AQUAPORINS IN PLANTS

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Maurel C, Boursiac Y, Luu D-T, Santoni V, Shahzad Z, Verdoucq L. Aquaporins in Plants. Physiol Rev 95: 1321–1358, 2015. Published September 2, 2015; doi:10.1152/physrev.00008.2015.—Aquaporins are membrane channels that facilitate the transport of water and small neutral molecules across biological membranes of most living organisms. In plants, aquaporins occur as multiple isoforms reflecting a high diversity of cellular localizations, transport selectivity, and regulation properties. Plant aquaporins are localized in the plasma membrane, endoplasmic reticulum, vacuoles, plastids and, in some species, in membrane compartments interacting with symbiotic organisms. Plant aquaporins can transport various physiological substrates in addition to water. Of particular relevance for plants is the transport of dissolved gases such as carbon dioxide and ammonia or metalloids such as boron and silicon. Structure-function studies are developed to address the molecular and cellular mechanisms of plant aquaporin gating and subcellular trafficking. Phosphorylation plays a central role in these two processes. These mechanisms allow aquaporin regulation in response to signaling intermediates such as cytosolic pH and calcium, and reactive oxygen species. Combined genetic and physiological approaches are now integrating this knowledge, showing that aquaporins play key roles in hydraulic regulation in roots and leaves, during drought but also in response to stimuli as diverse as flooding, nutrient availability, temperature, or light. A general hydraulic control of plant tissue expansion by aquaporins is emerging, and their role in key developmental processes (seed germination, emergence of lateral roots) has been established. Plants with genetically altered aquaporin functions are now tested for their ability to improve plant tolerance to stresses. In conclusion, research on aquaporins delineates ever expanding fields in plant integrative biology thereby establishing their crucial role in plants.

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I. INTRODUCTION

In their large majority, plants are autotrophic organisms that just require light and a carbon source (usually carbon dioxide, CO2), on the one hand, and water and mineral nutrients, on the other hand, to achieve their life cycle. Because they are sessile, plants have, however, to efficiently absorb water and mineral nutrients from their close surroundings. This function is mostly fulfilled by the root system, which shows a remarkable ability to grow continuously and explore the soil for available resources. With no short-term escape strategies, plants also have to continuously face severe constraints from both the soil and aerial environments. These include abiotic stresses such as a lack or excess of water (drought, flooding) or beneficial or toxic mineral ions at varying concentrations in the soil. High or low extremes in temperature or light intensity also impose severe stresses, on the shoot especially. In addition, plants are under constant attack by a myriad of herbivores and pathogens including viruses, bacteria, insects, or fungi. Many plant species are also able to develop symbioses with specific soil micro-organisms which, in particular, optimize plant mineral nutrition. Plants therefore continuously adjust their metabolic functions, growth and development, to adapt to an ever-changing abiotic and biotic environment.

With respect to their water status, plants exhibit remarkable features. Their aerial parts mediate a tricky trade-off with the atmosphere, by absorbing CO2, a fundamental brick for photosynthesis, and releasing water by transpiration. This exchange is realized and tightly controlled by stomata, microscopic pores located in the epidermis of the plant’s aerial parts (FIGURE 1). Transpiration is made possible by an intense flow of water (sap) traveling throughout the plant body, from the roots to the substomatal chambers where it evaporates. This stream is particularly useful to drive water and nutrient ascent to the uppermost parts of
the shoots. Whereas long-distance water transport mostly occurs through the xylem, which is formed of specialized dead vessels, water uptake by roots and delivery to shoots requires transport through living tissues (FIGURE 1). Controlling the intensity and direction of these flows is particularly important for maintaining the whole plant water status. At the cellular level, the presence of a cell wall allows buildup of intracellular hydrostatic pressure (turgor) of several atmospheres which largely supports the erect shape of the plant. In addition, plant growth and development are determined by cell divisions in well-defined territories called meristems and subsequent expansion growth of the living cells. Cell turgor provides the motive force for the latter process. The metabolic activity of the plant and its growth and development are therefore highly dependent on its water status.

Despite a few pioneering works (reviewed in Ref. 185), the identification of water channel proteins in plants in the early 1990s (187), shortly after their discovery in animals, was fairly unexpected and provided a strong momentum for studies on plant water transport. Early studies of these so-called aquaporins contributed to a general boost in the molecular characterization of membrane transport systems in plants and brought new paradigms to address the molecular bases of plant water relations. After 20 years, plant aquaporins are recognized as multifunctional proteins transporting water but also gases such as CO₂, nutriments [e.g., boron (B), silicon (Si)] or reactive oxygen species (ROS). Thus aquaporin studies have spread to many fields of plant biology, from molecular membrane biophysics to cell biology and signaling. Their function also appears central for the physiology of plant growth and responses to abiotic stresses. This review covers the many facets of aquaporin functions in plants and stresses their importance and functional originality in these organisms.

II. PLANT AQUAPORIN DIVERSITY

A. Phylogeny of Plant Aquaporins

Aquaporins are now assimilated in a broad sense to the ancient superfamily of major intrinsic proteins (MIPs). MIPs are present throughout the living kingdom, with an exception for thermophilic Archaea and intracellular bacteria (1). MIP homologs have a reduced (~25%) overall sequence conservation but show a typical sequence signature, with six putative membrane spanning domains and two highly conserved Asn-Pro-Ala (NPA) motifs. In addition, MIP coding sequences are formed from a direct sequence repeat, which creates an internal symmetry of the

FIGURE 1. Short- and long-distance transport of water in a vascular plant. During transpiration, an intense stream of water occurs from the roots to the shoots. Soil water flows radially across concentric cell layers in the root before reaching the xylem vessels (middle panel, X) and being exported to the shoots. In leaves, water flows out of vascular tissues and reaches substomatal chambers where it diffuses as a gas (vapor) through the stomata.
channel protein, with its two halves (each with 3 transmembrane domains) showing an inverted insertion in the membrane. With an increasing number of plant genome sequences available, aquaporin genes have now been fully described in several herbaceous (Arabidopsis thaliana, maize, rice, soybean, tomato, and cotton) and ligneous (poplar) higher plant species (45, 93, 121, 224, 235, 239, 252, 334). Thorough sequence analyses have also been performed in other plant species of agronomical interest such as wheat (76) or grapevine (265).

These studies have revealed a great diversity of aquaporins in higher plants, with more than 30 isoforms in all examined species (TABLE 1). Due to a higher degree of ploidy, the genomes of soybean and upland cotton even encode 66 and 71 homologs, respectively. Higher plant aquaporins fall into five subfamilies. Three of these, the plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), and the nodulin26-like intrinsic proteins (NIPs) are now well-described, with respect to protein localization and function. Two additional subfamilies, the small basic intrinsic proteins (SIPs) (120) and the uncategorized (X) intrinsic proteins (XIPs) (59) were discovered more recently. The latter are absent in some higher plant species such as the monocots or the Brassicaceae (FIGURE 2).

Genomic studies have also provided insights into the aquaporin family in algae or early branched land plants (1, 7, 8, 59) (TABLE 1). With respect to seed plants, mosses (e.g., Physcomitrella patens) have two additional subfamilies, the hybrid intrinsic proteins (HIPs) and GIPF-like intrinsic proteins (GIPs), whereas spike mosses (e.g., Selaginella moellendorffii) only have HIPs, in addition to the PIPs, TIPs, NIPs, SIPs, and XIPs (8, 59). Algae (e.g., Chlamydomonas reinhardtii, Chlorella, Ostreococcus tauri) reside upstream in the plant lineage. They also have PIP and GIP homologs in addition to five subclasses (MIP A-E) that, in contrast, seem largely unrelated to other plant aquaporin subclasses (7). Whereas animal and bacterial MIPs fall into both the water channel (aquaporin) and aquaglyceroporin clades, all plant subfamilies except GIPs belong to the first clade (1).

B. Plant Aquaporin Evolution

1. Interspecific variations

Phylogenetic analyses have drawn a general scheme of plant aquaporin evolution. The GIPs may originate by horizontal gene transfer from an ancestral bacterial gene (94) and together with the PIPs have been transmitted from algae to land plants. Whereas XIPs and SIPs likely trace back to early plant (algae) ancestors, other subclasses (HIPs, TIPs) seem to have emerged during land plant evolution, possibly from a PIP ancestor. Interestingly, plant TIPs and mammalian aquaporin-8 share sequence homology, and it is as yet unclear whether this reflects a distant evolutionary relationship (1). The origin of NIPs is also uncertain. They may derive by horizontal gene transfer from an ancestral bacterial gene encoding an aquaglyceroporin with solute transport functions. Alternatively, NIPs may reflect an evolutionary convergence toward this subclass of aquaporins (1, 330). Finally, some subclasses (such as XIPs, HIPs, or GIPs) were lost during evolution of certain plant lineages pointing to functional redundancies.

