (Maurel *et al.*, 1997*b*; Niemietz and Tyerman, 1997). For PIP1b, an antisense construct has been transformed into *Arabidopsis* (Kaldenhoff *et al.*, 1998). These transgenic plants showed an increased root : shoot ratio and reduced protoplast water permeability, suggesting that this PIP functions as an aquaporin *in vivo*.

Measuring water channels

Unfortunately, there is no technique equivalent to patch-clamping that can be used to detect water flow through individual water channels. Two characteristics of water channels in native membranes make the development of such a technique difficult. One is that water, when flowing across the membrane through an aquaporin molecule, does not carry any charge, so electrical current cannot be detected. The other is that the background water permeability of the lipid bilayer is relatively high.

In general, water channels only confer an increase in membrane water permeability relative to the lipid bilayer of one to two orders of magnitude, compared with an increase of over three orders of magnitude in the case of ion channels. This makes it difficult to detect small changes in net water flow resulting from the opening of a small number of water channels against an already high background flow. Techniques available for detecting water (volume) flow are far less sensitive than those available for the measurement of electrical current. The result of the flow of 10^6 ions s^{-1} through an ion channel, a value typical for an ion channel under a physiological gradient, can be measured. For a water channel with an osmotic permeability (P_{os}) of 6×10^{-20} m^3 s^{-1} per channel subunit (Zeidel *et al.*, 1994), an equivalent hydraulic conductivity of 4.4×10^{-22} m^3 s^{-1} MPa^{-1} per channel subunit can be calculated. Therefore, for a gradient of 0.1 MPa (1 bar) in osmotic pressure, which is physiologically realistic, 1.5×10^6 water molecules will flow through each channel subunit per second[1]. This is similar to the flux through an ion channel for a typical driving force.

Water flow in walled plant cells can be assayed by measuring pressure changes over time using the pressure probe (Steudle, 1993). For membrane vesicles or wall-less cells, it is necessary to rely on monitoring changes in volume generally measured on a large population of cells or vesicles using optical methods such as light scattering (Verkman, 1995). In both of these cases, it is not possible to resolve flow to the level of 10^6 water molecules per second. Moreover, for relatively large changes in the volume of cells, the volume change is necessarily accompanied by a significant change in membrane surface area. Since the lipid membrane is not very extensible (Wolf and Steponkus, 1983), water flow must necessarily be accompanied by incorporation or deletion of material into the bounding membrane of the cell by exo- or endocytosis, respectively.

Although the water permeability of individual channels is difficult to measure, there is another important osmotic parameter that can be determined more easily. This is the reflection coefficient (σ), which is a measure of the passive selectivity of the membrane for solutes relative to water (Tyerman and Steudle, 1982; Steudle, 1993; Murphy and Smith, 1994). Reflection coefficients are highly relevant when considering the selectivity of aquaporins to test solutes such as low-molecular-weight organic substances. Reflection coefficients of aquaporins may be measured by titrating water channels with mercurial reagents and measuring the overall hydraulic conductivity of the cells (Henzler and Steudle, 1995; Steudle and Henzler, 1995). However, the method as it stands averages over the different aquaporins present in the cell membrane, and would also include other transporters that allow some passage of water as well as solutes (see below). One possibility to avoid the latter difficulty would be, in principle, to use selective inhibitors that specifically block these transporters without affecting water-channel activity.

Characteristics of water channel activity in membranes

Since it is not yet possible to assay the function of individual water channels in membranes, it is necessary to determine activity from the ensemble activity of many water channels. This creates problems in native membranes because there may be a number of water-permeable proteins, including proteins that are not in the MIP superfamily, co-residing in the same membrane. Below are listed some of the observations and techniques that have been used to assess the role of water channels in plant membranes. These are also summarized in Fig. 1.

High water permeability

A high value of osmotic water permeability is the first indication of water-channel activity, i.e. P_{os} much greater than 10×10^{-6} m s^{-1},which is representative of reported water permeabilities across lipid vesicles, although this depends on the exact lipid composition (Lande *et al.*, 1995). Typical values for membranes with water channels are between 100 and 200×10^{-6} m s^{-1} (Zeidel *et al.*, 1994). For tonoplast membranes from higher plants, values between 86 and 600×10^{-6} m s^{-1}: have been

[1] Note that this is a different parameter to the P_{os} quoted by Nielson and Agre (1995) in terms of water molecules per second. They give 10^9 H_2O molecules per second per subunit. The reason is that a hydraulic conductivity is presented for our calculation which is $(P_{os} \times V_w)/RT$, where V_w is the partial molar volume of water and R and T have their usual values. This allows us to give a value for water flow per unit pressure gradient.

measured (Maurel *et al*., 1997*b*; Niemietz and Tyerman, 1997). For comparison, charophyte cells give values between 100×10^{-6} m s^{-1} and 300×10^{-6} m s^{-1} (Wayne and Tazawa, 1990; Henzler and Steudle, 1995). The erythrocyte membrane, studded with AQP1 (CHIP28), has a value of 200×10^{-6} m s^{-1} (Macey, 1984).

Low activation energy for water transport

Provided that the flow of water through water channels is essentially a viscous flow across a pore, the temperature dependence of P_{os} should be comparable to that for the self-diffusion of water or for the viscosity of water. This has been found for plant membranes in the presence of water channels (activation energies between about 17 and 25 kJ mol^{-1}, or 4.1 and 6.0 kcal mol^{-1}). The reason for the low activation energy is that water moving across a channel does not have to overcome a large energy barrier, i.e. water molecules crossing the pore do not 'see' an environment much different from that of bulk water. On the other hand, water movement through the membrane would need to surmount the high energy barrier of water partitioning into hydrophobic lipid phase. In practice, the activation energy has been observed to be close to that for viscous flow of water. When water channels are blocked, the activation energy increases substantially as predicted (Wayne and Tazawa, 1990; Schütz and Tyerman, 1997). It should be noted that temperature effects on the membrane lipid, i.e. phase transitions, or temperature effects on the water channels via gating, i.e. conformational changes of the protein caused by temperature, may conceal the actual temperature dependence of water flow through the channel (Hertel and Steudle, 1997). A small effect of lipid composition on the activation energy for water flow has been observed in proteoliposomes containing the expressed mammalian aquaporin AQP1 (Zeidel *et al*., 1994).

Osmotic water permeability (P_{os}, L_P) should be larger than diffusional water permeability (P_d)

The P_{os}/P_d ratio characterizes the predominant pathway of water movement. A ratio of unity is typical for independent diffusion into phospholipid vesicles, while membranes containing water channels show ratios greater than unity (Ye and Verkman, 1989). This ratio has been used to calculate the number of water molecules that line up in a file across the pore or the effective radius of the pore (Levitt, 1974; Hill, 1994). The ratio can be further examined by the use of solutes that cannot enter the pore and those that are permeant (Hill, 1994). One problem with determining the P_{os}/P_d ratio is the differential effect of unstirred layers in the measurements of P_{os} and P_d. The latter is more affected by unstirred layers, which tends to lead to an overestimation of P_{os}/P_d (Dainty, 1963). For small cells or membrane vesicles subject to turbulent mixing, e.g. in a stopped-flow spectrofluorimeter, it is likely that unstirred-layer effects are negligible (see discussion in Niemietz and Tyerman, 1997). Using stopped-flow, Niemietz and Tyerman (1997) found a P_{os}/P_d ratio of 7 for tonoplast-enriched vesicles from wheat roots. This is similar to the values reported for red blood cells that contain AQP1 water channels, but differs from the higher value of 27 obtained by comparing L_P (P_{os}) with the permeability coefficient of heavy water (HDO), both measured with a pressure probe (Henzler and Steudle, 1995). To some extent, the reflection coefficient of isotopic water is a measure of P_{os}/P_d. For *Chara*, it has been shown that the low σ values for HDO increase with increasing temperature, which suggests that temperature affects the hydraulic (viscous) flow across water channels more than diffusional transport (Hertel and Steudle, 1997). In contrast, the reflection coefficients for small solutes that cross the membrane via the lipid phase show the opposite temperature dependence, i.e. σ decreases with increasing temperature.

