Plant Aquaporins: Membrane Channels with Multiple Integrated Functions

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Abstract

Aquaporins are channel proteins present in the plasma and intracellular membranes of plant cells, where they facilitate the transport of water and/or small neutral solutes (urea, boric acid, silicic acid) or gases (ammonia, carbon dioxide). Recent progress was made in understanding the molecular bases of aquaporin transport selectivity and gating. The present review examines how a wide range of selectivity profiles and regulation properties allows aquaporins to be integrated in numerous functions, throughout plant development, and during adaptations to variable living conditions. Although they play a central role in water relations of roots, leaves, seeds, and flowers, aquaporins have also been linked to plant mineral nutrition and carbon and nitrogen fixation.

Contents

INTRODUCTION

Aquaporins are small integral membrane proteins that belong to the ancient family of major intrinsic proteins (MIPs), with members in animals, microbes, and plants. Fifteen years after their discovery in plants, it now appears that studies on aquaporins have provided unique perspectives into multiple integrated aspects of plant biology. Aquaporins first raised considerable interest because of their water channel activity. This finding was unexpected in plants and, although it may not have induced a real paradigm shift in understanding of membrane water transport (124, 140), it led researchers to revisit many aspects of plant water relations and to link these aspects to novel physiological contexts. More recently, MIPs were proved to be more than water channels (141), and other transport substrates of great physiological significance have been identified.

MIP: major intrinsic protein

PIP: plasma membrane intrinsic protein

TIP: tonoplast intrinsic protein

NIP: nodulin-26–like intrinsic protein

SIP: small basic intrinsic protein

Although the term aquaporin was initially restricted to water-transporting MIPs, we now use this term in a broader sense, referring to all plant MIPs as aquaporins. The molecular and cellular properties of aquaporins were recently reviewed in detail (18, 91). The aim of the present review is to examine how a wide range of selectivity profiles and regulation properties allows aquaporins to be integrated in numerous functions throughout plant development and during adaptations to variable environmental conditions.

MOLECULAR AND CELLULAR PROPERTIES

The Plant Aquaporin Family

Subfamilies. Plant aquaporins show a high multiplicity of isoforms, with 35 and 33 homologs in *Arabidopsis* and rice, respectively (62, 113, 120). On the basis of sequence homology, aquaporins in most plant species can be divided into four subgroups. The plasma membrane intrinsic proteins (PIP) (with two phylogenic subgroups, PIP1 and PIP2, and 13 isoforms in *Arabidopsis*) and the tonoplast intrinsic proteins (TIP) (10 homologs in *Arabidopsis*) are the most abundant aquaporins in the plasma membrane and vacuolar membrane (tonoplast), respectively (62, 113). The third subfamily comprises the nodulin-26–like intrinsic membrane proteins (NIPs), which were named after soybean (*Glycine max*) nodulin-26 (*Gm*NOD26), an abundant aquaporin expressed in the peribacteroid membrane of N_2-fixing symbiotic root nodules. NIPs are also present in nonlegume plant species (9 homologs in *Arabidopsis*) (149). A fourth class comprises small basic intrinsic proteins (SIPs) (3 homologs in *Arabidopsis*) (56, 62, 113). Although these four classes are conserved among all plant species, the aquaporin gene family shows signs of rapid and recent evolution and orthologs cannot necessarily be distinguished between species (120). In addition, some plant species have acquired additional, novel types of aquaporins.

For instance, a homolog of the bacterial glycerol facilitator GlpF has been acquired by the moss *Physcomitrella patens* by horizontal gene transfer (45), and the genome of this organism and some higher plants (such as poplar) encodes a fifth class of aquaporins, which are closely related to but yet clearly distinct from PIPs (139; U. Johanson, personal communication).

Subcellular localization. Plant aquaporins localize in all subcellular compartments forming or derived from the secretory pathway. This broad localization pattern reflects the high degree of compartmentation of the plant cell and the need for the cell to control water and solute transport not only across the plasma membrane but also across intracellular membranes. Similar to PIPs, some NIPs localize in the plasma membrane (82, 134). By contrast, the three *Arabidopsis* SIP homologs reside mainly in the endoplasmic reticulum (56).

However, aquaporins cannot simply be assigned to homogeneous subcellular compartments. For instance, immunocytochemical studies using isoform-specific anti-TIP antibodies revealed that distinct types of vacuole that can coexist in the same cell are equipped with specific combinations of TIP isoforms; TIP1 and TIP2 isoforms are preferentially associated with the large lytic vacuoles and vacuoles accumulating vegetative storage proteins, respectively (59). More recently, *Arabidopsis thaliana At*TIP1;1 was shown to accumulate in spherical structures named bulbs, tentatively identified as intravacuolar invaginations made of a double tonoplast membrane (118). Preferential expression of PIPs in plasmalemmasomes (convoluted plasma membrane invaginations that dip into the vacuole) has also been observed in *Arabidopsis* leaves (116). Finally, preferential expression of a PIP and a NIP homolog on the distal side of root exo- and endodermal cells has been described in maize and rice, respectively (46, 82). Such cell polarization is consistent with the uptake and centripetal transport of water and solute in roots (see below). A future challenge is to understand how aquaporins can be specifically targeted to membrane subdomains in the plant cell and how targeting contributes to their functional specialization.

Plasmalemmasome: convoluted plasma membrane invagination

Mechanisms of Transport

Pore structure and transport mechanisms. X-ray crystallography determination of atomic structures of microbial, animal, and plant homologs points to highly conserved structural features in the aquaporin family (38, 137). Aquaporins are 23–31 kDa proteins comprising six membrane-spanning domains tilted along the plane of the membrane and linked by five loops (*A* to *E*) located on the intra- (*B*, *D*) or extracytoplasmic (*A*, *C*, *E*) side of the membrane. The N- and C-terminal extremities are both exposed to the cytosol (**Figure 1**). A central aqueous pore is delineated by the transmembrane domains and loops *B* and *E*, which both carry a conserved Asn-Pro-Ala (NPA) motif and dip from either side of the membrane into the center of the molecule. Projection structures determined by cryo-electron microscopy indicate that, similar to their animal and microbial counterparts, PIPs and TIPs occur as tetramers in their native membranes (24, 34). X-ray structures have confirmed this type of assembly (38, 137) and in combination with molecular dynamics simulations have provided critical insights into the fundamental principles of aquaporin transport selectivity (38, 133) (**Figure 1**). In brief, the substrate specificity of aquaporins can be explained by several mechanisms, including size exclusion at two main pore constrictions [aromatic/Arg (Ar/R) and NPA] and stereospecific recognition of the substrate mediated by spatially defined H-bonding and hydrophobic interactions within the pore. The remarkable impermeability of aquaporins to protons is explained by electrostatic repulsion, dipole orientation, and transient isolation of the water molecule as it passes within a single

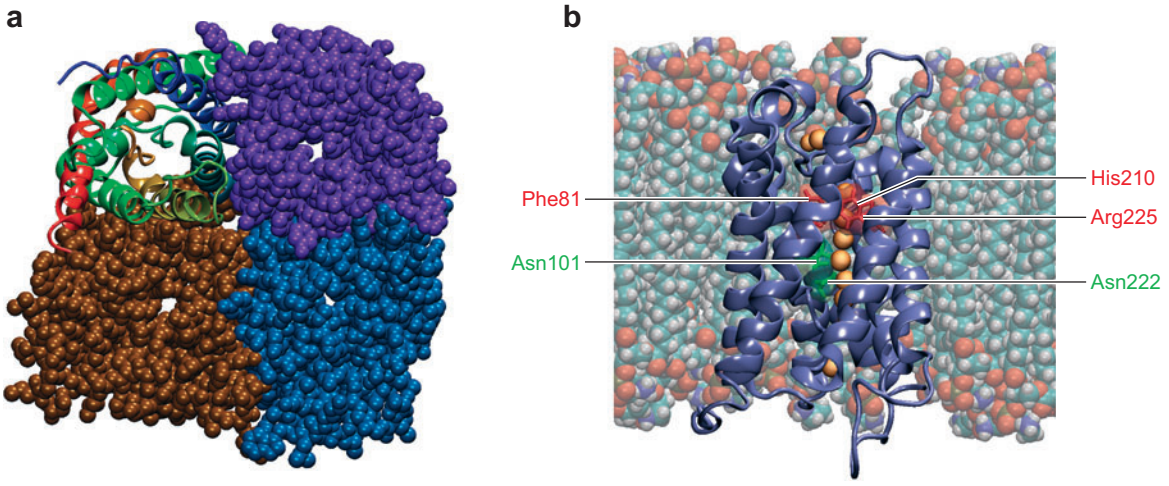


Figure 1

Representative atomic structure of a plant aquaporin (*a*) and general molecular mechanisms of transport selectivity (*b*). (*a*) Structure of the open conformation of *Spinacia oleracea* plasma membrane intrinsic protein 2;1 (*So*PIP2;1) [Protein Data Bank (PDB) ID 2B5F] (137) showing a typical tetrameric arrangement. Each monomer is composed of six tilted transmembrane helices; the N-terminal (*red*) and C-terminal (*green*) helices of the top left monomer are shown. The pores of individual monomers are emphasized by the space-filling representation of the three other monomers. (*b*) The two highly conserved Asn-Pro-Ala (NPA) motifs (represented by Asn101 and Asn222, *green*) are in close proximity to form one of the main pore constrictions. Another constriction called Ar/R (*red*) is formed on the extracytoplasmic side of the membrane by a spatial arrangement of aromatic (Ar) residues, such as Phe81 and His210, facing an Arg (R) residue, here Arg225. Proton transport is blocked by electrostatic repulsion in the Ar/R constriction and the dipole orientation of the water molecule by the two Asn residues of the NPA motifs. This results in a transient isolation of the water molecule within the single file of water molecules that fills the pore (*orange spheres*).

file of water molecules through the center of the pore (11, 38, 133) (**Figure 1**).