The genetic diversity of aquaporins in plants also reflects the great dynamics of their genomes. While some subfamilies were lost in certain plant lineages (e.g., the XIPs in monocots), key genomic rearrangements have shaped the general expansion of the plant aquaporin family. These events have occurred at different stages during plant evolution. The subdivision of PIPs in PIP1s and PIP2s, or of TIPs in five subfamilies may have occurred early since they are conserved throughout all higher plants (FIGURE 2). In contrast, the XIP subfamily may have evolved later as it shows taxon-specific clade divergences (162). In poplar (52), 48 of 54 aquaporin genes belong to pairs of genes. At variance to what is found in vertebrates (1), most of these pairs (17) are due to

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>PIPs</th>
<th>TIPs</th>
<th>NIPs</th>
<th>SIPs</th>
<th>XIPs</th>
<th>HIPs</th>
<th>GIPs</th>
<th>Total</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selaginella moellendorffii</td>
<td>Spike moss</td>
<td>3</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td></td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Physcomitrella patens</td>
<td>Moss</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>23</td>
<td>59</td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>Rice</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>33</td>
<td>252</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>Mouse ear-cress</td>
<td>13</td>
<td>10</td>
<td>9</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>121, 235</td>
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<tr>
<td>Solanum lycopersicum</td>
<td>Garden tomato</td>
<td>14</td>
<td>11</td>
<td>12</td>
<td>4</td>
<td>6</td>
<td></td>
<td></td>
<td>47</td>
<td>239</td>
</tr>
<tr>
<td>Populus trichocarpa</td>
<td>Black cottonwood</td>
<td>15</td>
<td>17</td>
<td>11</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
<td>55</td>
<td>93</td>
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<tr>
<td>Glycine max</td>
<td>Soybean</td>
<td>22</td>
<td>23</td>
<td>13</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
<td>66</td>
<td>334</td>
</tr>
<tr>
<td>Gossypium hirsutum</td>
<td>Upland cotton</td>
<td>28</td>
<td>23</td>
<td>12</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
<td>71</td>
<td>224</td>
</tr>
</tbody>
</table>

Shown for all plant species, the genome of which was fully sequenced, are the number of homologs present in each of the indicated aquaporin subclasses.
genomic duplication, whereas a few of them (3) appeared through tandem gene duplications.

Regarding regulation of MIP expression, some of duplicated genes (principally TIPs) present in poplar showed a significant coregulation whereas others (PIPs) mostly showed divergent gene regulation (52). A precise analysis of tissue-specific expression of the 35 aquaporins in Arabidopsis showed that, in this species, there is no strict overlap in expression between isoforms (1), pointing to quick genetic divergence. Evidence of balancing selection has even been provided in PIPs of five tropical trees species, providing a putative response to variable environmental conditions (11).
2. Intraspecific variations

Plant aquaporin evolution is still at work as indicated by intraspecific variations. In particular, marked differences in aquaporin gene expression between rice cultivars or Arabidopsis natural accessions exist under normal or water stress conditions (4, 152, 275). In rice cultivars, differences in aquaporin gene regulation seem to underlie distinct adaptive mechanisms to water deficit (152). The natural variation of plant aquaporin expression and function provides a promising avenue for exploring their integrated function. For instance, genome-wide association studies have pointed to the putative contribution of OsTIP3;1 to grain width in rice (109) or to the role of AtTIP2;1 in adaptation of wild Arabidopsis to local habitats (77).

III. MOLECULAR FUNCTIONS OF PLANT AQUAPORINS

A. Functional Expression and Selectivity

1. Expression systems

Xenopus oocytes have been the first expression system used to investigate plant aquaporin function (128, 187). These huge cells are prone to intracellular microinjection of complementary RNAs and allow simple transport assays based on measurements of cell volume, intracellular pH, or content in radiolabeled molecules. The oocyte system allows addressing transport by aquaporins of water and many other substrates such as organic compounds (glycerol, urea), metalloids (B, Si), or even dissolved gases [CO₂, ammonia (NH₃)]. The oocyte system is still frequently used but displays some disadvantages. Although some intracellular plant aquaporins such as TIPs can be efficiently expressed at the cell surface, some other plant aquaporin subclasses (e.g., PIP1s, SIPs) seem to be recalcitrant to functional expression. In addition, an accurate quantification of proteins present at the plasma membrane remains difficult, restricting the quantitative resolution of transport measurements.

Yeast cells provide an alternative and have been successfully used for characterization of all five plant aquaporin subclasses. Growth assays can easily be performed, revealing how expression of an aquaporin can enhance the assimilation of some compounds (urea, NH₃) or the toxicity of others. Toxic compounds include hydrogen peroxide (H₂O₂) (29), germanium dioxide (GeO₂) used as a toxic analog of Si (17), boric acid [B(OH)₃], arsenous acid [As(OH)₃], and antimonite [Sb(OH)₃] (31). However, growth assays provide a rather indirect evidence for transport. Genuine transport measurements on whole cells or spheroplasts are more difficult to develop, but were applied to CO₂ (221), ROS (29), NH₃ (22), or selenous acid [H₂SeO₃] (337). Even more challenging is functional reconstitution of recombinant aquaporins in proteoliposomes. This approach was used to dissect the transport activities of PIPs, NIPs, and SIPs for water (60, 214, 304), glycerol (60), and NH₃ (112). Reconstitution of aquaporins in gas-tight triblock-copolymer membranes is particularly efficient to detect their CO₂ transport activity (294).

2. Selectivity in five subclasses

All expression systems have revealed that plant aquaporins are multifunctional channels, with a wide range of selectivity profiles (TABLE 2). Most of the PIPs and TIPs function as efficient water channels with additional substrates such as H₂O₂ (28, 105) and CO₂ for PIPs, or NH₃ (115, 164) and urea (86) for TIPs. Glycerol has also been investigated as a test solute, by reference to studies in animal and microbial aquaporins (25, 86). Yet, the physiological significance of this transport in plants, for osmotic tolerance in particular, remains uncertain.

In contrast to PIPs and TIPs, all NIPs investigated showed at most a reduced water transport activity. NIPs are mostly permeable to small organic solutes and mineral nutrients (169, 278). In particular, they mediate the transport of beneficial [B, Si, selenium (Se)] or toxic [arsenic (As), antimony (Sb)] metalloids (30, 337).

Although SIPs potentially display an original pore conformation (see sect. IIIA3) (120, 214), their functional characterization has only revealed a moderate water transport activity. In contrast, XIPs appear as multifunctional channels (26, 162) permeable to water, metalloids, and ROS.

Whereas mammalian aquaporin-6 is truly permeable to ions (113), no such transport function could be established for plant aquaporins. The electrical conductance induction upon reconstitution of a soybean NIP (nodulin-26) in lipid bilayers was likely due to protein aggregation artefacts (315) and could not be reproduced in other expression systems.

3. Overall molecular organization and pore conformations

Whereas aquaporin structures have been mostly investigated in animal or microbial homologs, a handful of studies have established crucial structural features of plant aquaporins. Cryoelectron microscopy of PIPs (139) and TIPs (57) confirmed their overall organization as tetramers. More importantly, X-ray crystallography of spinach SoPIP2;1 revealed with an unprecedented resolution the structure of an aquaporin in its closed or open states (217, 286), shedding light onto the original gating properties of PIPs.

These data and information deduced by homology modeling have established that plant aquaporins are formed by an
assembly of four monomers, each with six transmembrane spanning domains (1-6) and five connecting loops (A–E) localized on the intra- (B, D) or extracytosolic (A, C, E) sides of the membrane (Figures 3 and 4). Each monomer is able to form an individual transmembrane pore, and remarkably, the A and D loops, which both carry a NPA motif, fold as half-membrane-spanning H9251 helices and dip into the membrane to position each of their NPA motif at the center of the pore. These two motifs contribute to a central pore constriction and, in conjunction with the dipole moment of the two half-membrane-spanning H9251 helices, prevent proton (H+) permeation. Furthermore, four conserved residues form, close to the extracytosolic mouth of the pore, a typical aromatic/arginine (Ar/R) constriction functioning as the main selectivity filter.