Block of water channels by mercurial reagents

For over 30 years, there have been indications in the literature that water permeation across plant cell membranes may occur via pores (Dainty, 1963; Dainty and Ginzburg, 1964; Steudle and Tyerman, 1983). However, the connection to proteinaceous pores was not really made until Wayne and Tazawa (1990) showed that the osmotic water permeability of *Nitellopsis* was inhibited by the mercurial agent *p*-chloromercuribenzenesulphonate (*p*CMBS), which inhibits aquaporins in animal cells (Naccache and Sha'afi, 1974). Subsequently, mercurial reagents have been tested for their effects on water permeation in a variety of membranes, cells and tissues (Maggio and Jolly, 1995; Carvajal *et al*., 1996; Tazawa *et al*., 1996, 1997; Henzler and Steudle, 1995; Maurel *et al*., 1997*b*; Niemietz and Tyerman, 1997; Schütz and Tyerman, 1997).

Mercurial and sulphydryl reagents in general block aquaporins, but there are notable exceptions (Daniels *et al*., 1994). Agents that block aquaporins are those that appear to be able to oxidize cysteine residues associated with the pore region of the protein, but this is not the case for mercurials, which act by binding to SH-groups of proteins. Block can usually be reversed with a scavenging agent such as the reducing SH-compound 2-mercaptoethanol. Different mercurials have different effects depending on the ability of the substance to diffuse across the membrane, which may be influenced by pH and temperature (Naccache and Sha'afi, 1974). Mercuric chloride, $HgCl_2$, is able to attack intramembraneous sites because it forms a relatively lipophilic ion pair that can diffuse across the membrane. On the other hand, *p*CMBS, which bears a net negative charge in aqueous solution, is

much less permeant and often does not show block (Schütz and Tyerman, 1997; CM Niemietz and SD Tyerman, unpublished results).

The mercury-sensitive sites on aquaporins have been probed by molecular techniques and some interesting results have emerged. One is that the sensitive cysteine residue in animal aquaporins (Preston *et al*., 1993; Zhang *et al*., 1993) is somewhat removed from the sensitive residues in plant aquaporins (Daniels *et al*., 1996). Also, the mercury-insensitive aquaporin RD28 of *Arabidopsis* can acquire mercury sensitivity by introduction of a cysteine residue next to the NPA motif facing the extracellular side of the membrane (Daniels *et al*., 1994).

The analysis of aquaporin function at the tissue level is made difficult by the lack of specificity of mercurial agents. Mercury has been tentatively used as a water channel blocker in whole-plant experiments (Maggio and Joly, 1995; Carvajal *et al*., 1996; Tazawa *et al*., 1997), but great care has to be taken because of the numerous side-effects of this substance (Schütz and Tyerman, 1997). Mercurial compounds are not good pharmacological agents for testing aquaporin activity in living cells, because some are general metabolic poisons and are very membrane permeant (e.g. the $HgCl_2^0$ species). They may also react with many other transport proteins, some of which may be permeable to water (such as the animal Na^+-glucose cotransporter: Loo *et al*., 1996). Another problem is that their effect on membrane water permeability could be indirect, especially when used on living cells, since it has been shown that water permeation via some aquaporins is controlled by phosphorylation (Daniels *et al*., 1994; Maurel *et al*., 1995; Johansson *et al*., 1998). For intact wheat-root cortical cells, metabolic inhibitors immediately reduce P_{os} by about 10-fold (Zhang and Tyerman, 1991). Anoxia, azide and $HgCl_2$ reduce P_{os} by the same amount and also drastically depolarize the plasma membrane voltage (WH Zhang and SD Tyerman, unpublished results). Given that plasma membrane vesicles from the same tissue did not show $HgCl_2$ sensitivity (Niemietz and Tyerman, 1997), this suggests that $HgCl_2$ may be having an indirect metabolic effect in intact tissue, or that upon isolation of plasma membrane vesicles the water channels are inactivated.

It is interesting that, in the *Chara* internode, the reversible blockage of water channels by $HgCl_2$ caused similar effects to those caused by high concentrations of permeating solutes. This was worked out by measuring both the decrease in membrane L_P and in the reflection coefficient (σ) caused by either treatment. The experiments suggested that in both cases reversible changes in the conformation of the channel protein take place which result in a complete blockage of the path. These results indicate (i) that the membrane may be treated as a system composite of sub-systems (water channel array, lipid array, and others) and (ii) that reflection coefficients of water channels (i.e. their selectivity pattern) may be determined in this way. The example shows that the measurement of additional membrane parameters (besides L_P [P_{os}]) such as σ or of the permeability coefficient of suitable test solutes may be a powerful tool in investigating the selectivity of water channels.

Gating of water channels

Currently, it is not known if water channels are gated, i.e. whether they open and close stochastically in the same manner as ion channels, such that the average flow of water through a channel is governed by the time-averaged probability of opening. The NOD26 and bovine lens MIPs form ion channels in lipid bilayers and these channels show gating (Ehring *et al*., 1990; Weaver *et al*., 1994; Lee *et al*., 1995). NOD26 is also water permeable, but the osmotic permeability is $3.2 \times 10^{-21}\ m^3\ s^{-1}$ per channel subunit, over an order of magnitude lower than that for AQP1 (Rivers *et al*., 1997). Furthermore, it is not known if the water and ions that move through NOD26 go via the same pathway (see discussion in Schäffner, 1998). For ion channels, one of the first indications of gating was voltage- and time-dependent conductances (e.g. in the action potential). Voltage dependence and concentration dependence of ion-channel gating leads to the phenomenon of rectification of current flow, in which particular channels only seem to open when flow is directed in one direction, outward or inward to the cell. Does similar evidence exist for water flow across native membranes?

There are some examples in the literature showing that P_{os} (L_P) can be a function of turgor pressure or osmotic pressure, components of the gradients that determine water flow analogous to ion concentration and voltage for ion flow. In *Chara*, P_{os} is reduced at increased osmotic pressure (Dainty and Ginzburg, 1964; Kiyosawa and Tazawa, 1972; Steudle and Tyerman, 1983). Recently, Niemietz and Tyerman (1997) showed that the P_{os} of tonoplast-enriched vesicles declined as the imposed gradient was increased. There are also examples of rectification of flow that appear to depend on the type of gradient imposed to create water flow (Kamiya and Tazawa, 1956; Dainty and Ginzburg, 1964; Steudle and Tyerman, 1983). Wayne and Tazawa (1990) found that a K^+ channel blocker inhibited endosmotic flow but did not affect exosmosis. Apart from these observations, there are also examples of time-dependent changes in P_{os} that have been interpreted as the result of changes in the hydraulic conductance of plasmodesmata (Cosgrove and Steudle, 1981; Zhang and Tyerman, 1991; Murphy and Smith, 1998).