The molecular basis of plant aquaporin selectivity has been investigated more specifically by homology modeling of pore structures at the Ar/R constriction (8, 150). Analysis of all 35 *Arabidopsis* homologs yielded up to nine pore types (150) and additional types exist in maize and rice (8). Whereas all PIPs exhibit a narrow pore structure typical of orthodox, water-selective aquaporins, larger substrate specificity was predicted for other plant homologs. According to this analysis, *At*NIP6;1 belongs to one of two NIP subgroups and as such exhibits a low and high permeability to water and urea, respectively (151). An Ala119Trp substitution, made to mimic the pore configuration of members of the other NIP subgroup, also confers novel permeability properties, i.e., higher permeability to water and failure to transport urea. This result and other examples in animal aquaporins (11) show that point mutations can drastically alter transport specificity and that these proteins may be engineered to accommodate novel substrates of interest.

Transport assays and aquaporin substrates. Functional expression in *Xenopus* oocytes or yeast was essential to show that plant MIP homologs of all four subclasses can function as water channels (56, 66, 94, 115). Enhanced water permeability of proteoliposomes containing a purified aquaporin provides the ultimate proof of water channel activity. Such functional reconstitution has been performed with *Gm*NOD26 purified

from native peribacteroid membranes (27) or after production of *Spinacia oleracea So*PIP2;1 in *Pichia pastoris* (67). Although strict comparative measurements have not been performed in plants, plant aquaporins may, similar to their animal homologs, exhibit marked differences (up to 30-fold) in intrinsic water transport activity (154).

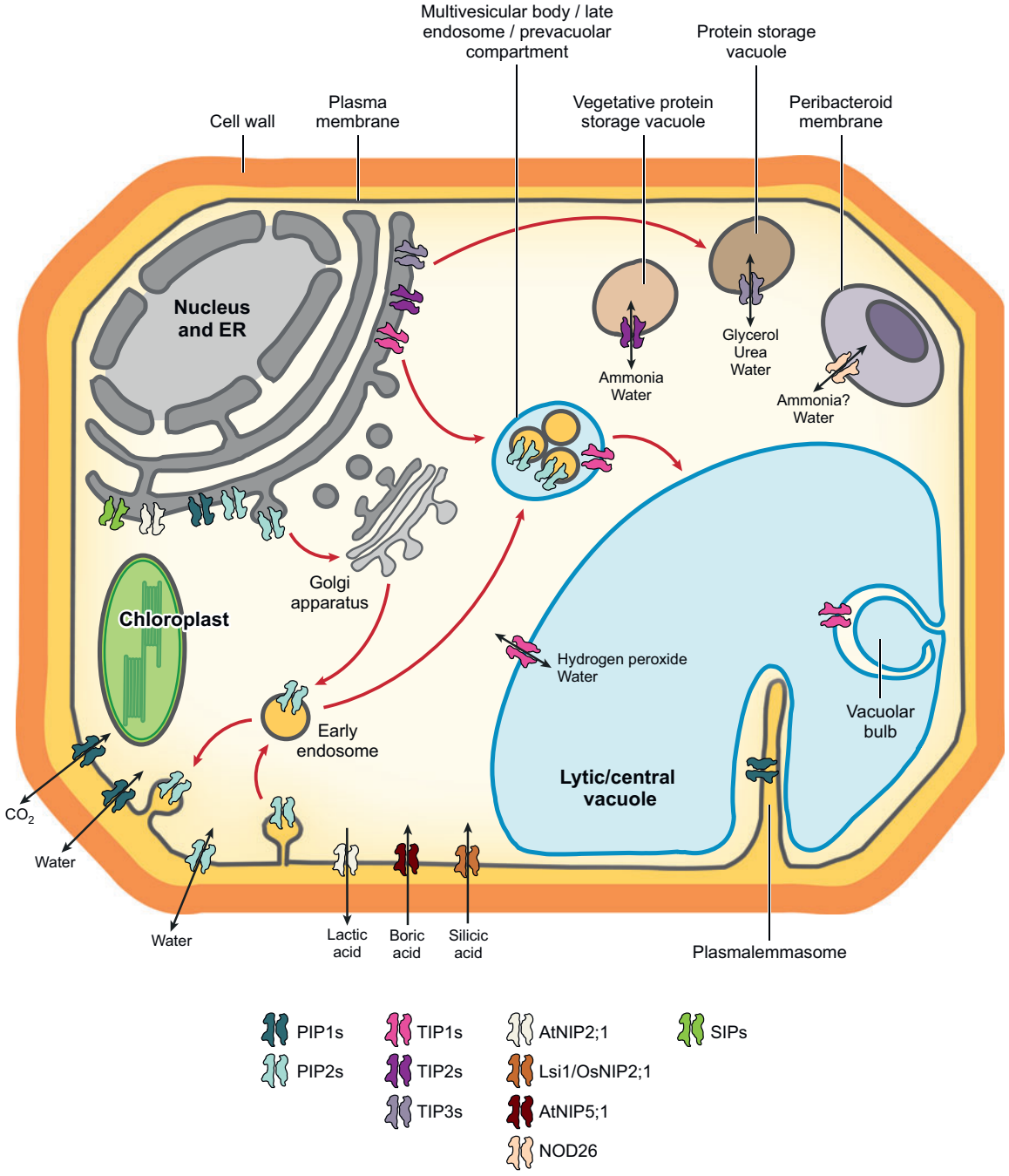
Expression studies in *Xenopus* oocytes also show that, similar to animal and bacterial aquaglyceroporins, some plant aquaporin isoforms can transport small neutral solutes such as glycerol (12), urea (42), formamide, acetamide (115), methylammonium (53), boric acid (134), silicic acid (82), or lactic acid (20). Ammonia (NH_3) and CO_2 transport is detected using substrate-induced extra- and intracellular acidification, respectively, whereas ammonium (NH_4^+) transport by *Triticum aestivum Ta*TIP2;1 results in inward currents (53, 144). Finally, expression in yeast cells deficient in endogenous systems responsible for urea or hydrogen peroxide uptake has proved efficient to screen, on the basis of a survival assay, aquaporin isoforms that possibly transport these molecules; these properties are subsequently confirmed by true transport assays (13, 77).

Several approaches have established that aquaporins contribute significantly to the permeability of plant membranes to water and small neutral solutes. In most studies, mercury derivatives, which act through oxidation and binding to Cys residues, were used as common aquaporin blockers. Plant aquaporins do not have Cys residues at conserved positions and various residues may be involved in plant aquaporin inhibition (23). We also note that mercury-resistant PIPs have been described in *Arabidopsis* and tobacco (12, 25). In some studies, the permeability profiles of the vacuolar, peribacteroid, and plasma membranes were characterized by stopped-flow spectrophotometry on purified membrane vesicles, and mercury induced a marked (50%–90%) inhibition of water transport in the first two types of membranes (42, 95, 104, 105, 115). In addition, a good parallel was established between the high permeability of the tobacco tonoplast and soybean peribacteroid membrane to urea and formamide, respectively, and the capacity of *Nicotiana tabacum Nt*TIPa and *Gm*NOD26 to transport these solutes (42, 115). In other studies, the respective water permeabilities of the plasma membrane and the tonoplast and their sensitivity to mercury were inferred from independent osmotic swelling assays on protoplasts and isolated vacuoles and calculations using a three-compartment model (92, 99, 102). **Figure 2** summarizes the contribution of plant aquaporins to water and solute transport in multiple subcellular compartments.