Whereas functional selectivity assays have been restricted to a few representative plant isoforms, homology modeling was used to explore in a broader sense the transport selectivity of plant aquaporins, with a main emphasis on the Ar/R selectivity filter (16, 309). Each aquaporin subfamily was found to show specific pore profile(s) and could be further refined in subclasses with distinct predicted selectivities. For instance, three putative selectivity subclasses can be identified in NIPs (194, 242, 308). A critical residue of transmembrane spanning domain 2 differs between subclasses I and II. Reverse substitution of this residue in a class I (nodulin-26) and class II (AtNIP6;1) homolog showed how, in class I, a Ala residue enhances permeation of large solutes at the expense of water transport, whereas in class II, a Trp residue has opposite effects (308). Class III NIPs have small size residues in the Ar/R selectivity filter allowing the passage of large diameter solutes such as silicic acid (194, 242). TIPs also display a great diversity of substrates and Ar/R selectivity filter configurations. A Val-to-Ile substitution at a critical position in loop E was shown to determine the range of transported solutes (14). In barley PIPs, a Ile residue at the end of loop E is also crucial for transport and would confer CO2 permeability through interaction with a Leu residue in loop C (203). There are also concerns that, in addition to the four individual monomer pores, a fifth pore may be formed at the center of the aquaporin tetramer. The differential inhibition by mercury (see sect. IIIB2) of water and NH3 transport in a wheat TIP was interpreted to mean that the preferential permeation pathways for these two substrates would be the four monomer pores and the central pore, respectively (22). In X-ray structures, the central region of spinach SoPIP2;1 tetramers can

<table>
<thead>
<tr>
<th>Subclass</th>
<th>Isoform</th>
<th>Substrate</th>
<th>Expression System</th>
<th>Transport Assay</th>
<th>Reference Nos.</th>
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<td>AtPIP2;1</td>
<td>Water</td>
<td>Proteoliposome</td>
<td>Shrinkage</td>
<td>304</td>
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<td>AtPIP2;1</td>
<td>H2O2</td>
<td>Yeast</td>
<td>Toxicity growth assay</td>
<td>66</td>
</tr>
<tr>
<td>PIP</td>
<td>AtPIP2;2</td>
<td>Water</td>
<td>Xenopus oocyte</td>
<td>Swelling</td>
<td>287</td>
</tr>
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<td>NtAQG1</td>
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<td>Xenopus oocyte</td>
<td>Radio labeling</td>
<td>25</td>
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<td>NtAQG1</td>
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<td>Xenopus oocyte</td>
<td>Intracellular pH</td>
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<td>NtAQG1</td>
<td>CO2</td>
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<td>NIP</td>
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<td>As(OH)3</td>
<td>Xenopus oocyte</td>
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<td>Yeast</td>
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<td>117</td>
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<td>VvSIP1</td>
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<td>162</td>
</tr>
</tbody>
</table>

This table is not intended to be exhaustive. It illustrates, using selected examples, the diversity of expression systems and transport assays used to determine the wide range of substrates found for members of each plant aquaporin subclass.
accommodate a detergent molecule instead of a lipid, indicating that CO₂ permeation through this pathway may be possible, at least from a steric point of view (78).

B. Gating

1. Molecular mechanisms

In vitro studies on purified plant membranes or proteoliposomes have demonstrated the role of cations, H⁺, and phosphorylation in controlling plant aquaporin activity (85, 90, 304). In the case of PIPs, a unifying model of pore opening and closing (gating) was proposed, based on SoPIP2;1 molecular structure (286) (FIGURE 3). This model indicates how the reversible motion of a hydrophobic residue (Leu197 in SoPIP2;1) into and out of the cytoplasmic opening of the pore controls the aquaporin water permeability. This hydrophobic gating residue is carried by the second cytosolic loop (D), the conformational changes of which are mediated by ionic and H-bond interactions with the NH₂-terminal tail (Asp28 and Glu31) and first cytosolic loop (B; Ser115 and Arg118).

The pH-dependent gating of PIPs primary relies on protonation of a perfectly conserved His residue of loop D (His193 in SoPIP2;1) (287) (FIGURE 3). At acidic pH, charged His193 interacts with Asp28, Glu31, and loop B (Ser115) to stabilize loop D in a closed pore conformation (79, 286). There also are reports of H⁺-dependent inhibition of TIPs (146, 272), which however involves a His residue located in the second extracytoplasmic (intravacuolar) loop C. The structural basis of this gating mechanism is as yet unknown.

Similar to H⁺-dependent gating, the inhibition of PIP water transport by divalent cations can be explained by a direct binding of the cation, which in turn mediates H-bonding between the NH₂ terminus (Gly30 and Asp31) and loop D through loop B, thereby stabilizing the closed pore conformation (FIGURE 3). Although this mechanism was revealed by Cd²⁺ binding in the crystal structure of SoPIP2;1, the binding site may rather be occupied by Ca²⁺ in vivo. In agreement with the biphasic dose-dependent effects of Ca²⁺ on the water permeability of certain plant plasma membranes (6), a second binding site was recently identified between loop D and the COOH terminus of SoPIP2;1 (78). This second binding site may reflect a stabilizing role of the COOH terminus on loop D folding.

Structure-function analyses of TIPs, PIPs, and NIPs in Xenopus oocytes (90, 122, 186) have pointed to the role of several cytosol-exposed phosphorylation sites in controlling water transport. Structural models of PIPs now support this view indicating how phosphorylation of a conserved Ser residue in loop B (Ser115 in SoPIP2;1) would destabilize the loop D-loop B anchor, thereby favoring the open-pore conformation. Interestingly, phosphorylation of Ser274 on SoPIP2;1 COOH-terminal tail would act similarly but through a transactivation process, whereby interaction between the COOH-terminal tail and the loop D of an adja-
cent monomer would be destabilized after Ser274 phosphorylation.

Due to these great advances, PIP gating is emerging as a paradigm in the aquaporin field. Although distinct protein domains may be involved, similar mechanisms seem to be at work in other plant, animal, or microbial aquaporins. For instance, the gating of Arabidopsis NIP7;1 is largely controlled by a unique Tyr residue (Tyr81), located in transmembrane spanning domain 2 and interacting with neighboring Arg220 of the Ar/R selectivity filter to stabilize closed channel conformation (148). In mammalian aquaporin-0 and bacterial aquaporin-z, the corresponding Arg189 residue was shown to adopt distinct conformational states leading to channel gating (102).

Despite recent progress, a refined structural model of plant aquaporin gating is still needed. For instance, SoPIP2;1 mutants carrying phosphomimetic mutations at Ser115 and Ser274 did not show enhanced water transport activity after reconstitution in proteoliposomes and, accordingly, their X-ray structures displayed a closed conformation (217). Thus these mutations may not be sufficient to stabilize the open pore conformation in vitro, whereas they have an activating role in vivo (232). Also, a control of aquaporin activity by osmotic or hydrostatic pressures has been reported in some plant cells or purified membranes (206, 212, 310, 327). It is not yet clear whether these effects are mediated through solute- or pressure-induced changes in aquaporin conformation or through membrane-associated cell signaling events (327). Finally, the role for membrane lipids in aquaporin gating is emerging in animals but has not yet been explored in plants (284, 285). In particular, its significance with respect to protein partitioning in membrane microdomains and stress-induced changes in lipid composition will have to be carefully examined (see sect. IVB2).