In intact higher plants, there are so far only a few observations that may indicate a similar action of water channels. In the aquatic species *Elodea densa*, P_{os}

increased when cell turgor pressure decreases below a certain value (Steudle *et al.*, 1982). For maize roots, it was shown that high salinity caused a considerable reduction in water permeability at the level of individual cortical cells (Azaizeh *et al.*, 1992). This was not observed for tobacco root cortical cells (Tyerman *et al.*, 1989). In maize, cell L_P was reduced by as much as a factor of four to five, which is much greater than the effects caused by high external osmotic pressure in the *Chara* internode. Changes in cell L_P were reflected in changes in root hydraulic conductivity of 30–60%, which was due to the fact that, in the root cylinder, most of the water was flowing around cells (Azaizeh and Steudle, 1991). It is conceivable that the changes were caused by either reducing the probability of opening of water channels or by a change in their number in the membrane. However, the exact mechanisms by which high salinity exerts these effects are not yet known.

Such species- and stress-specific changes in L_P may be caused by phosphorylation of aquaporins, as demonstrated by Johansson *et al.* (1996, 1998). Low water potential reduces phosphorylation of PM28A in spinach leaf plasma membrane. Phosphorylation is carried out by a Ca^{2+}-dependent membrane-bound protein kinase (Johansson *et al.*, 1996). When expressed in oocytes PM28A, can also be phosphorylated at the same site phosphorylated *in vivo*, and decreased phosphorylation reduces the water permeability of oocytes expressing PM28A (Johansson *et al.*, 1998). Taken together, these results suggest that PM28A is important in regulating water flow through leaf tissue. It is not known, however, whether phosphorylation increases the water permeation through single subunits or increases the number of subunits incorporated into the membrane.

Reverse genetics

Reverse genetics may provide another route to studying aquaporin function in plants, but the number of aquaporin genes renders this approach highly challenging. The task to be achieved is indeed enormous and phenotypic compensation in single-gene mutants by close homologues may occur. Nevertheless, genetic evidence that aquaporins play a crucial role in plant growth and development has started to emerge over the last two years. Ikeda *et al.* (1997) discovered that the *mod* mutation, which confers a recessive loss of self-incompatibility in *Brassica*, is the result of a mutation in an aquaporin-like gene. Also, Kaldenhoff *et al.* (1998) have used a PIP1b antisense transgene to reduce the expression of PIP1 genes in *Arabidopsis*. The result was a decrease in water permeability of isolated leaf protoplasts and an increase in root mass, which could reflect a compensatory mechanism resulting from reduced root hydraulic conductivity. While these reports provide compelling evidence for a critical role of aquaporins in plants, the molecular and cellular processes underlying the observed phenotypes remain to be dissected in detail.

Selectivity

It is generally accepted that aquaporins function as narrow pores or channels through which water flows passively down a free energy gradient. Aquaporins consequently confer a high osmotic water permeability (P_{os}) or hydraulic conductivity (L_P) on the membrane. Some aquaporins are highly selective for water, so justifying the name *aquaporin*, e.g. γ-TIP and α-TIP (Maurel *et al.*, 1993, 1995). Others allow non-electrolytes to permeate such as urea (e.g. AQP3: Echevarria *et al.*, 1994; Ishibashi *et al.*, 1997), or they are selective for specific non-electrolytes over water (e.g. the glycerol facilitator protein of *Escherichia coli*, GlpF: Maurel *et al.*, 1994). Some MIPs are relatively non-selective and also allow ion conduction (e.g. NOD26, a nodulin from the symbiosome membrane of soybean: Weaver *et al.*, 1994).

The designation 'aquaporin' derives from the observation that increased water permeability can be conferred on a membrane by functional expression of a MIP, either by translation of heterologous mRNA injected into the cell, as for example in *Xenopus* oocytes (Preston *et al.*, 1992; Maurel *et al.*, 1993) or in yeast (Laizé *et al.*, 1995), or by the incorporation of purified protein into proteoliposomes (Zeidel *et al.*, 1994). It is necessary to check for increased permeability to other non-electrolytes and ions, since increased water permeability may be secondary to a primary function that has little to do with water permeation. This has been done in some cases for plant MIPs (Maurel *et al.*, 1993; Rivers *et al.*, 1997), but more often only water permeation is tested. Assigning a physiological function based on permeability characteristics alone is fraught with the same dangers that beset the interpretation of patch-clamp data. For example, several ion channels permeable to Ca^{2+} have been identified at the electrophysiological level in plants, but determining which ones make the principal contributions to Ca^{2+} transport under cellular conditions is highly complex (Allen and Sanders, 1997; Piñeros and Tester, 1997).

Another important parameter that measures the selectivity of aquaporins is the reflection coefficient, mentioned above, which represents a quantitative index of the interaction between water and solutes as they traverse the membrane. Interactions should be very strong in pores, and the knowledge of reflection coefficients could help indicate the slippage of certain test solutes through aquaporins. The size and chemical structure of test solutes can be varied systematically in such experiments, as has been done for the *Chara* internode (Steudle and Tyerman, 1983; Steudle and Henzler, 1995; Hertel and Steudle, 1997).

Heterologous expression of MIPs in *Xenopus* oocytes has provided a convenient and powerful experimental system for functional characterization of aquaporins. Swelling experiments in hypotonic solutions, uptake of radiolabelled solutes, or two-electrode voltage clamp measurements have been used to investigate the capacity of proteins from the MIP superfamily to transport water, small non-electrolytes or ions, respectively. By means of these techniques, the γ-TIP protein of *Arabidopsis thaliana* and, subsequently, other MIP homologues from the plasma membrane and tonoplast of various plant species, have been shown to function as highly selective water channels (Maurel, 1997; Schäffner, 1998). In these experiments, γ-TIP in particular showed a very low permeability (if any) to H^+ (Maurel *et al*., 1993). These results agree with the high selectivity for water observed for the human red-cell aquaporin-1 (AQP1) after reconstitution into proteoliposomes (Zeidel *et al*., 1994).

In contrast, some MIP homologues in micro-organisms have been unambiguously identified as solute-specific transporters (Maurel *et al*., 1994; Luyten *et al*., 1995). The archetype of such proteins is GlpF, a glycerol facilitator of the inner membrane of *Escherichia coli*, which provides an important mechanism for glycerol import into the cell (Maurel *et al*., 1994). The *glpF* gene is in fact part of an operon encoding enzymes of glycerol metabolism (Sweet *et al*., 1990). *E. coli* also expresses a water-selective channel, AqpZ, with negligible glycerol transport activity, suggesting that solute and water transport can be independently regulated in this organism (Calamita *et al*., 1995, 1998). Analysis of the *E. coli* genome suggests that these are the only two members of the MIP superfamily in this organism (Agre *et al*., 1998). Genomic sequencing has also revealed sequences with similarity to the *E. coli* aquaporin genes in *Haemophilus influenzae*, *Mycoplasma genitalium* and *Synechocystis* sp. PCC6803 (Calamita *et al*., 1998), suggesting that aquaporins are a common feature of bacteria.