Molecular Mechanisms of Regulation

Cotranslational and posttranslational modifications. Because of their high abundance in plant membranes, and despite their high hydrophobicity, some aquaporins have proved to be particularly amenable to biochemical analysis, in comparison with other membrane proteins (34, 48, 63). Proteomics, and mass spectrometry techniques in particular, have recently been added to more classical techniques to produce a thorough description of aquaporin co- and posttranslational modifications (26, 121, 122). For instance, N-terminal maturation of PIP1s and PIP2s occurs through N-α-acetylation or cleavage of the initiating residue, respectively (121). In vivo and in vitro labeling studies, experiments with antiphosphopeptide antibodies, and mass spectrometry analyses have provided direct evidence for phosphorylation of Ser residues in the N-terminal and C-terminal tails of *Phaseolus vulgaris Pv*TIP3;1, *Gm*NOD26, and *So*PIP2;1 (26, 43, 63, 64, 96). PIPs show a conserved phosphorylation site in loop *B* and multiple (up to three) and interdependent phosphorylations occur in adjacent sites of their C-terminal tail (63, 64; S. Prak, S. Hem, J. Boudet, N. Sommerer, G. Viennois, M. Rossignol, C. Maurel & V. Santoni, unpublished results). Purification of calcium-dependent protein

kinases acting on aquaporins has been undertaken by several laboratories (48, 129). Although most plant aquaporins do not exhibit glycosylation, this type of modification has been observed in *Gm*NOD26 and in an ice plant TIP (96, 146). In the latter case, glycosylation was required for subcellular redistribution (described below). Aquaporins were also



the first plant membrane proteins found to be methylated (121). For instance, *At*PIP2;1 can carry one or two methyl groups on its Lys3 and Glu6 residues, respectively. These data show that, in addition to a high isoform multiplicity, plant aquaporins occur in a large variety of modified forms, which suggests intricate co- and posttranslational regulation mechanisms.

Gating. The gating of aquaporins, i.e., the opening and closing of the pore, can be regulated by multiple factors. A role for phosphorylation in gating *Pv*TIP3;1, *Gm*NOD26, and *So*PIP2;1 was first deduced from functional expression in oocytes of these aquaporins, either wild-type or with point mutations at their phosphorylation sites (43, 63, 93), and by using pharmacological alterations of endogenous protein phosphatases and kinases. A role for phosphorylation in *Gm*NOD26 gating has been unambiguously established by stopped-flow measurements in purified peribacteroid membranes, showing that alkaline phosphatase-mediated dephosphorylation leads to reduced water permeability (43). Water transport measurements in plasma membrane vesicles purified from *Arabidopsis* suspension cells or *Beta vulgaris* roots also suggest that PIPs can be gated from the cytosolic side by protons and divalent cations (4, 41). A half-inhibition of water transport is observed at ~pH 7.5 and for free Ca^{2+} concentrations in the 100 μM range (4, 41). Beet plasma membranes exhibit an additional affinity component in the 10 nM range (4).

Gating: opening and closing of a membrane channel pore

The molecular bases of aquaporin gating have been elucidated from structure-function analyses in *Xenopus* oocytes and more recently from the atomic structures of *So*PIP2;1 in its open and closed conformations (137, 138). These studies established that protons are sensed by a His residue that is perfectly conserved in loop *D* of all PIPs (138). The molecular mechanisms that lead to a conformational change of loop *D* and occlusion of the pore upon protonation of the His residue or binding of divalent cations are detailed in **Figure 3**. The atomic structure of *So*PIP2;1 also indicates how phosphorylation of loop *B* would unlock loop *D* to allow the open conformation. By contrast, phosphorylation of the C-terminal tail would act in trans to prevent loop *D* of an adjacent monomer from adopting a closed-pore conformation (137).

A role for solutes in gating aquaporins has been proposed, based mainly on pressure probe measurements in *Chara* cells (155). Inhibition of cell water permeability is linked to the presence of the solute on either side of the membrane and is strongly dependent on solute molecular size. A tension/cohesion model

Figure 2

The multiple cellular functions of plant aquaporins. The figure illustrates the variety of transport functions achieved by aquaporins in various subcellular compartments. The different aquaporin subclasses or isoforms are identified below the illustration in distinct colors. Isoforms of the plasma membrane intrinsic protein 1 (PIP1) and PIP2 subfamilies are thought to follow the secretory pathway, which carries cargo from the endoplasmic reticulum (ER) toward the plasma membrane through the Golgi apparatus. PIPs also undergo repeated cycles of endocytosis and recycling through endosomal compartments before being eventually targeted to the lytic vacuole through the multivesicular body. In *Arabidopsis* leaves, PIP1s label plasmalemmasomes (116). Tonoplast intrinsic protein 1s (TIP1s) are found in the lytic vacuole membrane. *At*TIP1;1 localizes in spherical structures named bulbs in epidermal cells of young cotyledons or salt-treated roots (15, 118). TIP2s and TIP3s are preferentially associated with vacuoles that accumulate vegetative storage proteins and seed protein storage vacuoles, respectively. Nodulin-26–like intrinsic membrane proteins (NIPs) show a broad range of subcellular localization patterns. *At*NIP2;1 is localized in the endoplasmic reticulum and the plasma membrane (20, 97), the *Oryza sativa* silicon influx transporter low silicon rice 1 (Lsi1, also named *Os*NIP2;1) and the *Arabidopsis thaliana* boric acid channel *At*NIP5;1 are localized in the plasma membrane, whereas *Glycine max* nodulin-26 (*Gm*NOD26) is exclusively expressed in the peribacteroid membrane.

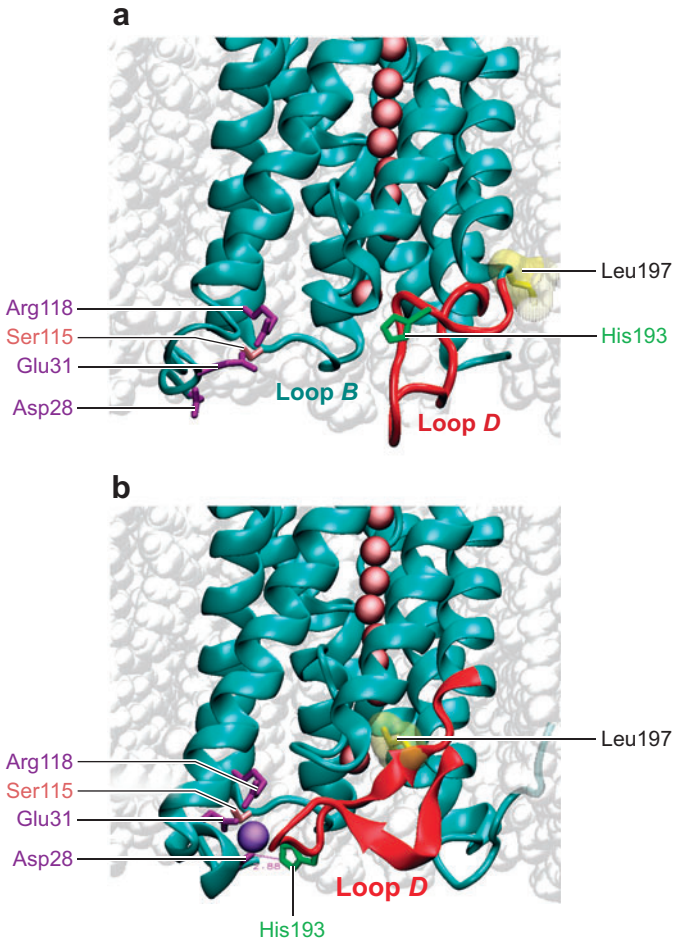


Figure 3

Molecular mechanisms of plasma membrane intrinsic protein (PIP) gating. The *Spinacia oleracea So*PIP2;1 structure was solved in an open (*a*) [Protein Data Bank (PDB) ID 2B5F] and in a closed (*b*) (PDB ID 1Z98) conformation (137). The His193 residue (*green*) is perfectly conserved in loop *D* of all PIPs. In the open conformation (*a*), His193 is not protonated and loop *D* is distal from the other cytoplasmic loop *B*. By contrast, the protonation of His193 (*b*) allows interaction with an acidic residue of the N terminus, Asp28 (*purple*). This in turn drives a conformational change of loop *D* and occlusion of the pore by displacement of the hydrophobic side chain of Leu197 (*yellow*) into the cytosolic pore mouth. Binding of divalent cations [Cd^{2+} in the atomic structure, (*purple sphere*)] would also involve Asp28 and an adjacent acidic residue (Glu31). Loop *D* would then be stabilized in the closed pore conformation through a network of H-bond and hydrostatic interactions, involving Arg118. In this model, phosphorylation of loop *B*, at Ser115 (*pink*), would disrupt this network of interactions and unlock loop *D* to allow the open conformation.

was proposed in which exclusion of the solute from the narrow vestibule of the pore would result in osmotic forces and tensions, which in turn would collapse the pore (155). Hydroxyl radicals also induce a marked (≥90%) and reversible inhibition of water transport in *Chara* cells, which was interpreted in terms of direct oxidative gating of aquaporins (50). By contrast, the inhibition of aquaporins by reactive oxygen species (ROS) in the *Arabidopsis* root seems to involve cell signaling mechanisms (Y. Boursiac, J. Boudet, O. Postaire, D.-T. Luu, C. Tournaire-Roux & C. Maurel, unpublished results).