**FIGURE 4.** Multiple covalent posttranslational mechanisms acting on plant plasma membrane aquaporins. The figure shows a schematic representation of Arabidopsis AtPIP2;1 with its six transmembrane domains (1–6), five connecting loops (A–E), and NH2- and COOH-terminal tails bathing in the cytosol. The indicated modifications were mostly determined by mass spectrometry. They include cleavage of the initiating methionine (cross), dimethylation (Met Met) of a Lys residue (K3), deamidation (Dea) of Gln and Asn residues (Q14, Q19, N286) (62), and ubiquitination (Ub), the ubiquitinated residue(s) being unknown (143). Phosphorylation of some Ser residues (S280 and S283) was established experimentally (62, 234). In contrast, the phosphorylation of loop B at S121 was inferred from studies on spinach SoPIP2;1 (122, 286). Some enzymes involved in covalent modification of PIPs have been identified. They include methyl-transferases SDG7 (At2g44150) and OMTF3 (At3g61990) (247), a U3 ligase (143), and several protein kinases including Mg2+- and Ca2+-dependent protein kinases (268) and SIRK1 (At5g10020) (318). The figure is color-coded to show the enzymatic targets and the main molecular and cellular function associated with the posttranslational modification. Thus posttranslational modifications interfere with the subcellular localization (red), gating (green), and degradation of the aquaporin (purple). The functional role of PIP deamidation is unknown.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Role</th>
</tr>
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<tbody>
<tr>
<td>SDG7</td>
<td>Subcellular localization</td>
</tr>
<tr>
<td>OMTF3</td>
<td>Gating</td>
</tr>
<tr>
<td>Mg2+-dependent protein kinase</td>
<td>Degradation</td>
</tr>
<tr>
<td>U3 ligase</td>
<td>Gating</td>
</tr>
<tr>
<td>Ca2+-dependent protein kinase</td>
<td>Subcellular localization</td>
</tr>
<tr>
<td>SIRK1</td>
<td></td>
</tr>
</tbody>
</table>
2. Mercury and other aquaporin blockers

Mercury is the most common aquaporin blocker used in plants and animals. It is thought to inhibit the water permeability of purified membranes, cells, or tissues through binding to the thiol groups of crucial aquaporin Cys residues. Mercury may also target His residues as recently revealed in rice OsNIP3;3 (130). The use of mercury must be considered with care, because of its strong cellular toxicity (see sect. VIB1) (175, 336). The generality of its inhibiting effects has also been questioned. First, some plant aquaporins are insensitive to mercury because they do not harbor the critical Cys residues identified in other isoforms (58). In addition, mercury did not block but rather activated SoPIP2;1 in proteoliposomes (78). This activation, which was Cys independent, may reflect a mechanical gating through effects on the lipid environment. In plants as in other organisms (328), there is therefore a strong need for new aquaporin blockers with reduced cellular toxicity. Weak acids or respiration inhibitors (azide, cyanide) decrease cytosolic pH, thereby inducing a H⁺-dependent PIP closure. Although they can potentially confirm mercury inhibition (275), these treatments are also toxic. Silver and gold compounds offer an interesting alternative (213), but proper application to living cells and tissues seems to be challenging.

C. Posttranslational Modifications

Although their high hydrophobicity hinders classical biochemical analyses, plant aquaporins are, together with H⁺-ATPases, the most abundant intrinsic proteins of plant plasma and vacuolar membranes. Aquaporins have therefore been prone to extensive proteomic characterizations. In-depth mass spectrometry analyses have revealed that plant aquaporins carry numerous co- and posttranslational modifications, including phosphorylation, methylation, deamidation, NH₂-terminal acetylation, and ubiquitination (44, 62, 132, 216, 254, 255, 297) (FIGURE 4). The latter modification and N-glycosylation were also revealed using immunodetection techniques (143, 303). Thus the molecular diversity of plant aquaporins goes far beyond a high number of isoforms. The multiple posttranslational modifications of aquaporins point to a variety of regulatory mechanisms targeting aquaporin expression and function. Quantitative proteomics is now revealing how these modifications can vary in abundance, depending on plant tissues or physiological contexts (62, 232). Whereas a general role of phosphorylation in aquaporin gating and trafficking (see sect. IVB2) is now well established, the significance of other aquaporin posttranslational modifications is still elusive. Ubiquitination of Arabidopsis AtPIP2;1 and N-acetylation of ice plant (Mesembryanthemum crystallinum) McTIP1;2 seem to act on endoplasmic reticulum (ER) degradation and redistribution to endosomal compartments, respectively (143, 303). AtPIP2;1 was the first plant membrane protein and first aquaporin shown to be methylated. Two adjacent residues, Lys3 and Asp6, serve as methylation sites on its cytosolic NH₂-terminal tail (254). These residues overlap with a diacidic motif involved in ER export of the protein (see sect. IVB1), suggesting a role for AtPIP2;1 methylation in protein subcellular trafficking. Yet, any mutation at these sites had dominating effect on aquaporin trafficking, thereby preventing proper structure-function analyses (254). Although deamidation of PIPs shows multiple stimulus-induced changes in Arabidopsis roots, the mechanisms leading to this modification and its role are as yet unknown (62).

D. Heteromerization

1. Oligomeric structures

Due to their high structural similarity, members of a same plant aquaporin subclass may physically assemble as heterotetramers, thereby enabling multiple molecular and functional combinations. Although their existence remains to be formally demonstrated, heteromers formed of either PIPs or TIPs have been suggested from functional coexpression in Xenopus oocytes or yeast, or protein-protein interaction assays such as bimolecular fluorescence complementation (72, 209, 282, 331). Furthermore, molecular modeling of PIPs pointed to a critical role of first extracytosolic loop (loop A) in oligomer interactions. This loop contributes to a disulfide bond that stabilizes PIP dimers, which in turn may associate as tetramers (27). Accordingly, site-directed mutations within this loop were able to modify the interaction properties of Beta vulgaris (common beet) PIP1s and PIP2s (124).

2. Functional effects

In plants, heteromerization may be critical for combining PIP1s and PIP2s with distinct functional properties (221). Coexpression studies in Xenopus oocytes and maize protoplasts have revealed a clear incidence on trafficking. Whereas singly expressed PIP1s were retained intracellularly, interactions with PIP2s allowed them to reach the plasma membrane (72, 331). Converse effects, whereby PIP1s may alter the overall tetramer pH sensitivity (20) and transactivate their interacting PIP2 partner (324), have also been revealed using oocyte expression. Functional expression in yeast showed that heteromeric assembly of PIP isoforms with a preferential role in water or gas (CO₂) transport may also provide means for adjusting the overall PIP tetramer selectivity (221). Whereas these studies point to very complex combinatorial regulations, their significance in the whole plant remains difficult to assess. For instance, overexpression in transgenic Arabidopsis of a PIP2 mutant form deficient in trafficking to the plasma membrane induced an intracellular retention of native PIPs (and a decrease in root water transport) (270). Although this was not strictly demonstrated, these effects...
may be due to molecular interactions between PIPs in the plant.

E. Perspectives

The present section shows that we have got a fair understanding of plant aquaporin molecular functions. This knowledge is central for addressing aquaporin function and regulations at the plant cell and whole organism level. A powerful, but challenging integrative approach is expression in transgenic plants of site-directed mutant forms of aquaporins. The sections below exemplify how aquaporin phosphorylation and trafficking are now analyzed along these lines. By comparison, in planta analysis of aquaporin properties such as transport specificity or H⁺- or Ca²⁺- dependent gating is lagging behind. Addressing the functions and regulations emerging from aquaporin heteromeric assembly also represents an important but highly challenging objective.

IV. CELL BIOLOGY OF PLANT AQUAPORINS

A. Subcellular Localization Patterns

In relation to their unique metabolic capacities (e.g., photosynthesis, N₂-fixation through endosymbiosis), plant cells are characterized by a great diversity of intracellular compartments and subcellular membranes. Accordingly, plant cells display a wide palette of aquaporin subcellular localization patterns.

1. Plasma membrane

As in animals, the plant plasma membrane represents a crucial barrier and exchange platform. Accordingly, this membrane harbors three subclasses of aquaporins, namely, the PIPs (FIGURE 5A), NIPs, and XIPs (26). Fusions with fluorescent protein reporters have indicated that most of these aquaporins are expressed on the entire cell surface. Yet, some isoforms can be confined to membrane subdomains. For instance, two NIP homologs, Arabidopsis At-NIP5;1 (277) and rice Lsi1 (OsNIP2;1) (277), are specifically expressed on the endofacial side of root endodermal cells, to mediate a passive cellular efflux of B and Si, respectively. Transporters specialized in the cellular influx of the same solutes (BOR1 and Lsi2, respectively) show an opposite polar localization. Thus these complementary arrangements create an efficient path for transcellular transport of B and Si, and centripetal transfer into vascular tissues (xylem vessels).