The situation seems to be more complex in multicellular organisms. Ten mammalian aquaporins have so far been identified (Agre *et al*., 1998), three of which (AQP3, AQP7 and AQP9) have been found to transport small non-electrolytes such as urea or glycerol in addition to water (Ishibashi *et al*., 1997; Kuriyama *et al*., 1997; Yang and Verkman, 1997). This is also the case for a recently characterized tonoplast aquaporin from tobacco; parallel measurements in isolated tonoplast vesicles indicate that this aquaporin may account for the very high permeability of the tobacco cell tonoplast to solutes such as urea (P Gerbeau, P Ripoche and C Maurel, unpublished results). Tonoplast-enriched vesicles from wheat also show a high urea and glycerol permeability that is mercury-sensitive, in contrast to the lower, mercury-insensitive permeability of the plasma membrane to these molecules (Fig. 2; CM Niemietz and SD Tyerman, unpublished results).

The ability of certain members of the aquaporin superfamily to transport small solutes may also have an evolutionary basis. Sequence analysis suggests that the aquaporins able to transport glycerol, namely the mammalian AQP3, AQP7 and AQP9 together with the *E. coli* glpF, are orthologous proteins phylogenetically distinct from the true water-selective aquaporins (Park and Saier, 1996; Agre *et al*., 1998; Sasaki *et al*., 1998). The term 'aquaglyceroporin' has been suggested for this subgroup of proteins capable of transporting glycerol, but more extensive studies of the substrate selectivity of these aquaporins are certainly needed before the functional significance of this distinction is properly understood. For example, it was recently reported that the 'aquaglyceroporin' AQP9 isoform from human leukocytes facilitates transport of water and urea when expressed in *Xenopus* oocytes, but not glycerol (Ishibashi *et al*., 1998). On the other hand, another AQP9 isoform cloned from liver apparently has a very broad substrate selectivity when expressed in oocytes, transporting both water and a wide range of neutral solutes that includes carbamides, polyols, purines, and pyrimidines (Tsukaguchi *et al*., 1998). Interestingly, the *Saccharomyces cerevisiae* genome contains two open-reading frames related to the aquaglyceroporins and two others more closely related to the true aquaporins (Agre *et al*., 1998). Studies with an *fps1* deletion mutant of *S. cerevisiae* have indicated that this gene encodes a glycerol transporter; moreover, the mutant had altered passive diffusion characteristics and contained a lower ratio of phospholipids to glycerolipids than the wild type, suggesting that the Fps1p protein might also play a role in regulation of lipid metabolism which may, in turn, affect membrane permeability (Sutherland *et al*., 1997).

Further complexity is also likely to be uncovered in attempts to define the physiological substrates of the aquaporins. Recently, the human AQP1 protein, which has been generally accepted as the functional aquaporin that accounts for the high water permeability of red cells, has been shown to facilitate CO_2 diffusion across the *Xenopus* oocyte membrane (Nakhoul *et al*., 1998). It will be interesting to see in subsequent experiments if the CO_2 permeability of AQP1 is also mercury-sensitive, which would be anticipated if CO_2 moves through the protein via the same pathway as water. From the data presented by Nakhoul *et al*. (1998), and assuming that CO_2 moves through individual aquaporin subunits as assumed for water, it is possible to calculate a CO_2 permeability per subunit. Although the predicted CO_2 permeability per subunit (approximately 6×10^{-21} m^3 s^{-1}) is 10-fold lower than that for water, a relatively high CO_2 permeability for the red cell membrane is functionally important for this membrane. In respiring tissue, CO_2 must diffuse into the red cell to be converted to bicarbonate; this is then exchanged for Cl^-, so that bicarbonate can be stored in