Tetramer assembly and cellular trafficking of PIPs. Recent studies have pointed to aquaporin trafficking as a critical point for regulating aquaporin expression and function. The inability of some PIP1 isoforms to be functionally expressed in *Xenopus* oocytes has been reported by several laboratories. Fetter and coworkers (31) explained this inability by a failure of these aquaporins to traffic to the oocyte plasma membrane, and showed that coexpression of maize PIP1s in *Xenopus* oocytes with reduced amounts of PIP2 isoforms could alleviate this defect. Affinity copurification and coimmunopurification studies provided the first biochemical evidence that PIP1s and PIP2s physically interact, both in oocytes and plants (31, 158). The results of fluorescence resonance energy transfer (FRET) imaging in living maize protoplasts coexpressing PIP1s and PIP2s further support a model in which aquaporins of the two classes directly interact, very likely by heterotetramerization, to facilitate PIP1 trafficking (158). A possible role for PIP1 phosphorylation was recently added to this model (135). Phosphorylation on loop *B* of a *Mimosa* PIP1 is not necessary for aquaporin interaction but enhances the overall water transport activity of PIP1-PIP2 complexes in oocytes (135). Whereas interaction-dependent trafficking of PIP1s and PIP2s offers a broad range of combinatorial regulations, a future challenge is to determine to what extent this

process can dominate the expression of PIP1 or PIP2 homotetramers. Antisense inhibition experiments in *Arabidopsis* of PIP1s and PIP2s, alone or in combination, have suggested that the two classes of aquaporins contribute to the same functional water transport units (90).

Similar to other membrane proteins, PIP2 aquaporins are subjected to constitutive cycling. Their endocytosis is clathrin-dependent (28) and reduced by auxin (108). Export of PIP2 aquaporins from the endoplasmic reticulum is also critically controlled and the role of a di-acidic motif contained in the N-terminal tail of PIP2s was recently uncovered in maize and *Arabidopsis* (F. Chaumont, personal communication; M. Sorieul, D.-T. Luu, V. Santoni & C. Maurel, unpublished results). The cellular mechanisms that determine aquaporin trafficking and their subcellular relocalization in response to stimuli will surely fuel intense investigations in the coming years.

AQUAPORIN FUNCTIONS THROUGHOUT PLANT GROWTH AND DEVELOPMENT

Water Transport

Principles of plant water transport. A wide range of cell water permeabilities can be observed between distinct cell types and throughout plant development. For instance, cell pressure probe measurements indicate that, in growing epicotyls of pea, cortical cells have a ~30-fold higher hydraulic conductivity than epidermal cells (130). Also, swelling assays on isolated protoplasts from rape roots indicate that their osmotic water permeability coefficient increases from 10 to 500 μm sec^{-1} within less than two days (114). Although the contribution of the lipid membranes should be taken into account, one challenge will be to determine how the aquaporin equipment of each individual cell can determine such strikingly different water transport properties. In these respects, attempts have been made to relate the cell-specific expression of aquaporin isoforms in radish taproots and maize primary roots and the water permeability of protoplasts derived from the various cell types of these materials (46, 132).

In the whole plant, long-distance transport of fluids occurs mostly through vascular tissues, which do not present significant membrane barriers. Yet, living tissues can be the site of intense flows of water during transpiration or expansion growth. For this, water can flow along various paths: (*i*) the apoplastic path, i.e., within the cell wall continuum, (*ii*) the symplastic route through cytoplasmic continuities and plasmodesmata, and (*iii*) the transcellular path across cell membranes (mainly plasma membranes), which in many tissues is mostly mediated by aquaporins. Although it is not very specific and is inactive on certain aquaporins (see above), mercury represents one of the very few tools available to evaluate the contribution of aquaporins to water transport in plant tissues. The general toxicity of this compound in vivo must be carefully evaluated (reviewed in 61), and researchers checked that mercury does not perturb xylem solute transport and respiration in aspen roots. By contrast, mercury depolarizes wheat root cells in parallel to inhibition of water transport (61). A reversibility of mercury effects by reducing agents is also required to show that the blocking effects are due to oxidation mechanisms and not to irreversible damage of the cells. Despite all these restrictions, the effects of mercury on water transport have been characterized in a large variety of physiological contexts. Overall, these studies provide a consistent picture of the role of aquaporins, in particular during root water transport (61). Yet, new specific aquaporin blockers are critically needed. Gold and silver ions have been described as potent aquaporin blockers in vitro but the use of these compounds in vivo seems to be problematic and their mode of action is as yet unknown (106).

Transpiration. Because it induces an intense renewal of water throughout the plant,

Hydraulic conductivity: water permeability (i.e., intrinsic capacity to transport water) of a membrane, cell, or tissue

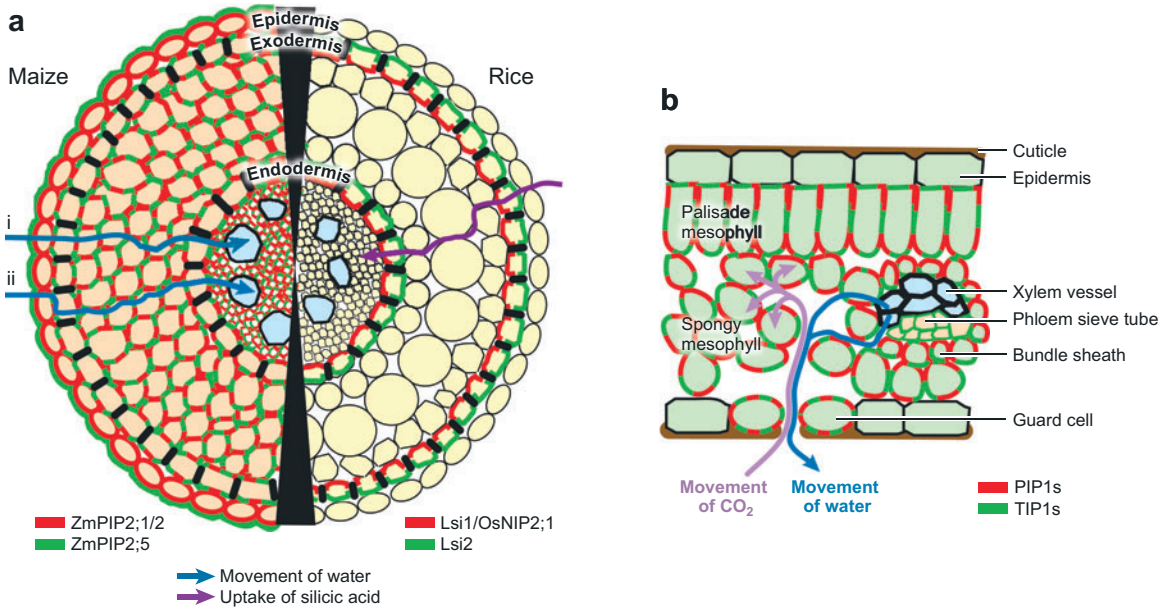


Figure 4

Aquaporin-mediated transport of water and solutes in roots (*a*) and leaves (*b*). Schematic cross sections with representations of the tissue-specific expression patterns of aquaporins and paths of transport are shown. Aquaporin expression and water transport in maize roots is summarized according to Reference 46, whereas uptake of silicic acid in rice roots by *Oryza sativa* Lsi1 (*Os*NIP2;1) in combination with the efflux transporter Low silicon rice 2 (Lsi2) is drawn according to References 82 and 83. Expression of plasma membrane intrinsic protein 1s (PIP1s) and tonoplast intrinsic protein 1s (TIP1s) in *Brassica napus* leaves was summarized according to Reference 35. The movement of water can follow the cell-to-cell (symplastic and transcellular) (*i*) or apoplastic (*ii*) path.

transpiration represents an obvious context in which to investigate aquaporin function in roots and leaves. A thorough description of cell-specific expression of aquaporins in roots was recently performed in maize using in situ and quantitative RT-PCR and immunolocalization (46). Strong expression of *Zm*PIP2;1 and *Zm*PIP2;5 is observed in both the exodermis and endodermis of the mature zone, suggesting that because of the presence of Casparian strips a bypass of the apoplastic path may be necessary in these cell layers (46) (**Figure 4*a***). Also, a strong expression in the stele and vascular tissues is consistently observed in roots of several plant species (46, 124, 132). Several lines of functional evidence also show that aquaporins significantly contribute to water uptake by roots. Firstly, mercury inhibits the root hydraulic conductivity (Lp_r) by 30%–90% in more than ten plant species (61). In addition, antisense inhibition of aquaporins of the PIP1 and/or PIP2 subclasses reduced Lp_r by approximately 50% in tobacco or *Arabidopsis* (90, 128). Finally, two allelic *Arabidopsis pip2;2* knockout mutants show, with respect to wild-type plants, a reduction of 25%–30% in hydraulic conductivity of root cortex cells (60). In addition, the osmotic hydraulic conductivity of entire roots, as derived from free exudation measurements, is decreased by 14% in the mutants, pointing to PIP2;2 as an aquaporin specialized in osmotic fluid transport in the *Arabidopsis* root (60). Further analysis of mutants corresponding to aquaporin genes with distinct cell-specific expression patterns may help

Lp_r: root hydraulic conductivity

dissect the contribution of the various cell layers to distinct modes (i.e., osmotic or hydrostatic) of water transport within the root.