A few PIP isoforms also show a somewhat polar expression, with preferential accumulation on the exofacial and endofacial sides of root cells in rice and maize, respectively (95, 250). The PIP isoform counterparts that must be expressed

FIGURE 5. Localization of aquaporins in the plant plasma and vacuolar membranes. Transgenic Arabidopsis plants expressing AtPIP2;1 fused to the green fluorescent protein (PIP2;1-GFP) (A), or coexpressing PIP2;1-GFP and AtTIP1;1 fused to the fluorescent protein mCherry (TIP1;1-mCherry) (B), were observed by laser scanning confocal microscopy. A: plasma membrane localization of PIP2;1-GFP in a cross section of a root (left panel) with indicated cell layers (ep, epidermis; co, cortex; en, endodermis; st, stele) and in epidermal cells of cotyledons (right panel). B: localization in root epidermal cells of PIP2;1-GFP and TIP1;1-mCherry, in the plasma membrane and vacuolar membrane (tonoplast), respectively. The arrows indicate cytoplasmic strands formed within grooves at the surface of the invaginated vacuole, and the asterisk indicates a nucleus skirted by the tonoplast. Bar size = 20 μm.
on the opposite cell side to mediate transcellular water transport remain to be identified.

Recent imaging of PIPs using fluorescence recovery after photobleaching (FRAP) or evanescent wave microscopy techniques also revealed their extremely low lateral mobility at the cell surface (107, 150, 167, 181). This feature may partly result from hindering interactions with the cell wall (181). Single particle tracking further showed a confinement of PIPs in microdomains associated with specific sterol compositions (150). Accordingly, PIPs were found to be enriched in detergent-resistant membrane fractions tentatively associated with membrane lipid rafts (131, 199, 202). This membrane partitioning likely underlies the dynamics of PIP cycling between the cell surface and endosomal compartment (see sect. IVB2).

2. Vacuoles

Vacuoles represent the most voluminous intracellular organelles of plant cells. Vacuoles are not uniform, with distinct subtypes present in a same cell and showing specialized lytic or protein storage functions. Although vacuoles harbor a single subclass of aquaporins, the so-called TIPs (319) (FIGURE 5B), each vacuolar subtype is characterized by a specific set of isoforms (118). It has been argued that these patterns must be determined by targeting pathways specific of each vacuolar subtype. However, they are more likely generated through one or several common trafficking machinery(ies) and simply result from distinct developmental expression of TIP isoforms during the course of cell differentiation (111, 118). The vacuolar membrane (tonoplast) is more fluid than the plasma membrane and, by comparison to PIPs, TIPs show a much higher (>10-fold) lateral membrane mobility (107, 167). Yet, their expression is not uniform and preferential expression of TIP fused to GFP has been observed in intravacuolar invaginations (bulbs) and in apposing tonoplast regions of two adjacent vacuoles (19, 248). This pattern may allow privileged exchanges between neighboring vacuoles. It may also partly result from antiparallel dimerization of GFP expressed in two apposing membranes (262).

3. Other intracellular compartments

Whereas most plant aquaporins can be observed in the ER, during biogenesis and transfer to their destination membrane, some isoforms such as SIs (114, 214) and some NIs (197) seem to be resident of this compartment. Their mode of targeting and cellular function in this compartment are as yet unknown.

The chloroplast is a plant specific organelle, delineated by an envelope formed by a double membrane. The chloroplast also contains numerous stacks of thylakoid membranes, which harbor the light-collecting antenna and electron transfer chains crucial for photosynthesis. Thorough proteomic analyses in Arabidopsis (70, 71) have suggested the presence of PIPs and TIPs, in the inner envelope and thylakoids, respectively. Localization of a PIP1 (NtAQPI) in the chloroplast envelope of tobacco leaves has also been revealed by immunocytochemistry (295). Whereas these localizations point to highly relevant plant specific function (see sect. VD), they now call for complementary data and confirmation in other plant materials.

Mammalian aquaporin-8, which shows a somewhat atypical sequence among other animal aquaporins and significant homology to TIPs, has been localized to mitochondria (38). A putative mitochondrial targeting signal was also detected in pollen-specific Arabidopsis AtTIP5;1 (272). Ectopic expression in the vegetative cell of transgenic pollen of AtTIP5;1 fused to a fluorescent reporter led to the proposal that AtTIP5;1 localizes to mitochondria. Yet, more adequate reporter fusion and colocalization studies revealed that this conclusion is erroneous and that AtTIP5;1 actually localizes to the tiny vacuoles of pollen sperm cells (320). Thus, as far as we know, plant mitochondria seem to be deprived of aquaporins.

4. Dual localization patterns

It is assumed that the high degree of cell compartmentalization in plants has represented a major driving force during the molecular and functional diversification of aquaporins in these organisms. In summary, plant aquaporins have been localized throughout the cell secretory system including ER, Golgi, endosomes, autophagosomes, and vacuoles (98, 168). Specific isoforms are also expressed in chloroplasts and in the symbiosome membrane that surrounds, in legumes, the intracellular N2-fixing bacteroids of Rhizobaceae-infected roots (see sect. VIII A). In contrast, and for some unknown reasons, aquaporins seem to be excluded from some compartments such as plant mitochondria and peroxisomes.

Interestingly, examples of dual subcellular localizations of aquaporins have recently emerged in plants. For instance, the PIP1 homolog NtAQPI is expressed in the plasma membrane and chloroplast of tobacco leaves (295), while TIP3s have been localized in both the plasma membrane and tonoplast of maturing Arabidopsis seeds (82). A vacuolar TIP1 homolog can also be transiently expressed on the symbiosome membrane of Medicago truncatula (barrel clover) root nodules (84). As discussed below, these patterns must be determined by exquisite trafficking signals, which most often remain to be identified.

B. Aquaporin Trafficking

1. Multiple pathways in the secretory system

The modes of plant aquaporin targeting to their destination membranes have been addressed using various ap-
proaches. First, site-directed mutations of maize and *Arabidopsis* PIPs revealed that inner sequence motifs direct their trafficking to the plasma membrane. In particular, diacidic motifs located on their NH2-terminal tail likely interact with the COPII sorting machinery and critically determine their export from the ER (270, 332) (FIGURE 6A). Anterograde transport of PIP2s in the secretory pathway also involves a LxxxA motif (48) in the third membrane spanning helix and a phosphorylation site located on the COOH-terminal tail (234). Most PIP1s do not have these motifs but are likely dragged to the plasma membrane through heteromer assembly with PIP2s.

The motifs that determine TIP routing to the vacuole remain largely unknown. A pharmacological screening in *Arabidopsis* (241) revealed that TIP1 targeting is sensitive to Brefeldin A and likely involves a Golgi-dependent route (FIGURE 6A). In contrast, trafficking of TIP2s and TIP3s does not pass through the same route and is sensitive to a newly identified compound named C834 (241). In the future, a chemical genetic approach may be used to identify the molecular components of these different pathways.

Q-SNAREs of the syntaxin family are well identified molecular players of vesicular trafficking in eukaryotes. In plants, they mediate the trafficking of PIPs and TIPs at specific stages along the secretory pathway (24, 291). In particular, post-Golgi trafficking of *Arabidopsis* AtPIP2;7 was shown to depend on a direct physical interaction with two specific syntaxins, SYP61 and SYP121 (96).

**FIGURE 6.** Subcellular dynamics of PIPs and TIPs. A: targeting of aquaporins to the plasma membrane and tonoplast. Both PIPs and TIPs follow the secretory system to be targeted to their final destinations. All isoforms are synthesized in the endoplasmic reticulum (ER). Specific signals or mechanisms are requested for the exit of PIPs out of this compartment. These include diacidic (D/EXD/E) or LXXXA motifs, or phosphorylation (Phosp) for PIP2s, or physical interactions with PIP2s (PIP1s/PIP2s) for PIP1s. The existence of similar mechanisms or motifs is not documented for TIPs. The targeting pathways followed by TIP1s and TIP2/3s are sensitive to Brefeldin A (BFA) or C834, respectively. The Golgi apparatus (GA) and trans-Golgi network (TGN) continuum provide intermediate compartments for trafficking of PIPs, prior to their exocytosis towards the PM. In contrast, TIP1s traffic through the GA/TGN and multivesicular body (MVB) compartments before reaching the tonoplast. Note that, in plants, MVB is also termed prevacuolar compartment (PVC) or late endosomal compartment (LE). The precise targeting path followed by TIP2/3s is not yet known. Once at the plasma membrane, PIPs undergo constitutive cycling, through endocytosis from the cell surface towards early endosomes (EE) using a clathrin-dependent pathway and subsequent exocytosis back to the PM. The TGN and the EE are considered to form a single compartment. The syntaxins (SYP) that are known to play a role in some steps of PIP or TIP trafficking are indicated. B: subcellular dynamics of PIPs, with mobilization from the plasma membrane to intracellular compartments, in salt-stressed root cells. The effects of salt are mediated through ROS which enhance the lateral mobility of PIPs at the cell surface and their partitioning in membrane microdomains. PIP cycling, by endocytosis through clathrin-dependent and clathrin-independent pathways and exocytosis, is also enhanced by ROS. Some of the internalized PIPs are directed toward the MVB and spherical bodies. Abbreviations are as in A. C: osmotic stress-induced degradation of PIPs. In addition to enhanced cycling, PIPs undergo ubiquitination or interaction with multi stress regulator proteins. As a consequence, PIPs are directed toward the proteasome and vacuolar degradation pathways. The latter pathway involves autophagosomes (see main text for details). Abbreviations are as in A.
2. Constitutive cycling of PIPs and response to environmental stresses