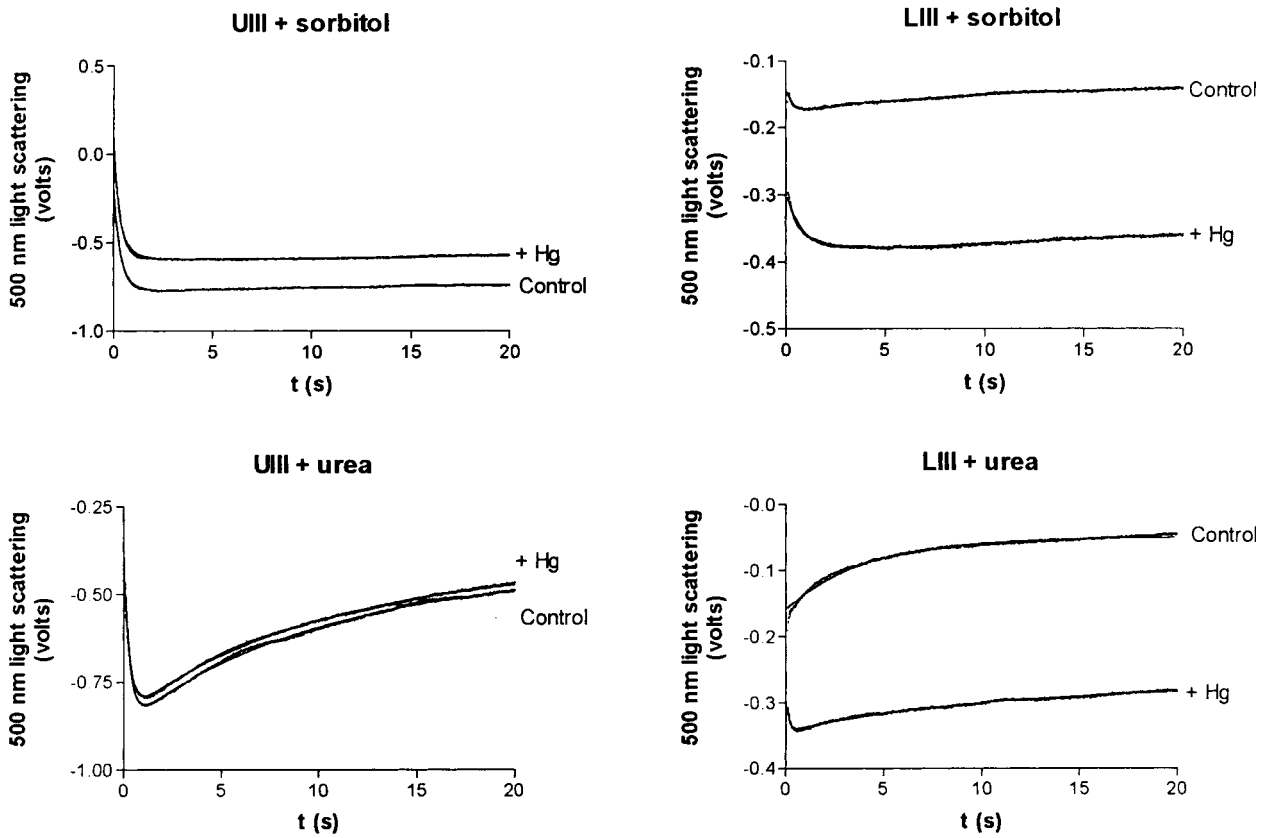


Fig. 2. Permeation of water and non-electrolytes through membrane vesicles isolated from wheat roots. Membrane vesicles were isolated using phase partitioning and light scattering was recorded as a measure of vesicle volume changes after a sudden increase in external concentration using the test solutes, sorbitol and urea (Niemietz and Tyerman, 1997). Plasma-membrane-enriched vesicles (UIII) and endomembrane vesicles, largely tonoplast (LIII), show very different permeation characteristics. Sorbitol barely enters the plasma membrane vesicles (top left) over a period of 20 s, but clearly permeates the tonoplast-enriched vesicles (top right). This can be assessed by the degree to which the vesicles re-swell (upward movement of trace) after the initial osmotically induced shrinking (downward movement of trace). For tonoplast-enriched vesicles, the initial shrinking due to water efflux is very fast and difficult to resolve on the time scale used in these graphs. It is more clearly seen when inhibited by mercury. Urea permeates both plasma membrane- (bottom left) and tonoplast-enriched vesicles (bottom right), but the permeability coefficient for urea in tonoplast-enriched vesicles is higher than for plasma membrane vesicles (5.5×10^{-9} m s^{-1} compared with 1.7×10^{-9} m s^{-1}). Addition of 100 μM mercuric chloride does not inhibit water or urea permeation in plasma membrane vesicles (left), but strongly inhibits permeation of water, sorbitol and urea in tonoplast-enriched vesicles (right). In the presence of mercury, the tonoplast urea permeability is reduced to 2×10^{-9} m s^{-1}, similar to the plasma membrane urea permeability. The traces have been fitted to the sum of two exponentials to obtain the rate constants for water efflux and solute influx. From these rate constants and the vesicle volume to surface area ratio, the water and solute permeability can be calculated (CM Niemietz and SD Tyerman, unpublished results).

the plasma for transport to the lungs. It might be argued that the lipid bilayer should have a sufficiently high permeability to CO_2 to allow adequate rates of transport by passive diffusion, although this incidentally is not always the case for small non-electrolytes (Kikeri *et al.*, 1989; Boron *et al.*, 1994; Walsbren *et al.*, 1994). This argument is reminiscent of that used by some biophysicists regarding facilitated water flow across plant membranes at the 9th Membrane Transport Workshop in Monterey in 1992, when Maarten Chrispeels described the functional characterization of γ-TIP. But clearly, the possibility that aquaporins play a role in transport of CO_2, and perhaps even other non-electrolytes, in plant cells is one that merits further investigation.

The physiological relevance of the capacity of certain aquaporins to transport both water and solutes, if any, remains to be determined in plants. Future work should consider not only critical compounds like CO_2 and NH_3, but also compatible solutes of relatively small size such as proline and perhaps even polyhydric alcohols. Given that NOD26 allows permeation of water, glycerol and formamide (Rivers *et al.*, 1997), it would be interesting to measure the NH_3 permeability of this MIP, since the symbiosome membrane in which NOD26 resides must allow both NH_3 and NH_4^+ to escape from the bacteroid to the plant cytosol to be assimilated. An NH_4^+-permeable channel resides on the symbiosome membrane as determined by patch-clamp (Tyerman *et al.*, 1995).

The molecular basis of aquaporin selectivity is currently unknown. Substrate exclusion by size in a narrow aqueous pore has long been considered to be the basis of water channel selectivity (Macey, 1984). This mechanism is

unlikely to be sufficient to account for the various selectivity profiles observed among all the different members of the MIP superfamily. Nevertheless, recent progress in determining the 3-D structure of aquaporins (Cheng *et al*., 1997; Walz *et al*., 1997) suggests that an atomic understanding of water and solute permeation in the aquaporin pore is approaching.

Role of aquaporins at the cell, tissue and organ levels

Tissues and organs

At the level of individual cells and organelles, water movement is usually very fast. For cells of higher plants, time constants are typically of the order of several seconds (Steudle, 1993). Even in the absence of water channels, water transport would not be expected to rate-limit processes at the cell level such as osmoregulation, extension growth or other processes that originate from solute movement within tissues or from other 'endogenous' changes in water potential. However, at the tissue and organ levels the situation may be different. On purely geometric grounds, diffusion theory predicts that the time constants for water-potential equilibration increase substantially in cell aggregates (Philip, 1958). Also, the pathways of water flow through tissues need to be considered. In tissues, three different parallel components of water flow are possible, i.e. symplastic movement of water via plasmodesmata, transcellular movement across cell membranes, and apoplastic flow around protoplasts (Steudle *et al*., 1993; Steudle, 1994). To date, the symplastic and transcellular pathways cannot be measured separately and thus they are treated as a 'cell-to-cell' component or path, the contribution of which can be determined experimentally.

Provided there are no special apoplastic barriers in the tissue (such as the Casparian bands and suberin lamellae in the root's exo- and endodermis), the apoplastic pathway has often been considered to be that of least hydraulic resistance. However, despite the relatively high L_P of the cell-wall material, the fractional cross-sectional area of the apoplast pathway is rather small in many tissues. Furthermore, extensive studies on many cell types with the pressure probe have shown that cell-membrane L_P is high (presumably on account of abundant water channels!). The net result appears to be that, in at least some parenchymatous tissues, water flow probably occurs mainly via the cell-to-cell pathway rather than through the apoplast (Boyer, 1985; Smith and Nobel, 1986).

One difficulty in quantifying water flow through different pathways is that the relative contribution of apoplastic and cellular components can vary. It has been proposed that this may explain most of the variability in tissue/organ hydraulic properties that is frequently observed (Brouwer, 1954; Weatherley, 1982; Passioura, 1988; Steudle, 1994). To date, the greatest amount of data is available for root hydraulics (water uptake by roots), the variability in which has been known for more than 60 years (Brewig, 1937). For the root, there are data for both the overall hydraulic conductivity as well as for the cell (protoplast) level which allow quantification of the contribution of pathways and an explanation of the variability of tissue hydraulics (Steudle, 1994, 1997; Steudle and Frensch, 1996; Steudle and Peterson, 1998). The effects may have a direct bearing on the ability of plants to survive and grow under adverse conditions such as water shortage, high salinity, and heavy metal and nutrient stress.

Transient and steady water flow

In tissue water relations, two different situations have to be considered: (i) a non-steady-state in which a plant tissue has to respond transiently to a change in the water status (water potential) of its surroundings; (ii) cases in which a steady flow of water has been achieved across the tissue. In the case of transient changes, the storage capacitance of the system (cells/protoplasts and apoplast) and its detailed hydraulics (water permeabilities) play a role as well as the tissue/organ size and geometry. As noted above, these factors determine the time constant or half-time of the plant's response. Under steady-state conditions, such as during transpirational water flow across roots and leaves, only hydraulic (frictional) properties of the two parallel pathways are important, although these may change in response to endogenous and external signals or to alterations in tissue anatomy (Zimmermann and Steudle, 1998).

Under conditions in which the cell-to-cell pathway of water flow dominates, regulation of the activity of water channels could obviously have a major effect on the hydraulic properties of the tissue. This is indicated in some experiments showing a rapid 4–6-fold reduction in root L_P with $HgCl_2$ (Maggio and Joly, 1995; Carvajal *et al*., 1996; but note the discussion of the possible indirect effects of mercury on cell metabolism above). In other situations where the overall flow is dominated by the apoplastic component, the contribution of water channels to the control of water transport would be expected to be small. Here water channels may be important for cells in the pathway to be in local equilibrium with the apoplast.

Physiological meaning of variable root hydraulics

Considerable changes in water permeability (hydraulic conductivity) can occur in roots. During the day, transpiration generates high tensions in the root xylem and large gradients in water potential across the root cylinder; as the hydraulic conductivity of the root is rather high, these

gradients drive a large radial flow of water. At night, when transpirational flow is generally much reduced, hydraulic (pressure) gradients across the root cylinder decline. Gradients in water potential may even reverse and may be non-hydraulic (osmotic and/or matric) in nature. Under these conditions, the hydraulic conductivity of the root is low, which reduces water loss from the plant to the dry soil. This example shows that changes in the nature of the force driving water across a tissue may cause changes in the transport pathway (from apoplastic to cell-to-cell) and changes in hydraulic conductivities. Depending on the exact circumstances, the relative contribution of water channels to flow across the root could thus vary. This may provide a useful mechanism by which the plant can respond to changing environmental conditions.