The preferential expression of some aquaporin isoforms in vascular tissues and cell types such as tracheary elements, xylem parenchyma cells, and phloem-associated cells suggests a general role of aquaporins in sap transport throughout the plant body (reviewed in 92). In the leaf, the water supply at the evaporating sites is sustained by a flow of liquid water from the vascular system through the extravascular compartment, including the vascular bundles and the mesophyll (**Figure 4*b***). A role for aquaporins in mediating water transfer from the veins to the stomatal chamber has been proposed on the basis of two lines of evidence. Firstly, mercury can inhibit leaf hydraulic conductance (K_{leaf}) in sunflower and in six temperate deciduous trees (1, 103). Secondly, light-dependent changes in K_{leaf} in walnut occur within one hour, are associated with changes in expression of PIP2 aquaporin transcripts, and both are inhibited by 100 μM cyclohexemide, indicating a role for protein synthesis in K_{leaf} regulation (22). In a recent study, mesophyll protoplasts were isolated from *Arabidopsis* genotypes differing in stomatal aperture or from plants grown at varying relative air humidity (98). Surprisingly, researchers observed an inverse relationship between the rate of transpiration in the plant and the water permeability of the isolated protoplasts. These variations occur without any alteration in the leaf PIP content, suggesting that aquaporin function is controlled at the posttranslational level. The physiological significance of this control and its occurrence in other plant species are as yet unknown. Expression of aquaporins has also been reported in stomatal guard cells (36, 123). Despite the critical role played by these cells in maintaining the whole plant water status, the function of aquaporins in stomatal movements is as yet unclear and deserves more attention for future research.

Tissue expansion. Although some aquaporin isoforms seem to be specific to dividing cells (9), a strong link between PIP and TIP aquaporin expression and cell expansion has been observed in numerous plant materials. For instance, expression of the *AtTIP1;1* promoter is associated with cell enlargement in *Arabidopsis* roots, hypocotyls, leaves, and flower stems (81), and transcript accumulation is enhanced by the growth-promoting hormone gibberellic acid (GA3) (109), suggesting that *At*TIP1;1 may contribute to the differentiation of a large central vacuole in fully elongated cells (81).

Because most plant cells have short half-times of water exchange (130), water influx into a single plant cell can hardly be limiting during expansion growth. Membrane water transport in growing tissues should rather be envisioned in the context of a transcellular delivery of water from vascular tissues toward peripheral expanding tissues (148). The presence, in certain physiological conditions, of significant water potential gradients within growing tissues supports the idea that this type of water transport can be limiting. Accordingly, mercury blocks tissue growth in maize roots, but exclusively in the older cells, distal to the apex (55). These cells are characterized, with respect to younger cells, by a high, mercury-sensitive cell hydraulic conductivity and reduced symplastic connections with the phloem, suggesting that aquaporin-mediated transcellular water transport is necessary for delivery of water from the phloem into the cells. In castor bean hypocotyls, the transcript abundance of a specific PIP2 isoform and a high hydraulic conductivity of cortical cells both show the same light- and spatial-dependence as that of tissue expansion (30). Finally, diurnal epinastic movements (unfolding) of tobacco leaves can be accounted for by a differential growth of the upper and lower surfaces of the petiole. A limiting role for tobacco aquaporin 1 (*Nt*AQP1) in this process was proposed on the basis of observations that expression of *Nt*AQP1 in the petiole shows a diurnal rhythm that coincides

K_{leaf}: leaf hydraulic conductance

g_m: mesophyll conductance to CO_2

with leaf unfolding and that antisense inhibition of *Nt*AQP1 impairs leaf movement (127). These studies draw a convincing, but qualitative, picture of aquaporin function in expansion growth. Future studies will have to quantitatively integrate growth rates, cell and tissue water relation parameters, and aquaporin expression in time and space (148). Fruit development and ripening, which involve cell enlargement and cotransport of sugar and water, will also represent important processes in which to investigate aquaporin function (19, 110).

Tissue desiccation and imbibition. Plant reproduction requires the intense desiccation of certain organs, which then acquire specific dissemination and resistance properties. TIP isoforms specific to pollen grains have been reported in *Arabidopsis* but their function during pollen desiccation and/or pollen tube growth is as yet unclear (111). By contrast, antisense inhibition of PIP2 aquaporins in tobacco delays anther dehydration and dehiscence, suggesting that these aquaporins are involved in water flow out of the anther via the vascular bundle and/or evaporation (14). Seed germination represents another remarkable context, during early tissue imbibition and subsequent embryo growth, in which aquaporins may mediate a fine temporal and spatial control of water transport; evidence for aquaporin function in seeds is emerging. In *Brassica napus*, the germination rate of seeds that have gone through various priming treatments is strongly correlated with the transcript abundance of a PIP2 aquaporin (39). In pea and *Arabidopsis*, mercurials reduce the speed of seed imbibition and seed germination, respectively (145, 147). Finally, expression in tobacco and rice of sense and antisense *PIP1* transgenes shows that the speed and extent of germination of seeds in normal and/or water stress conditions is positively correlated with aquaporin expression (76, 156). Liu and coworkers (76) suggested a role for PIP1s in stimulating seed germination by nitric oxide (NO) in rice.

Nitrogen, Carbon, and Micronutrient Acquisition

Nitrogen fixation. A first link between aquaporins and nitrogen (N) assimilation came from the observation that expression of some aquaporin genes is dependent on N compounds; some genes, such as *ZmPIP1;5b*, are strongly induced by nitrate (40), whereas others, such as *AtTIP2;1*, are induced under long-term N starvation or short term NH_4^+ supply (79). This type of regulation was first interpreted as a reflection of well-known connections between water relations and N metabolism. However, evidence was recently presented that aquaporins of the PIP, NIP, and TIP subfamilies can transport N compounds. Transport of urea by TIPs (42, 70, 140) may contribute to urea equilibration within the cell and storage into and remobilization from the vacuole (70). Wheat and *Arabidopsis* TIP2 homologs also show a remarkable permeability to NH_3 and may therefore contribute to significant loading of this compound and acid-trapping of the protonated form (NH_4^+) in the vacuole (53, 57, 79). However, studies of transgenic *Arabidopsis* that overexpresses *At*TIP2;1 failed to establish any significant role for this aquaporin in NH_4^+ accumulation (79).

CO_2 transport and carbon metabolism. CO_2 transport by aquaporins in planta was first evaluated by treating *Vicia faba* and *Phaseolus vulgaris* leaves with mercury (136). In these two experiments, mercury altered the dependency of photosynthesis on intercellular but not on chloroplastic CO_2. This effect was interpreted to mean that CO_2 diffusion into the chloroplast [i.e., mesophyll conductance to CO_2 (g_m)] is blocked and therefore involves proteins, possibly aquaporins. Transgenic plants with altered aquaporin expression provide systems in which to explore this issue further (47, 144). In tobacco plants with antisense inhibition or an antibiotic-inducible overexpression of *Nt*AQP1, g_m positively correlates with the *Nt*AQP1 expression level

(33, 144). In view of the CO_2 transport activity of *Nt*AQP1 in oocytes, this result was interpreted as evidence that this PIP1 homolog serves as a CO_2 pore in tobacco leaves (33, 144). Interestingly, in various genetic and physiological contexts g_m is positively correlated with maximal stomatal conductance and CO_2 assimilation capacity (see 33 and references therein). Up to now, it was unclear whether changes in g_m can be accounted for by changes in leaf anatomy or by changes in cell permeability to CO_2 (47). Following evidence that PIP contributes to both g_m and leaf hydraulic conductance, the hypothesis that aquaporins coregulate CO_2 and H_2O transport in the mesophyll emerged (**Figure 4*b***).

A role for aquaporins in carbohydrate storage and compartmentation has also been suggested. In a first study, tomato fruits with antisense inhibition of a PIP homolog showed increases in organic acid content and decreases in sugar content; these defects are associated with a marked alteration of the ripening process (19). In another study, metabolomic analysis of *Arabidopsis* plants lacking *At*TIP1;1 expression revealed complex alterations in the accumulation of various sugars, organic acids, and starch (84). Although this provocative hypothesis remains to be confirmed, reduced expression of *At*TIP1;1 was proposed to alter vesicle trafficking and therefore carbohydrate compartmentation.

Nutrient uptake. Two recent studies unraveled novel functions of aquaporins in plant nutrient uptake. Transcriptome analysis of *Arabidopsis* roots revealed striking upregulation of *At*NIP5;1 in response to boron (B) deficiency (134). Interestingly, *At*NIP5;1 transports boric acid in *Xenopus* oocytes and significantly contributes to root B uptake, as shown in two independent *nip5;1* insertion lines. The physiological significance of *At*NIP5;1 was further underscored by mutant plants that, under B limitation, display a striking growth retardation of shoot and roots and an inhibition of flower and silique formation (134). We note that a role for membrane channels in B transport was first proposed in an early study on squash roots (29). However, the role of aquaporins had remained uncertain owing to the low B transport activity of the PIPs and NIPs investigated in that study.