Although they have a reduced lateral mobility, PIPs are far from static at the plasma membrane. Pharmacological and genetic studies have revealed that they continuously cycle between the plasma membrane and early endosomes (trans-Golgi network) (167) [FIGURE 6A]. Under resting conditions, this process is mediated through clathrin-mediated endocytosis (61) and is blocked in part by the auxin analog NAA using an as yet unknown mechanism. In root epidermal cells under osmotic or salt stress, the cycling rate of PIPs is enhanced due in part to the activation of a complementary endocytic pathway, independent of clathrin, and associated with membrane micro-domains (150, 167) [FIGURE 6B]. These processes are probably linked to the enhanced mobility of PIP molecules at the cell surface observed under osmotic and salt stress conditions (107, 150).

In connection to these processes, the same stresses induce a partial internalization of PIPs (34, 35) [FIGURE 6B]. This leads to a reduced abundance of PIPs at the root cell surface, which may contribute to the observed decrease of root water permeability. In addition, osmotic stresses induce functional and morphological rearrangements of the vacuolar apparatus. For instance, they trigger a relocalization of TIPs, from the vacuole to endosomal compartments or intravacuolar bulbs, in ice plant and Arabidopsis, respectively (34, 303).

These profound effects of stresses on aquaporin subcellular localization and dynamics are central in plant responses to their environment. Beyond their impact on plant water relations, they represent one of the earliest cellular responses to stresses and can possibly be extended to other membrane proteins. In addition, stimulus-induced PIP trafficking can be antagonized by ROS scavengers, in agreement with the central role played by ROS in stress and hormonal signaling in plants (35) [FIGURE 6B]. Deciphering the molecular and cellular mechanisms that govern PIP and TIP dynamics may therefore be central to understand the perception and transduction of stress signals in plants. In Arabidopsis roots, both salt and oxidative stresses induce quantitative changes in the double COOH-terminal phosphorylation of At-PIP2;1 (62, 234). Phosphorylation of a specific residue (Ser283) interferes with trafficking of internalized PIPs to fuzzy structures or spherical bodies. These structures have tentatively been identified as sorting endosomes and pre-vacuolar compartment (multivesicular bodies), respectively (234) [FIGURE 6B]. Whether this sorting permits a reversible sequestration of PIPs, prior to remobilization to the plasma membrane, or whether it directs them towards vacuolar degradation is unknown. Nevertheless, these mechanisms are reminiscent of the vasopressin- and phosphorylation-dependent trafficking of aquaporin-2 in renal epithelial cells (198).

3. Degradation pathways

Although aquaporin abundance can markedly vary in response to abiotic stresses or oscillate along circadian rhythms, information on the modes of plant aquaporin degradation has remained scarce. A RING membrane-anchor E3 ubiquitin ligase from pepper, and its Arabidopsis homologs, were shown to localize in the ER and ubiquitinate AtPIP2;1 leading to its retention in this compartment (143). This process was enhanced under osmotic stress, leading to aquaporin degradation through the proteasome pathway [FIGURE 6C]. A more recent study established a very novel link between aquaporin degradation and intracellular trafficking in Arabidopsis (98). AtPIP2;7 was shown to interact in the ER and Golgi, with a membrane protein, named TSPO for tryptophan-rich sensory protein/translocator and serving as multistress regulator [FIGURE 6C]. The complex was then directed towards vacuolar degradation, using the autophagosome pathway, this process being stimulated by drought-induced hormone, abscisic acid (ABA). Thus plant cells under water stress can use various pathways, for aquaporin degradation and long-term downregulation of plasma membrane water permeability.

C. Perspectives

The present section showed that, in addition to multiple substrates and selectivity profiles, plant aquaporins show very diverse subcellular localization patterns, and cell responses to hormonal and environmental stimuli. These properties can provide a first hint at their high genetic diversity. We also note that aquaporins have initially been used as mere reference markers for the plasma membrane or the vacuole. An accumulating body of data now reveals their remarkable trafficking properties. Aquaporins are therefore emerging as one of major membrane protein models in plant cell biology (168).

While evanescent wave fluorescent microscopy and associated single particle tracking techniques provide novel insights into plant aquaporin dynamics, much remains to be learned about the molecular and cellular mechanisms that direct their trafficking, both under resting and stress conditions. The recent characterization of individual aquaporin partners has provided insights into specific cell sorting or degradation steps (96, 98, 143) [TABLE 3]. Genomic studies based on classical (53) or split-ubiquitin (123, 140) yeast two-hybrid systems are now revealing a myriad of additional aquaporin partners [FIGURE 7]. These genome-wide aquaporin interactomes and their functional genomic characterization will undoubtedly reveal novel mechanisms related to aquaporin trafficking. It should also reveal hormonal and environmental signaling chains acting on aquaporins or large protein complexes coupling water transport to other membrane processes [TABLE 3].
V. CELL-AUTONOMOUS AND INTRACELLULAR FUNCTIONS

A. Osmoregulation

Addressing the cell-autonomous functions of aquaporins provides a useful step towards integration of their role at the organ and whole plant levels. One major cellular function of aquaporins is osmoregulation.

In agreement with the targeting of specific aquaporin isoforms to distinct subcellular compartments, water transport assays in purified membrane vesicles, isolated vacuoles, and protoplasts have revealed that membranes from a same cell can exhibit strikingly different permeability profiles. In tobacco cells for instance, purified tonoplast vesicles were 100-fold more permeable to water than their plasma membrane counterparts (188). An extremely high water permeability in the tonoplast seems to be common to most plant cells (188, 204, 212). According to mathematical models, this property would provide the cell with the capacity of reducing peak fluctuations of cytosolic volume in case of a sudden change in extracellular water potential (208, 290). Thus plant cells use their large vacuoles as buffering compartments during cell osmoregulation.

The delayed volume response of isolated protoplasts subjected to an osmotic challenge indicated that dynamic changes in water permeability may also contribute to plant cell responses to osmotic stresses (206). A direct dependence of water permeability on transmembrane osmotic gradients has been observed in tonoplast vesicles purified from wheat roots, but not in tobacco suspension cells (188, 212). Although the mechanisms leading to rapid and possibly direct changes in aquaporin activity have not yet been addressed in these contexts, we assume that they pertain to the general gating and trafficking properties described in the previous sections (see sects. III and IV).

The dynamic responses of aquaporins to osmotic stress have led to the idea that aquaporins, localized on the vacuolar or plasma membrane may serve as osmosensors, in guard cells during stomatal movements or in growing pollen tubes for instance (173, 263). These ideas remain highly speculative and rely at most on experiments showing that osmoregulation is altered by mercury, a nonselective aquaporin inhibitor. More solid genetic evidence is now needed to support such models. Despite the presence of aquaporins in most intracellular compartments, we also ignore the volume dynamics and osmotic or mechanical constraints at work on their membranes during volume or turgor regulation. Finally, most studies have focused on plasma membrane and vacuolar aquaporins, and a closer characterization of SIPS, aquaporins that are confined in the ER, may reveal novel aspects of plant cell osmoregulation (114, 214).