Composite transport model

It has been proposed (Steudle, 1994; Steudle and Frensch, 1996; Steudle and Peterson, 1998) that the composite structure of tissues and organs in plants results in a composite transport of water across them, which may be dealt with within the framework of irreversible thermodynamics. Composite transport allows for variability in tissue/organ hydraulics, which may be essential for the plant's survival under adverse conditions. As shown for the root, composite transport may result in a form of coarse regulation of water flow. Water-channel activity may be required for fine regulation, which could be influenced by cell metabolism, for example, via phosphorylation of the aquaporin protein (Daniels *et al.*, 1994; Maurel *et al.*, 1995; Johansson *et al.*, 1998). In the root, water-channel activity may be essential when the root is heavily suberized and the apoplastic path blocked.

Quantitative information on water flow at the level of individual cells (cell pressure probe) and the whole root (root pressure probe and other techniques) collected over the past decade appears to support the composite transport model, as well as suggesting explanations for differences between species (e.g. herbaceous versus woody) and the low reflection coefficients of plant roots. The root example illustrates how water-channel activity is only one component that has to be integrated into a complete description of the composite pattern of water transport in plants. Regulation of water-channel activity by itself is unlikely to be able to explain the large variations in hydraulic conductivity at the level of tissues and organs.

Cells

Root water transport, cell elongation, movement of stomatal guard cells, and many other non-steady-state physiological processes in plants with relatively slow kinetics (time constants of the order of minutes) involve transmembrane water transport. Indeed, the kinetics of water equilibration by whole organs can be of the order of hours or even days. This raises the question whether the involvement of aquaporins in transmembrane water flow at the cellular level—where time constants are of the order of seconds—can also be said to be significant for the water relations of the whole plant. As mentioned above, this situation is partly explained by the geometry and structural organization of plant tissues. Standard diffusion theory shows that the time constants for equilibration of cell aggregates can be orders of magnitude greater than those for individual cells. Presumably, if water channels did not contribute to transmembrane water flow, the time constants for whole tissues and organs would be even longer. In addition, certain conditions may result in very large and rapid changes in extracellular water potential and provide a rationale for a cellular role of aquaporins. This is the case in root cells, for instance, when a sudden moistening of the soil may follow a prolonged drought. Also, mechanically induced movements of aerial plant parts can create strong tensions in plant tissues, which may be transduced at the cell level as very rapid changes in hydrostatic pressure. Finally, the hydraulic coupling between xylem vessels and the neighbouring cells suggests that cavitation events in the xylem may result in drastic water-potential perturbations in these cells.

The equilibration of a cell in response to a water-potential perturbation could seriously disturb the cytoplasm. This is due to the cytoplasm having a small volume relative to the entire volume of a fully expanded cell, and its arrangement in series between the cell exterior and the vacuole. As water flows across the plasma membrane the volume of the cytoplasm will change. The concomitant change in cytosolic osmotic pressure will result in the cytoplasm being in osmotic disequilibrium with the vacuole. There will be a flow of water across the tonoplast in response to the osmotic gradient. Since water flow across the plasma membrane is driven by both osmotic and pressure gradients, the latter being rather large, the volume flow can be initially rapid across the plasma membrane. In contrast, the flow across the tonoplast can only be driven by a small osmotic-pressure gradient, constrained by the extent to which the cytoplasm changes volume. One way to compensate for this imbalance and to keep the cytoplasmic volume change to a minimum would be for the tonoplast to have a much higher L_P than that of the plasma membrane.

The extent to which the difference in tonoplast and plasma membrane L_p values affect the change in cytoplasmic volume during a change in external water potential can be modelled according to well-established equations describing the elastic properties of the cell wall and water movements across a membrane in response to hydrostatic and/or osmotic pressure gradients (for reviews see Steudle, 1993, 1994; and Maurel, 1997). Such a model,

illustrated in Fig. 3, shows that when the tonoplast and plasma membranes have equal L_P (1 : 1 situation) there is a rapid and large transient in cytoplasmic volume. This is abolished when the ratio of L_P (plasma membrane:tonoplast) becomes small (e.g. 1 : 100). Essentially, the higher water permeability of the tonoplast allows the vacuole to buffer the cytoplasm very rapidly, thereby preventing short-term volume changes in the cytoplasm that might otherwise damage the cytoskeleton and metabolism. The modelled situation of a higher water permeability of the tonoplast compared to the plasma membrane is supported by measured ratios of 7 for wheat root cells (Niemietz and Tyerman, 1997) and 100 for tobacco suspension cells (Maurel *et al*., 1997).

In an alternative interpretation of this model, one may consider the relaxation of water-potential gradients rather than the relaxation of compartmental volumes. Simulations show that a high P_{os} at the tonoplast provides an efficient mechanism for dissipating any water-potential (osmotic) gradient across this membrane. This would avoid any constraint within the cell and confine, at any time and in any condition, the drop in osmotic pressure to the plasma membrane.

Abundance and diversity

While as many as ten aquaporins have been identified in mammals (Koyama *et al*., 1997; Kuriyama *et al*., 1997; Agre *et al*., 1998), the count of MIP genes in plants seems to be even higher. Weig *et al*. (1997) identified 23 MIP homologues in *Arabidopsis* (Fig. 4), mostly from the analysis of expressed sequence tags (ESTs), and a few additional genes from this family are likely to be identified once the sequence of the *Arabidopsis* genome is completed. In comparison, the genome of fungi—the third major group of crown eukaryotes—appears to contain fewer MIP genes (Chrispeels and Agre, 1994; Yamada *et al*., 1995; Weig *et al*., 1997). In the yeast *Saccharomyces cerevisiae*, for example, one reading frame (YPR192W; length: 305 amino acids) with approximately 50% identity to a plant plasma membrane aquaporin (PIP1b of *Arabidopsis*) has been identified. Two other reading frames, YLL053C (152 amino acids) and YLL052C (149 amino acids), exist that encode similar but smaller proteins consisting of only three membrane-spanning regions, but the presence of the encoded proteins in yeast has not yet been documented. In addition, another member of the MIP family encodes the yeast glycerol facilitator Fps1. Deletion of the yeast YPR192W aquaporin does not produce a phenotype under normal growth conditions (H Li and HJ Bohnert, unpublished results). The percentage identity between plant and animal MIPs ranges from 30–40%, as opposed to 70–90% when comparing MIPs between plant species. In animals, extensive expression analyses have been carried out at both the transcript and protein levels for individual members of the MIP superfamily. An analysis of the EST databank of human and mouse transcripts indicates that the aquaporins already identified might represent the entire mammalian gene family.

Although *Arabidopsis* contains in excess of 23 MIP homologues, other data indicate that the maize genome includes even more MIP genes. An analysis of EST sequences, most of which are not yet in the public domain, suggests the presence of at least 31 MIP genes in maize (F Barrieu and R Jung, personal communication). In all probability, however, these are likely to represent only the most abundant transcripts, and the presence of even more MIP genes is to be expected. In *Mesembryanthemum crystallinum*, 14 MIP transcripts have been partially characterized (Yamada *et al*., 1995, 1997; HH Kirch, F Quigley and HJ Bohnert, unpublished results). Based on sequence similarities and biochemical studies on the localization of MIPs, the plant MIP family can be divided into three groups: two subfamilies of approximately equal gene copy number encoding plasma membrane (PIP) and tonoplast (TIP) proteins. A third 'subfamily' comprises a single sequence, NLM1, related to a MIP expressed in soybean nodules (Weig *et al*., 1997).