Silicon is a major mineral component of certain plants, such as Gramineae, and more generally, helps plants withstand abiotic stresses and pathogen attacks. Molecular characterization of *low silicon rice1* (*lsi1*), a mutant of rice defective in silicon uptake into roots, led to the unexpected finding that Lsi1 encodes a NIP homolog (82). Lsi1 transports silicon after heterologous expression in oocytes. Lsi1 is expressed on the distal side of exodermal and endodermal root cells and may contribute, in combination with the efflux transporter Lsi2 (83), to a vectorial transport of silicic acid from the soil solution into the xylem, a limiting step for translocation of silicon to the aerial parts (**Figure 4*a***). A similar function can be expected in other important crops such as maize, which also accumulates significant amounts of silicon and has close Lsi1 homologs (82).

AQUAPORINS IN A VARIABLE ENVIRONMENT

Changes in Irradiance

Light is a key environmental parameter that, besides its long-term effects on plant growth and development, diurnally affects the plant metabolic regime and therefore affects water relations. A primary effect of light is to control stomatal aperture, and therefore transpiration. In guard cells of sunflower leaves, the transcript abundance of a TIP homolog (SunTIP7) is under diurnal regulation and is maximal at the end of the day, during stomatal closure, suggesting that this aquaporin contributes to water efflux from the guard cell (123). Light also enhances K_{leaf} in many plant species (22, 103, 142). For instance in sunflower, the ~ 50% increase in K_{leaf} induced by light is fully sensitive to mercury inhibition,

suggesting that the variations in K_{leaf} are due to changes in aquaporin activity (103). This was confirmed in walnut twigs, where K_{leaf} shows a very tight kinetic correlation with the abundance of two major *PIP2* transcripts during a transition from dark to high light (22). Enhanced activity of leaf aquaporins during the day, and therefore increased K_{leaf}, may favor water transport into the inner leaf tissues when transpiration is maximal. This process would avoid excessive drops in leaf water potential, reduce xylem tensions, and therefore prevent possible xylem embolization.

Light interception is optimized by diurnal movements of leaves, a process in which aquaporins also participate. In the Mimosaceae, the movement of leaves and leaflets is determined by coordinate swelling and shrinking of cells on opposing sides of a motor organ, the pulvinus. In *Samanea saman*, the osmotic water permeability of protoplasts isolated from the pulvinus shows diurnal regulation and is maximal in the mornings and evenings, concomitant with leaf movement (100). Accumulation of a PIP2 homolog in these cells is under circadian control and in phase with these rhythmic changes in water transport. In *Mimosa pudica*, motor cells harbor both a tannin and a central aqueous vacuole. Immunocytochemistry experiments indicate that the latter type of vacuoles shows an approximate tenfold higher density of TIP1 aquaporins as compared with the former type, in agreement with the major contribution of the central vacuole to water exchanges during cell volume regulation (32).

Diurnal variations of root Lp_r, with a two- to threefold increase during the day, have been observed in many plant species and may contribute, together with light-dependent regulation of K_{leaf}, to reducing xylem tension under conditions of high transpiration demand (16, 49, 78). In *Lotus japonicus* and maize, for instance, root water transport is maximally enhanced around midday and is matched or slightly preceded by an increase in the abundance of *PIP1* and *PIP2* transcripts (49, 78). In maize, *Zm*PIP1;5 transcripts are detected in all root cell types during the day but restricted to the epidermis during the night (40). However, the abundance of PIP proteins in maize roots shows a more complex diurnal variation profile than that of transcripts, suggesting a role for posttranslational regulation (78). The mechanisms that allow light perception and long-distance control of aquaporins in the plant root deserve more precise investigation.

Water, Salt, and Nutrient Stresses

Regulation of turgor and intracellular water movements. Because it is central for plant water relations, the regulation of water transport during water deficit has been the object of extensive research. Understanding the role of aquaporins in this context now requires integration of numerous observations made at the molecular, cell, and tissue levels. Fundamental regulation properties that explain the remarkable ability of plant cells to withstand water deprivation have emerged from basic knowledge of plant cell water relations and from more recent research on aquaporins. Water deficit induces primarily an efflux of water, which can result in a marked drop in cell turgor and ultimately, but more rarely, in cell plasmolysis or cytorrhysis. In this context, the cytosol, which contributes to a minor fraction of the plant cell volume, may be very sensitive to differential flow of water across the plasma membrane and the tonoplast. Abrupt changes in cytosolic volume can theoretically be avoided if mobilization of water from or into the vacuole is nonlimiting (92, 140). Studies with membranes purified from wheat and tobacco have confirmed that, in these preparations at least, the tonoplast shows much higher water permeability and aquaporin activity than the plasma membrane (95, 104).

Osmotic stress also requires an adjustment of plasma membrane surface area; recent results link this process to the regulation of membrane water permeability. In *Vicia faba* guard cells, a pretreatment with inhibitors

of membrane trafficking (wortmannin, cytochalasin D) slows down cell shrinkage in response to hypertonicity, suggesting that a reduction of cell hydraulic conductivity and possibly aquaporin downregulation is induced (126). When protoplasts isolated from maize suspension cells are hypotonically challenged, a subset of these protoplasts exhibits a retarded swelling, which was interpreted to mean that their initial water permeability is extremely low and is dynamically adjusted during the course of cell swelling and mobilization of membrane material at the cell surface (101). In agreement with these functional data, dynamic changes in aquaporin subcellular localization were observed in osmotically challenged cells. These processes may also reflect transfer of aquaporins to subcellular compartments devoted to protein degradation. For instance, mannitol-induced osmotic stress in ice plant suspension cells induces the relocalization of *Mc*TIP1;2 from the tonoplast to a putative endosomal compartment (146). This process is dependent on aquaporin glycosylation and a cAMP-dependent pathway. In salt-treated *Arabidopsis* roots, *At*TIP1;1, but not the *At*TIP2;1 homolog, is relocalized in vacuolar bulbs (15). In addition, redistribution of PIPs from the plasma membrane to internal compartments contributes to the downregulation of root water uptake (15).

The regulation of water transport in *Chara* cells in response to changing osmotic or hydrostatic pressures has been interpreted as the result of a direct gating of aquaporins by these factors (152, 155). In higher plant cells, aquaporins are more likely under the control of osmo- and pressure-sensing molecules and downstream signaling cascades. For instance, the downregulation of water permeability in melon protoplasts by salt can be counteracted by okadaic acid, a protein phosphatase inhibitor (89). More specifically, phosphorylation of *So*PIP2;1 in spinach leaf fragments decreases in response to a hyperosmotic treatment (64). A general model of cell osmoregulation involving stretch-activated Ca^{2+} channel and Ca^{2+}-dependent phosphorylation of *So*PIP2;1 was proposed to explain that aquaporin phosphorylation, and therefore cell hydraulic conductivity, would be maximal at high water potential to favor water entry in fully turgid cells (63). Finally, several authors proposed that aquaporins themselves may function as osmosensors, but the molecular and cellular mechanisms involved remain elusive (51, 86).

Whole plant level. At the whole plant level, a major effect of drought is to reduce transpiration through stomatal closure. Yet, in extreme drought conditions, high tension in the xylem can lead to vessel occlusion by embolization. A specific role for aquaporins in embolism refilling and recovery of stem axial conductance after drought was proposed in grapevine on the basis of mercury inhibition experiments (80). In roots of most plant species investigated, drought or salt stresses also result in a marked decrease in L_{p_r} (61). In *Arabidopsis* for instance, exposure to 100 mM NaCl reduces L_{p_r} by 70% with a half-time of approximately 45 min. The fact that residual L_{p_r} of salt-stressed *Arabidopsis* or paprika roots becomes resistant to mercury was interpreted to mean that aquaporin activity is downregulated in these conditions (17, 88). During the day, this early response may provide a hydraulic signal to the leaf to trigger stomatal closure, whereas during the night, it may avoid a backflow of water to the drying soil.

Numerous early studies reported on water stress–dependent expression of aquaporin genes and a large variety of individual regulation profiles were described (92). A more comprehensive understanding of the processes involved has emerged from recent studies in maize, rice, radish, and *Arabidopsis*, in which expression of the whole aquaporin family was considered (3, 15, 44, 58, 73, 85, 131, 159). In salt-stressed roots of *Arabidopsis* and maize, a coordinated downregulation of most aquaporin transcripts occurs, which over the first 24 h of stress can contribute to L_{p_r} downregulation (3, 15, 85). A recovery toward initial

transcript abundance occurs over longer term stresses (85, 159). Transcriptional control of aquaporins in drought-stressed leaves appears to be more complex and, although a tendency to overall aquaporin gene downregulation is also observed, specific upregulation of certain *PIP* transcripts occurs in rice and *Arabidopsis* leaves (3, 44). Interestingly, the two transcripts that are upregulated in *Arabidopsis* are specifically expressed in aerial parts (3). Although their tissue expression pattern is as yet unknown, these isoforms may facilitate water flow toward critical cell types. Studies in *Hordeum vulgare* (barley) leaves suggest that increased abundance of *HvPIP1;6* transcripts in response to salt may reflect a role for this aquaporin in promoting residual growth of the leaf under stress (37).