### Table 3. Molecular and functional characterization of aquaporin-interacting proteins

<table>
<thead>
<tr>
<th>Aquaporin</th>
<th>Interacting Protein Name</th>
<th>Interacting Protein Function</th>
<th>Method</th>
<th>Reference Nos.</th>
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<td>ZmPIP1;1, ZmPIP1.2, ZmPIP1.6</td>
<td>ZmPIP2;1 and/or ZmPIP2;5</td>
<td>Aquaporin</td>
<td>FRET/FLIM</td>
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<td>RhPIP1;1</td>
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<td>GhPIP2;6</td>
<td>GhPIP1</td>
<td>Aquaporin</td>
<td>Y2H, BiFC</td>
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<td>AtPIP2;1</td>
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<td>E3 Ub ligase</td>
<td>Y2H Pull-down</td>
<td>143</td>
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<tr>
<td>ZmPIP2;5</td>
<td>ZmSYP61 (At1g28490)</td>
<td>Syntaxin</td>
<td>Pull-down, BiFC, Split-Ub</td>
<td>96</td>
</tr>
<tr>
<td>AtPIP2;7</td>
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<td>Syntaxin</td>
<td>Pull-down, BiFC, Split-Ub</td>
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</tr>
<tr>
<td>AtPIP2;7</td>
<td>TSP0 (At2g47770)</td>
<td>Tryptophan-rich sensory protein/translocator</td>
<td>Pull-down, BiFC</td>
<td>98</td>
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<td>SIRK1 (At5g10020)</td>
<td>LRR-receptor-like kinase family</td>
<td>Pull-down</td>
<td>318</td>
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<tr>
<td>GmNOD26</td>
<td>Soybean GS1 β1, β2, γ1, γ2</td>
<td>Glutamine synthase</td>
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<td>Cucumber mosaic virus 1a</td>
<td>Y2H, SRS, co-IP</td>
<td>133</td>
</tr>
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</table>

The table lists all plant aquaporin-interacting proteins that have been individually validated. The molecular function of each partner is indicated. The methods used to demonstrate a protein-protein interaction are also shown: FRET, fluorescence resonance energy transfer; FLIM, fluorescence-lifetime imaging microscopy; BiFC, bimolecular fluorescence complementation; Split-Ub, split-ubiquitin; Y2H, yeast two-hybrid; SRS, Sos recruitment; co-IP, co-immunoprecipitation.
**B. ROS Detoxification and Signaling**

Consistent with the ability of TIPs to transport H$_2$O$_2$ in yeast (29), transgenic overexpression (312) or inactivation of these aquaporins (258) can confer plant tolerance or sensitivity to oxidative stress, respectively. Among ROS, H$_2$O$_2$ has a relatively long lifetime and its import by TIPs into the vacuole may efficiently contribute to ROS detoxication. This implies, however, that a detoxication machinery is expressed in vacuoles. Up to now, most of these systems have been characterized in the cytoplasm.

PIP2s can also have a significant H$_2$O$_2$ transport capacity (66), and the expression of some of these aquaporins is reduced by H$_2$O$_2$ itself (105). Considering that H$_2$O$_2$ produced in the apoplasm by combined NADPH-oxidase and superoxide dismutase activities can serve as a signaling intermediate, a role of PIPs in plant cell signaling is very likely. Such signaling role, which has been established for mammalian aquaporin-1, aquaporin-3, and aquaporin-8 (28), still awaits experimental demonstration in plants. Guard cells, which respond to ABA through H$_2$O$_2$ production, may represent an interesting model to address this issue.

**C. Vacuolar Storage**

The permeability of tonoplast aquaporins to compounds such as NH$_3$ (164) or urea (86, 160) has suggested a role for TIPs in vacuolar storage and remobilization of nitrogen metabolites. However, this role could not be validated in planta using reverse genetic approaches (164). In addition, plant membranes are equipped with other transport systems that mediate a more specific and vectorial transport of NH$_3$/NH$_4^+$ and urea. Thus the role of TIPs in vacuolar partitioning of nitrogen metabolites seems at most minor.

**D. Chloroplast Transport**

Because of its roles in cell bioenergetics and photosynthesis, the plant chloroplast is a unique organelle in eukaryotic cells (FIGURE 8). One key reaction is water oxidation to molecular oxygen that occurs in the thylakoid lumen. It was calculated that this consumption of water molecules represents 60- to 170-fold the thylakoid lumen volume per day (18). Although NMR measurements indicate a moderate diffusional water permeability of the thyla-
koid membrane, a minute osmotic gradient (≈3 mosM) might be sufficient to support the steady uptake of water required for thylakoid lumen refilling (18). Thus aquaporins may be dispensable during this process. In contrast, the thylakoid lumen shows fast and marked volume increase in response to light, which may be facilitated by aquaporin activity.

CO₂ is another potentially relevant substrate of aquaporins in the chloroplast. Several PIPs and TIPs have been tentatively localized in the inner envelope and thylakoid membranes. They may contribute to water transport into the stroma and thylakoid lumen. In the latter compartment, photosynthesis leads to oxidation of water to molecular oxygen. Changes in incident light can result in adjustments of thylakoid and overall chloroplast volumes. A role of PIP1s in CO₂ transport across the inner envelope membrane is also indicated. Carbon fixation occurs in the stroma through carboxylation reactions within the Calvin-Benson cycle. Finally, ROS can be formed as by-products of photosynthetic activities. A putative role of PIP and TIP in facilitating H₂O₂ export from the chloroplast is shown.

CO₂ is another potentially relevant substrate of aquaporins in the chloroplast. Several physical and biochemical components of plant cells, such as cell wall composition and tortuosity, unstirred layers and carbonic anhydrase activities in the vicinity of membranes, or CO₂ permeability of the membranes themselves possibly restrict CO₂ diffusion throughout plant cells (69). The chloroplast envelope may represent a critical barrier, limiting carboxylation reactions performed in the stroma through the Calvin-Benson cycle. Interestingly, the CO₂ permeability of chloroplast envelopes purified from tobacco leaves was fivefold lower than that of PM vesicles and was reduced by ≈90% after antisense inhibition of NtAQP1 in transgenic tobacco (295). It is now critically needed that these data are extended to other plant materials.

Finally, aquaporins located on the chloroplast envelope may help dissipate ROS produced in the chloroplast under high-light conditions through H₂O₂ transport into the cytosol (207).
VI. WHOLE PLANT WATER TRANSPORT AND TRANSPIRATION

A. Context

Terrestrial plants establish a continuum of water between the soil and the atmosphere (273) (FIGURE 1). One illustration is plant transpiration which drives an ascending flow of water, its intensity being primarily determined by the hydraulic resistances crossed along the soil-plant-atmosphere continuum. The hydraulic processes operating at the soil-root (rhizosphere) and leaf-air (stomata) interfaces are crucial during transpiration but will not be discussed here, as they do not involve cell membranes and aquaporins. This section rather focuses on water transport within the plant. The functional characterization of aquaporins has brought a strong momentum to earlier studies on this topic (273), building up on their physical concepts and providing molecular and cellular insights into previously described physiological controls of cell and tissue hydraulics (46, 189).

Water transport in inner plant tissues is indeed targeted by multiple regulations, which tend to stabilize the plant water status and provide means for adaptation of the plant to its environment. For instance, the water permeability of roots (root hydraulic conductivity; $L_p$) is highly dependent on the soil content in water, nutrients, and oxygen, whereas leaf hydraulics is sensitive to air humidity and light regime. Temperatures and diurnal rhythms also exert a general impact on plant hydraulics. The coordinated transcriptional regulation of aquaporins by hormonal and environmental factors (5, 34, 116) and abiotic stresses in particular (92, 172, 340) has provided a first hint at the general role of aquaporins during these processes. As detailed below, multiple posttranscriptional mechanisms provide additional means for fine tuning aquaporins and water transport in roots and leaves.

B. Root Water Transport

1. Structure-function analyses

In all higher plants, root water uptake is mediated by radial (centripetal) transport from the soil into xylem vessels, through the epidermis, cortex, endodermis, and stele tissues (FIGURE 9A). Once in vessels, water (xylem sap) flows axially towards the plant shoots. During radial transport, water can flow along cell wall structures (apoplastic path) or from cell to cell, along cytoplasmic continuities formed by plasmodesmata (symplastic path) or across cell membranes (transcellular path). A so-called composite model of the root was developed to formalize water transport along this

![FIGURE 9. Water transport and aquaporin expression in roots. A: schematic representation of an Arabidopsis root cross section (pink, epidermis; blue, cortex and endodermis; green, pericycle; brown, stele and xylem vessels). Water flows through the cell-to-cell and apoplastic paths are represented by blue arrows. B–D: radial and longitudinal cuts of Arabidopsis roots expressing AtPIP1;2-GUS (B; Ref. 230), AtPIP2;2-GUS (C; Ref. 119) or AtPIP2;1-GUS (D; Ref. 226) transgenes, which allow expression of β-glucuronidase (GUS) reporter gene under the control of the indicated PIP promoters. These pictures show how various aquaporin isoforms exhibit different expression patterns although all are expressed in the stele. Bars = 50 μm (B), 50 μm (C), or 25 μm (D).]
network (274). The model can account for water transport under transpiring conditions, when hydrostatic driving forces (pressures and tensions) predominate, or under root pressure conditions when water transport is mostly driven by solute transport and osmotic forces. The model also explains how water transport paths and therefore \( L_p \) can vary depending on the plant water transport regime (274). While most studies have considered that water flows in root tissues along water potential gradients, by diffusion through cell walls, lipids, or water channels, a role of solute transporters for localized uphill water pumping has recently been hypothesized (316).