Reality, however, may be more complicated. Although the PIP/TIP dichotomy is striking at the sequence comparison level (Fig. 4), recent evidence suggests that the biological interpretation of its significance might be more complex. In particular, (i) plants possess more than one type of vacuole (Paris *et al*., 1996); (ii) endosomal traffic—as in animal and yeast cells—could play a role in the turnover of membranes and membrane proteins in plants (Dupree, 1996; Robinson *et al*., 1996; Takeuchi *et al*., 1998); and (iii) compared with the multitude of putative aquaporin genes in plants, their assignment as either PIP or TIP is based on a uncomfortably small number of experiments in which their localization has been studied. Both the location in intracellular membranes and aquaporin traffic through the plant membrane system need to be investigated for a better understanding of water-channel functions.

Figure 4 compares the deduced MIP amino-acid sequences from just two species of plants, but the comparison clearly reinforces the identification of the subfamilies of orthologous proteins defined by Weig *et al*. (1997). The species concerned are both dicotyledons, but they are representatives of two different subclasses, the Caryophyllidae (*Mesembryanthemum*) and Dilleniidae (*Arabidopsis*), that diverged tens of millions of years ago. Alignments of such evolutionarily distant sequences might be expected to separate into species-specific subfamilies, but this is not the case. The alignment clusters the sequences into at least three subgroups within subfamily 1 (PIP) and into at least two separate subgroups in subfamily 2 (TIP), while the deduced NML1 gene product

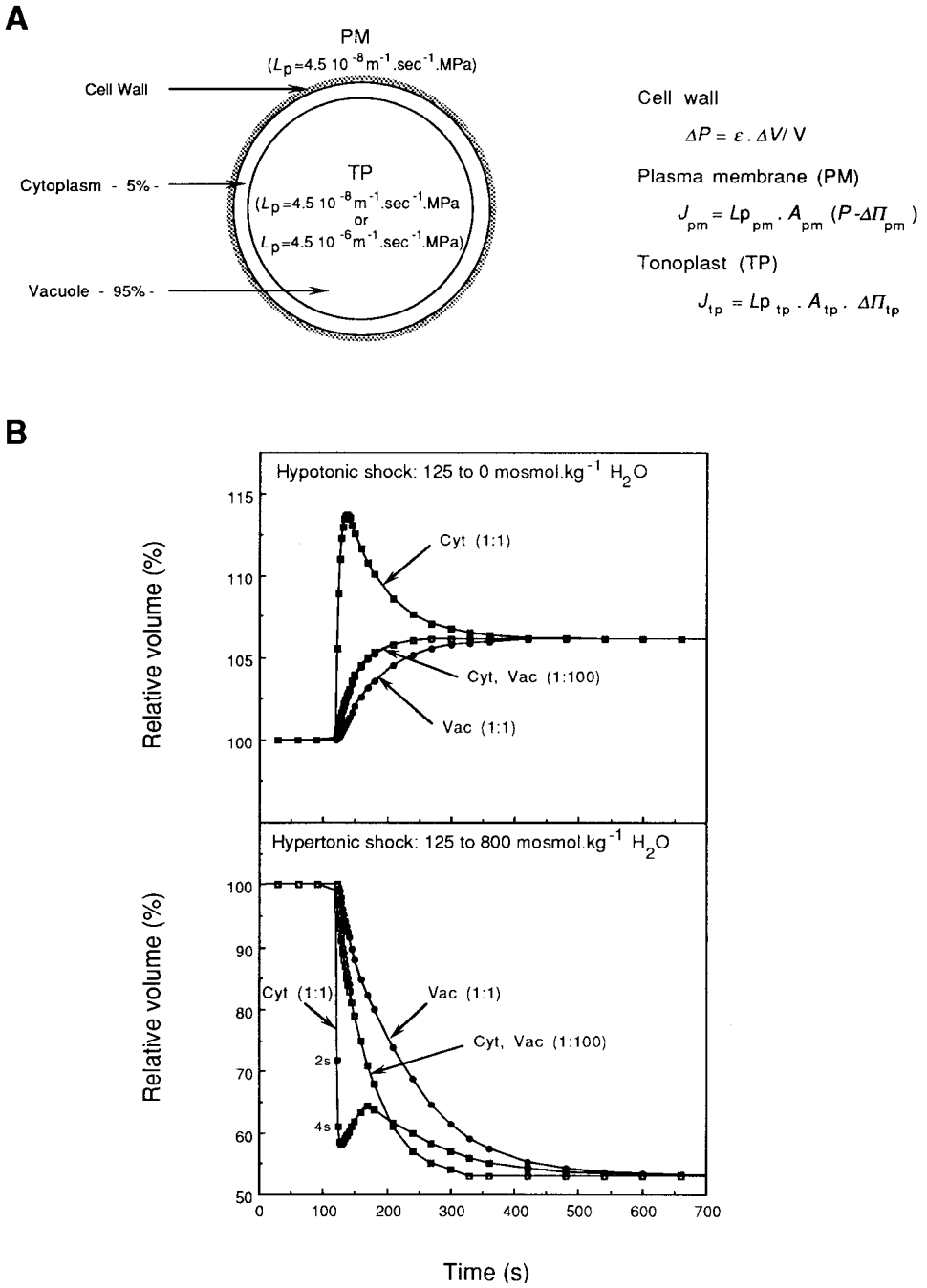
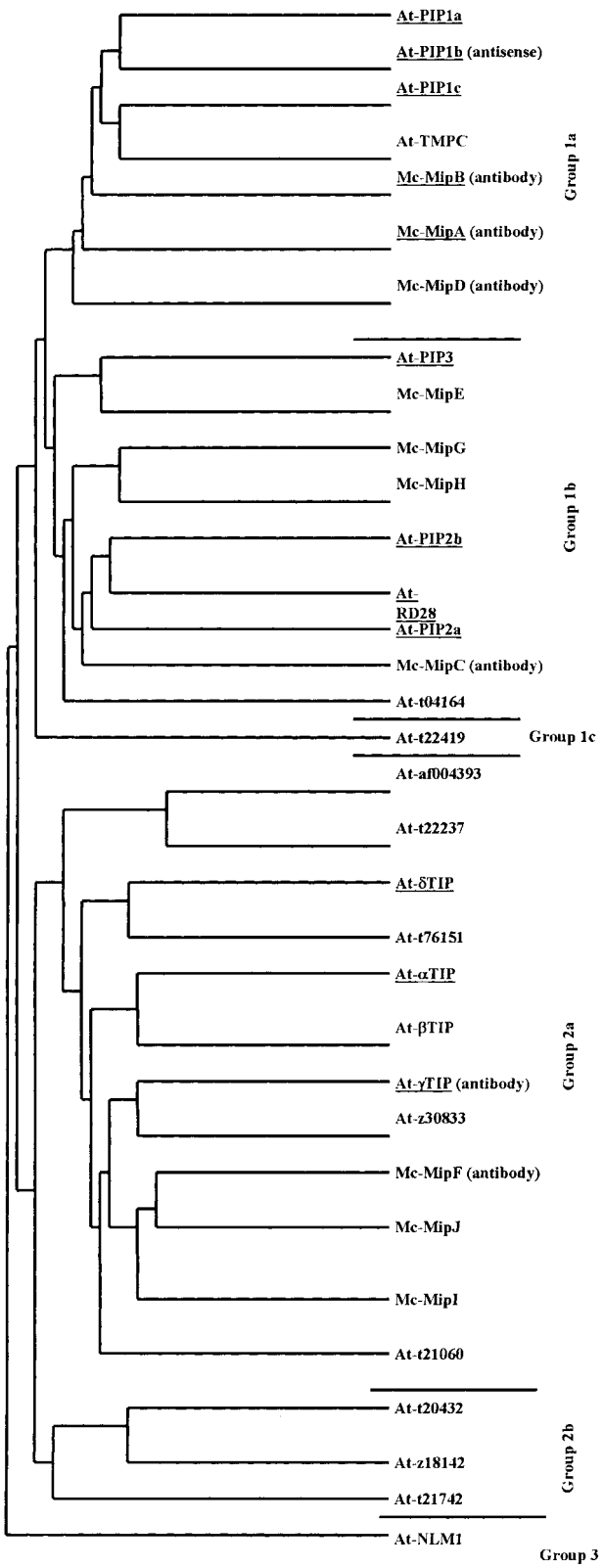