In agreement with its central role in plant responses to water stress, abscisic acid (ABA) seems to mediate, at least in part, drought- and salt stress-induced aquaporin regulation. For instance, treatment of maize roots with ABA results over 1–2 h in a transient increase in hydraulic conductivity of the whole organ and of cortical cells, by factors of 3–4 and 7–27, respectively (54). Consistent with these effects, ABA also rapidly enhances the expression of some PIP isoforms (159). In rice roots, a strong induction of several PIPs is observed in response to water deficit, specifically in an upland cultivar that shows an enhanced production of ABA (73). This response may optimize uptake of residual soil water at the onset of soil drying.

Genetic approaches have also been useful to investigate the function of aquaporins during drought. Transgenic tobacco plants with antisense inhibition of PIP1 and transgenic *Arabidopsis* plants with antisense inhibition of PIP1 expression and PIP2 expression showed lower leaf water potentials than wild-type plants under drought stress conditions (90, 128). Most strikingly, the recovery following rewatering of leaf wilting in tobacco and leaf water potential and plant hydraulic conductance in *Arabidopsis* is significantly delayed in the antisense plants (90, 128). Therefore, PIP aquaporins contribute to adaptation of the plants to drought by mechanisms that remain to be determined, and even more significantly, contribute to rehydration of the whole plant body after drought. Another genetic strategy is to enhance aquaporin expression in transgenic plants. Although spectacular phenotypes are observed in most studies, aquaporin overexpression has either beneficial (44, 72, 156) or adverse (2, 68) effects on drought tolerance, depending on the aquaporin gene or the plant species investigated. Therefore, the relevance of this approach for biotechnological improvement of plant tolerance to water stress remains uncertain. One reason may be that many studies relied on overexpression of an aquaporin in a heterologous plant species (2, 44, 68, 156). Inadequate regulation of the foreign aquaporin may disturb the endogenous stress response. In these respects, more relevant insights were provided by a study showing that *Oryza sativa Os*PIP1;3 is specifically induced by water stress in an upland, drought-avoidant cultivar of rice (72). Furthermore, the performance of a lowland cultivar under drought can be significantly enhanced by expression of this aquaporin under a stress-responsive promoter (72). In future studies it will be important to evaluate the capacity to recover from water stress after rewatering in transgenic plants that ectopically express an aquaporin.

Responses to nutrient stress. Strong interactions exist between the nutrient and water status of plants; integration of these two aspects seems to be critical for a deeper understanding of plant stress responses. For instance, deprivation of N, phosphorus (P), or sulfur (S) in plants results after a few days in a significant inhibition of water transport in whole roots or individual root cells; initial root water transport properties can be restored in the 24 h following nutrient resupply (16, 21, 125). A downregulation of water channels under N and P deprivation is invoked on the basis of the insensitivity of residual Lp_r to mercury (16). The adverse effects of nutrient starvation

on plant water relations have also been studied in sorghum under drought stress. As compared with replete conditions, P starvation enhances the inhibition of L_{p_r} by a polyethylene glycol treatment and slows down its recovery after water resupply (125).

The molecular and cellular mechanisms involved in these regulations remain unclear. Stimulation of maize L_{p_r} by NO_3^- is blocked in the presence of tungstate, an inhibitor of nitrate reductase, suggesting that products of the N assimilation pathway are required for activation of aquaporin functions (10). A general transcriptional control of aquaporins by nutrient stress is also observed in the *Arabidopsis* root and, for instance, calcium deprivation results in an overall transcriptional downregulation of aquaporins (85). The effects of potassium (K) starvation are more moderate but a downregulation of *Arabidopsis* PIPs is also induced in the long term. By contrast, K deprivation in rice induces a twofold stimulation of L_{p_r} after 4–6 h and enhances expression of some PIP isoforms, in parallel to expression of K channels (75). Coregulation of aquaporins and K transport systems has also been observed in roots treated with CsCl, which in addition to blocking K transport, reduces L_{p_r} and aquaporin expression (75). These data suggest tight interactions between water and K transport during cell turgor regulation.

Cold Stress

Chilling of plant roots (i.e., exposure to 4°C–8°C) reduces root pressure, sap flow, and L_{p_r} in a few hours (5, 71, 157). These effects in turn induce water deficit symptoms in shoots, such as decreased leaf water potential and stomatal closure (157). In maize, both a chilling-tolerant and a chilling-sensitive variety show an initial, >80% decline in L_{p_r} but the tolerant variety shows a unique capacity to spontaneously overcompensate L_{p_r} upon prolonged (>3 d) chilling (5). The speed and reversibility of inhibition of L_{p_r} by chilling in cucumber and rice and a concomitant six- to ninefold reduction in cortex cell hydraulic conductivity in cucumber roots suggest that inhibition of aquaporin activity is involved (71, 157).

Comprehensive gene expression analyses in roots and shoots of rice, maize, and *Arabidopsis* show that chilling induces a marked (two- to fourfold) decrease in abundance for most *PIP* transcripts (5, 58, 120, 157). Normal gene expression is restored in the 24 h following the return to permissive temperature. However, in maize and rice roots under cold stress or recovery, the abundance of aquaporin transcripts and proteins is not always correlated, suggesting the occurrence of posttranscriptional regulation (5, 157). In addition, the abundance of PIP1s and of phosphorylated PIP2 forms (as monitored by immunodetection) increases during prolonged chilling in roots of both a chilling-tolerant and a chilling-sensitive variety of maize; intriguingly, this response is not correlated to their differential L_{p_r} regulation (5). Because the sensitivities to chilling and H_2O_2 are correlated in the tolerant and sensitive varieties, it was proposed that ROS-induced damage probably dominates the aquaporin response and determines the poor performance of the sensitive variety during stress (5). A more direct relationship between aquaporins and ROS was determined in cucumber (71). In this species, H_2O_2 accumulates in response to chilling and treatment of roots by exogenous H_2O_2 inhibits L_{p_r} to the same extent as chilling.

Flowers can also perceive temperature. In tulip, diurnal movements of petals are controlled in part by changes in temperature (7). Petal opening and an associated water retention can be induced at 20°C and are linked to phosphorylation in a microsomal membrane fraction of a 31-kDa protein, tentatively identified as a PIP isoform. Petal closure is induced at 5°C and is associated with decreased phosphorylation of the putative aquaporin. The effects of a calcium chelator [1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid, or BAPTA] and a calcium channel blocker on petal

AM: arbuscular mycorrhiza

movement and the associated phosphorylation of the aquaporin suggest a role for calcium signaling in this process (7).

Finally, freeze-thaw cycles occurring during winter can, similar to severe drought stress, embolize xylem vessels of woody plants (119). Embolism repair may be achieved by hydrolysis of starch in adjacent parenchyma cells, exudation of the resulting sugars in the vessel, and concomitant water influx to chase out the air bubble. In walnut twigs, the transcripts and proteins corresponding to two PIP2 homologs show seasonal variations and preferentially accumulate in the xylem parenchyma during winter, suggesting a role for these aquaporins in embolism repair (119).

Anoxia

Flooding or compaction of soils results in acute oxygen deprivation (anoxia) of plant roots, which is a major stress for cultivated plants. Most plant species investigated show a rapid inhibition of L_{p_r} in response to anoxia. Tournaire-Roux and coworkers (138) delineated the organ and cell bases of this process in *Arabidopsis* roots. They showed that anoxic stress results in acidosis of root cells and that the L_{p_r} of excised roots diminishes in parallel to cytosolic pH. These observations can be linked to the molecular mechanism of PIP aquaporin gating by cytosolic protons, which is conserved in PIPs of all plants and therefore can explain how an anoxic stress results in a massive inhibition of root water uptake. This regulation may avoid excessive dilution of xylem sap after flooding or, on a longer term, favor ethylene accumulation, which in turn induces aerenchyma differentiation (52). Ethylene, which enhances L_{p_r} in hypoxic aspen roots, may also compensate for the initial inhibition of water transport in response to oxygen deprivation (65). Consistent with the physiological inhibition of L_{p_r}, a general downregulation of *PIP* and *TIP* genes in response to hypoxia occurs in *Arabidopsis* (74). By contrast, expression of *AtNIP2;1* is markedly induced upon flooding stress and hypoxia (20, 74). *At*NIP2;1 transports lactic acid and may therefore provide a path for release of this fermentation product from root cells, to contribute to cytosolic pH regulation and metabolic adaptation to long-term hypoxia (20).

Biotic Interactions

Rhizobia-legume symbiosis. Interactions of plants with soil microorganisms, which have long been known as central for plant mineral nutrition and metabolism, more recently appear to play an important role in plant water relations and tolerance to environmental stresses (6, 43, 143). Notably, *Gm*NOD26, the first plant aquaporin to be identified, is specifically expressed in symbiotic nitrogen-fixing nodules formed after infection of soybean by *Rhizobiaceae* bacteria (149). Similar nodulins have been identified in other legumes. *Gm*NOD26 is a major component of the peribacteroid membrane, a membrane of plant origin that surrounds the bacteroid and mediates exchanges with the root cell. Antibodies raised against either the native or phosphorylated form of *Gm*NOD26 reveal that maximal expression of the protein and its subsequent phosphorylation coincide with bacteroid maturation (43). Because of its solute transporting activity, *Gm*NOD26 has been tentatively linked to a channel-mediated import of NH_3 from the bacteroid, but unequivocal evidence for such function is still lacking (105, 149). The water transport activity of *Gm*NOD26 may also help the plant cell to couple osmoregulation of the plant cytosol and peribacteroid space. Accordingly, drought and salt stress result in a threefold increase in *Gm*NOD26 phosphorylation, suggesting that enhanced water permeability is required for nodule osmoregulation and adaptation to water stress. (43).