Fig. 3. Water transport kinetics in a model plant cell. (A) A simplified plant cell was modelled as two concentric spheres corresponding to, respectively, the cell wall and plasma membrane (PM) and the tonoplast (TP). In the simulations, P and ΔP refer to the turgor pressure and its variation, V and ΔV represent the cell volume and its variation, and ε is the cell volumetric elastic modulus. For each membrane (PM and TP), J is the net volume flow, L_P the hydraulic conductivity, A the membrane surface, and $\Delta\Pi$ the osmotic gradient. The water permeability of the PM was set to a constant value ($L_P = 4.5 \times 10^{-8}$ m s^{-1} MPa^{-1}, equivalent to an osmotic water permeability coefficient $P_{os} = 6 \times 10^{-6}$ m s^{-1}), as experimentally determined in plasma membrane vesicles purified from tobacco suspension cells (Maurel *et al.*, 1997). Two distinct water permeability values were considered for the TP ($L_P = 4.5 \times 10^{-8}$ m s^{-1} MPa^{-1} and $L_P = 4.45 \times 10^{-6}$ m s^{-1} MPa^{-1}), which define two distinct configurations for the cell (1 : 1 and 1 : 100, respectively). (B) The response of the model cell to a hypotonic (change in external Π from 125 to 0 mOsmol kg^{-1} H_2O) or a hypertonic shock (change in external Π from 125 to 800 mOsmol kg^{-1} H_2O) was simulated and the changes in relative volume of the cytosol (Cyt) and the vacuole (Vac) are shown. Note that the initial rate of cytosol shrinking or swelling is much higher in the (1 : 1) configuration than in the (1 : 100) configuration. Initial conditions were as follows: $\varepsilon = 4$ MPa; $P_{(0)} = 0.73$ MPa; $V_{(0)} = 3.35 \times 10^{-14}$ m^3; $V_{Cyt} = 0.05 \times V_{(0)}$.



(subfamily 3) has no *Mesembryanthemum* orthologue yet identified. This sequence comparison suggests that the gene duplication events which gave rise to the principal subgroups within the MIP gene family must have preceded the speciation events that separated these two subclasses of dicotyledonous plants. The resolution of this phylogeny, which is at present rudimentary, should become better as complete, or nearly complete, sets of MIP transcripts from different species emerge. Members of individual subgroups must then be analysed for subcellular location, expression characteristics and function. With the advent of complete gene sequences for a few species and complete transcript sequences for many more, functional assignments based on sequence alignments should eventually become possible.

In addition to the difficulties of resolving their subcellular localization, even the cell- and tissue-specific expression of the MIP gene products is largely unknown. In a few examples, root-, leaf-, or guard-cell-specific expression, or the presence of transcripts in vascular tissue, has been demonstrated (Yamada *et al.*, 1995; Sarda *et al.*, 1997). Recently, the presence of different MIP proteins has been documented in epidermis, root cortex, pericycle or endodermis, xylem parenchyma, phloem companion cells, mesophyll cells, guard cells, or meristematic cells by *in situ* hybridizations or immunological methods (Barrieu *et al.*, 1998*a*, *b*; Chaumont *et al.*, 1998; HH Kirch and HJ Bohnert, unpublished results). But one potentially serious difficulty with the immunological approach is the following. Since not all MIP-type genes are known, generating antibodies against either isolated proteins or (which is more typical) peptides from deduced amino-acid sequences provides an immuno-cytochemical localization that may represent the combined signal from several related aquaporins whose sequences have not yet been detected. Better resolution can be achieved by isolating the promoters for all the MIP genes from a species and using them as fusions with reporter- or indicator-coding regions in transgenic plants (Yamada *et al.*, 1997). This will be a difficult task, but only with such information will we be able to understand aquaporin function and the reason for the complexity of this gene family in plants.

Fig. 4. Alignment of *Arabidopsis* and *Mesembryanthemum* sequences with homology to major intrinsic proteins. The alignment (program: Pileup), which is not based on parsimony or nearest-neighbour analysis, shows the three subfamilies defined by Weig *et al.* (1997) with the *Arabidopsis* NLM1 sequence as the outgroup (Group 3). Groups 1 and 2 represent PIP and TIP sequences, respectively. Inclusion of the *Mesembryanthemum* sequences permits finer resolution into several subgroups within the PIP and TIP subfamilies. At, *Arabidopsis thaliana*; Mc, *Mesembryanthemum crystallinum*. Sequences are identified by common name or accession numbers (see Weig *et al.*, 1997). Not all *Mesembryanthemum* sequences have been deposited in the databases (F Quigley, HH Kirch and HJ Bohnert, unpublished results).

Conclusions

With respect to determining the function of aquaporins in native membranes, rapid progress could be made by combining reverse genetics with biophysical characterization of water flow across the native membranes of control and transformed plants. The effects of mercurials on water permeation across living cells and for whole organs should be interpreted with caution as some mercurials, particularly $HgCl_2$, are highly membrane-permeant and are powerful metabolic inhibitors. Reduced phosphorylation of water channels caused by metabolic inhibition is one possible outcome of $HgCl_2$ application.

Measurement of selectivity of water channels both in native membranes and in expression systems needs to be extended to include CO_2 and NH_3. It would also be interesting to examine MIP selectivity for compatible solutes used for balancing the cytoplasmic and vacuolar osmotic pressures in cells.

At the level of tissues and organs, factors other than membrane water permeability can lead to variations in overall hydraulic conductivity. At the cell level, high tonoplast water permeability compared to the plasma membrane will buffer the cytoplasm from experiencing large and rapid changes in volume.

The abundance of MIPs in plants is probably related to plants having to achieve a three-dimensional control of water exchange in living tissues. This control has to be exerted during all stages of plant growth and development and has to respond to various, and sometimes very challenging, environmental conditions. The typical compartmentation of highly vacuolate plant cells also requires tightly co-ordinated control of water and solute transport across both the plasma membrane and tonoplast. The combination of these requirements may explain why so many aquaporins are to be found in plants, each with distinctive properties.

Acknowledgements

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