Mycorrhiza. Arbuscular mycorrhizas (AM) represent the most common form of symbiosis between land plants and soil fungi. Similar to the *Rhizobia*-legume symbiosis, this

interaction results in deep anatomical changes of root cells, involving in this case the differentiation of convoluted, periarbuscular membrane structures that are the site of extensive exchanges of mineral nutrient (phosphate), carbohydrates (photosynthates), and water with the fungus. This membrane specialization results in marked changes in *PIP* and *TIP* gene expression, with a specific profile depending on the plant host or the symbiotic fungus (reviewed in 143). In poplar, mycorrhized plants show, with respect to non-mycorrhized plants, a 55% increase in L_{p_r}; besides changes in root anatomy (internal surface), this increase can be accounted for by enhanced expression of most of the PIPs expressed in roots (87). By contrast, the L_{p_r} of mycorrhized *Phaseolus vulgaris* plants is reduced approximately threefold and this reduction is associated with a decreased abundance of PIP2s and their phosphorylated forms (6). Plant aquaporins expressed during AM symbiosis may also contribute to NH_3 import from the fungus (143).

AM symbiosis also exerts beneficial effects on tolerance of plants to water stress, whether induced by drought, salinity, or chilling (6, 143). These effects may be mediated through alteration of both root water uptake and transpiration to promote water economy. A specific role for aquaporins was deduced in a study on transgenic tobacco, showing that antisense inhibition of *Nt*AQP1 reduces the positive effects of mycorrhiza on root and leaf growth under drought (112). Two recent studies in lettuce, soybean, and tomato drew an interesting parallel between the effects of AM and drought stress, which synergistically regulate *PIP* genes in roots and leaves (reviewed in 143).

Nematode and other infections. Specific aquaporins also seem to be involved in plant-pathogen interactions as an adaptive response to infection-induced changes in plant cell morphology. For instance, infection of roots by nematodes leads to the differentiation of giant cells that serve as feeding sites for the parasite. Regulatory sequences that are specifically responsive to nematode infection were identified in the promoter sequence of a tobacco *TIP* gene (107). Enhanced expression of this aquaporin might be necessary to achieve extensive delivery of water and solutes to the parasite, together with proper osmotic regulation of the giant cells. In tomato, incompatible interaction with the parasite *Cuscuta reflexa* induces the expression of a PIP1 homolog, probably in relation to the auxin-dependent elongation of hypodermal cells induced after pathogen attachment (153).

CONCLUSIONS

Aquaporins have provided a unique molecular entry into plant water relations and their study has significantly improved our understanding of integrated mechanisms of water transport, in roots in particular. Yet, in view of the complex expression and regulation profiles of aquaporins, their role in regulating water transport in many other physiological and developmental contexts, including seed germination, stomatal regulation, and leaf water transport, deserves further investigation (**Figure 5**). In most studies, aquaporin function is experimentally monitored through water flow intensities and kinetics. However, in the whole plant the overall flow of water across plant tissues is determined by stomatal regulation and/or solute transport. Therefore, it will be important to consider how, in this context, aquaporins may critically determine local water potential gradients rather than water flow intensities. These new considerations may lead to a better understanding of the role of aquaporins during cell elongation and water stress.

Studies on aquaporins have also led us far beyond membrane water transport. The transport of solutes of great physiological significance, such as CO_2, H_2O_2, B, or silicic acid, is now well established and has linked aquaporins to many functions, including carbon metabolism, oxidative stress responses, and plant mineral nutrition (**Figure 5**). Yet,

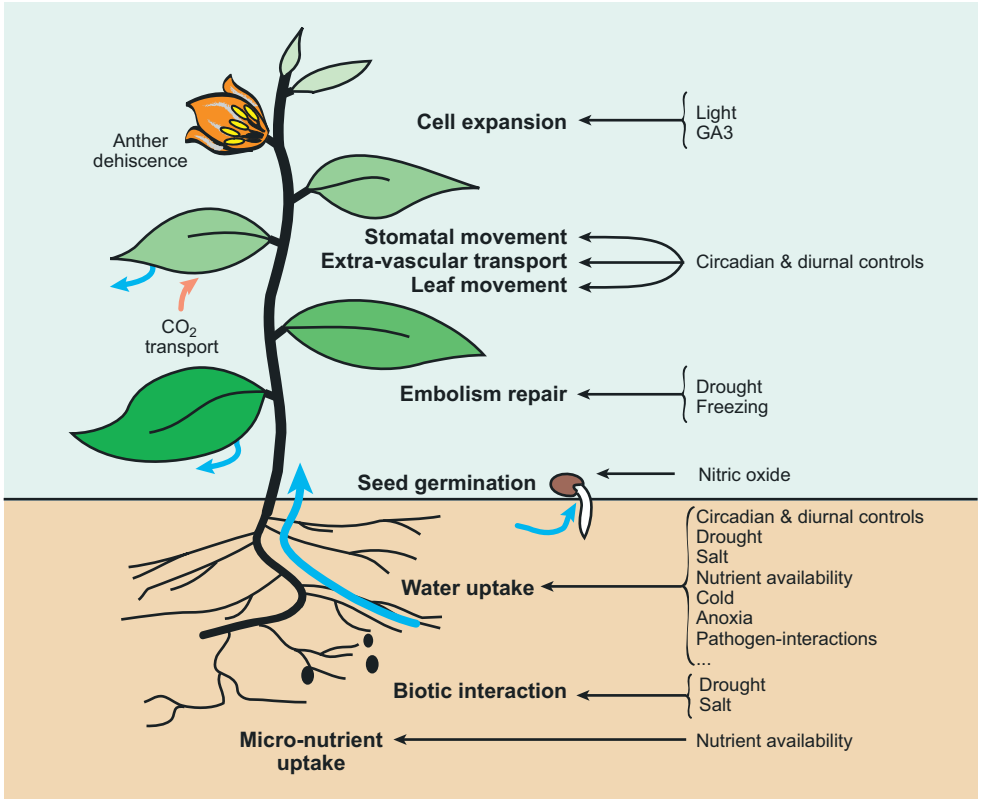


Figure 5

Integrated functions of plant aquaporins. The endogenous and environmental factors acting on each of the indicated aquaporin functions are shown. GA3, gibberellin.

novel putative substrates of plant aquaporins such as arsenate, NO, and NO_3^- await further investigation. We also note that several plant aquaporins, in the NIP and SIP subgroups in particular, have unknown functions and that new aquaporin subclasses are being discovered (139).

Finally, aquaporin functions need to be further integrated in the whole plant physiology. This will first require a better understanding of how the various transport activities of aquaporins are coupled with those of other transport proteins (75, 83, 134). The chains of events that lead to control of aquaporin functions by local or long-distance signals, during development or in response to biotic or abiotic signals, will also have to be elucidated. Finally, and although the field of aquaporin research has already enlarged considerably, we may not be at the end of our surprises because novel primary functions as diverse as cell proliferation (117) or virus replication (69) might be anticipated for plant aquaporins.

SUMMARY POINTS

1. Aquaporins are membrane channels that have a conserved structure and facilitate the transport of water and/or small neutral solutes (urea, boric acid, silicic acid) or gases (ammonia, carbon dioxide).

2. Aquaporins exhibit a high isoform multiplicity that reflects distinct transport specificities and subcellular localizations.
3. Aquaporin transport activity can be regulated by multiple mechanisms, including regulation of transcript or protein abundance, subcellular trafficking, or gating by phosphorylation or cytosolic protons.
4. Aquaporins play a central role in plant water relations. They mediate the regulation of root water transport in response to a variety of environmental stimuli. They facilitate water transport through inner leaf tissues during transpiration and in expanding tissues.
5. Multiple integrated roles of aquaporins in carbon and nitrogen assimilation and micronutrient uptake are being uncovered.

FUTURE ISSUES

1. The transport specificity of aquaporins lacking function and, in particular, of novel classes of aquaporins recently discovered in certain plant species should be investigated.
2. The mechanisms governing aquaporin subcellular trafficking should be investigated, and in particular it will be important to evaluate in planta how the functional expression of aquaporins of the plasma membrane intrinsic proteins 1 and 2 (PIP1 and PIP2) subclasses is determined by mutual physical interactions.
3. Investigation of the function and regulation of aquaporins in poorly explored physiological contexts, such as stomatal regulation or seed germination, will be required.
4. The mechanisms that determine regulation of aquaporins by light in inner leaf tissues should be dissected, and the role of aquaporins in coregulating CO_2 and H_2O transport in these tissues should be deciphered.
5. The relevance of altered aquaporin expression for biotechnological improvement of plant tolerance to water stress must be explored. The role of aquaporins during water stress recovery and in conditions similar to those experienced by crops in the field will have to be specified.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Errata

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