Aquaporin blockers, and mercury in particular, have been used as convenient tools for testing the contribution of aquaporins to the composite model of root water transport. Mercury inhibition of \( L_p \) indicated that the relative importance of the transcellular (aquaporin-dependent) path can vary between plant species and is predominant in tomato or \textit{Arabidopsis}, where it represents 57% and up to 64% of \( L_p \), respectively (175, 275). At present, plant aquaporin pharmacology remains highly limiting, all inhibitors described being potentially toxic and exerting numerous secondary effects. Nevertheless, a higher reliability can be obtained when inhibitors with different modes of action (mercury versus weak acids or azide) yield similar estimates of the aquaporin-dependent path (275).

Aquaporin expression patterns can also provide useful hints at the contribution of water channels to root water transport. These patterns have been investigated in roots of many plant species with most accurate descriptions in \textit{Arabidopsis} (83, 119, 230) (FIGURE 9, B–D) and cereals such as rice (251), maize (97), and barley (136). In addition to their agronomical importance, cereals offer an interesting context for studying root function, with seminal and shootborne roots showing distinct morphologies and functions. Aquaporin expression studies in roots have focused on PIPs, which supposedly play a crucial role in plasma membrane and transcellular water transport, and TIPs, which show high expression levels in roots. For instance, the \textit{Arabidopsis} root expresses at least seven PIP and six TIP isoforms with specific expression patterns, the latter being expressed in all tissues except the vasculature and the root tip meristem (83). In all plant species examined, very precise and distinct cell-specific expression patterns could be established for the numerous aquaporin isoforms present in roots (97, 251). Overall, the data stress the importance of controlling local hydraulic properties all along the radial pathway, with preferential expression of some isoforms in the exodermis or endodermis (97). Apoplastic barriers are formed in these two cell layers, through lignin deposition at Casparian strips and subsequent suberization of the whole apoplast, thereby creating specific limitation for water transport. In addition, high expression of some other isoforms in the stele is consistent with centripetal transport of water towards the root vasculature, whereby water flows have to be mediated by reduced exchange surfaces (80, 230) (FIGURE 9, B–D). Aquaporin expression profiles were also shown to vary along the root axis, indicating that the radial pathway itself varies during root growth and differentiation. In some cases, these studies were coupled to water transport measurements in well-defined root zones (80, 136). Recent comparisons of water transport and aquaporin expression patterns in woody perennial and herbaceous plants have indicated distinct root hydraulic differentiation profiles between these types of plants (80). In grapevine for instance, the radial pathway seems to be extremely tight in differentiated root segments, due to the formation of a periderm, suggesting that soil water is taken up through root tips, mostly.

In complement to expression analyses, reverse genetics have brought unequivocal evidence for the contribution of PIPs to \( L_p \) (119, 226, 230, 267). Although of modest amplitude (10–20% change in \( L_p \)), significant water transport phenotypes could be observed in single knockout mutants. Yet, these studies have remained restricted to a few isoforms and a genetic redundancy between close homologs has to be considered (226). Thus we still lack a comprehensive molecular view of root hydraulics, whereby each isoform may be associated with water transport at specific cell sites and under a given type of force.

Modeling of root water transport, along concentric cell layers and the root axis, or in distinct root subtypes (37, 137), can provide a significant support to these functional and genetic analyses. These modeling studies indicate that the site of water absorption (root tips versus whole root) may vary between species. A future challenge will be to integrate root hydraulic functioning in a soil to apprehend all kinetic and spatial refinement of water uptake in a natural environment (64). These studies should not be restricted to herbaceous species. For instance, the use of natural caves revealed that aquaporins of live oak and gum bumelia (\textit{Sideroxylon lanuginosum}) contribute to root water uptake deep into the soil (18–20 m) where they can respond to seasonal and diurnal rhythms (190).

2. Water stress

Direct exposure of roots to water stress usually results in inhibition of aquaporin activity and water transport at the cell and whole organ levels (34, 43, 99, 227, 275). Yet, some plant varieties or natural accessions show opposite responses with an enhancement of root cell hydraulic conductivity (99, 275). The effects of external (106, 176) or endogenous ABA (223) are also contrasting, during time and between species. For instance, ABA transiently enhances cell hydraulic conductivity in maize root cells (106), whereas it inhibits \( L_p \) in aspen (311). These observations highlight the variety of hydraulic strategies in response to soil drying. Whereas increased \( L_p \), during the early phase of
drought could help optimize the capture of soil water resources, a long-term inhibition provides a more conservative mechanism for the plant, to prevent a reverse flow of water, from the plant root into a dry soil. Regulation of whole root water transport can also have a dramatic hydraulic impact on leaves, to favor or reduce growth or act on stomatal aperture (transpiration) (see sect. VIIA3).

It is now required that we go beyond classical and abrupt water stress settings used in laboratories. In particular, the heterogeneity of soil water content in natural conditions will have to be considered more carefully in future experimental studies, to understand how plant roots sense soil water deficit and manage soil water on the long term. Partial root-zone drying in a riparian tree (Melaleuca argentea) resulted in threefold enhancement of root hydraulic conductance in the wet soil portion, with a slight increase in PIP1 expression, by a signaling mechanism that remains to be identified (191). Also, heterogeneities in local hydraulic conductivities could interfere in water redistribution (hydraulic lift) between soil layers varying in water content (40).

3. **Nutrient availability**

Root water transport and aquaporin activities are extremely sensitive to nutrient availability in the soil. Plant deprivation in most macronutrients (phosphorus, nitrogen, or sulfur) leads over a couple of days to a decrease in \( L_p \) (42, 62) through aquaporin downregulation (FIGURE 10). It is assumed that these regulations may favor whole water uptake in conditions favorable to plant growth. Considering possible soil heterogeneities, these regulations may also enhance nutrient drag to the root in soil zones that are the richest in nutrients. At variance with these effects, potassium (\( K^+ \)) starvation enhances \( L_p \) (157, 236). The modes of aquaporin regulation by nutrients seem to be complex since they involve mixed effects on aquaporin expression and phosphorylation (62, 88, 157, 313).

The signaling mechanisms that mediate these effects are as yet unknown but clearly involve hormone responses. Thus ethylene was shown to mediate the inhibiting effects of phosphorus starvation on \( L_p \) in Medicago falcata (151), whereas ABA enhanced \( L_p \); stimulation by \( K^+ \) starvation in sunflower (236). It will also be important to explore the respective contributions of local and systemic signals in these regulations (87).

4. **Other soil signals**

Both low temperatures and oxygen deprivation in the soil exert a general inhibiting effect on root water uptake through decrease of cell and whole root hydraulic conductivities. Cold exerts complex up- and downregulating effects on PIP expression in roots (144, 329). ROS and calcium, which accumulate in these conditions, and aquaporin phosphorylation may provide complementary mechanisms for cold-induced \( L_p \) regulation (144, 145). In rice, a long-term (2–5 days) exposure of roots to low temperature induced a compensatory increase in \( L_p \), due in large part to enhanced expression of OsPIP2;5 (2). Interestingly, this induction required that the shoot was maintained both at a control temperature and in its integrity, suggesting that a shoot-to-root signal was involved (see sect. VIB6).

Soil flooding and compaction prevent oxygen diffusion, thereby creating severe anoxic stress, especially onto actively growing root tips. This stress results in a strong metabolic imbalance, with a marked cytosolic acidosis, which in turn mediates \( H^+ \)-dependent gating of PIPs (287). The long-term effects of anoxia are mediated through a general inhibition of aquaporin gene expression (156). Interestingly, the AtNIP2;1 gene shows a strong induction in Arabidopsis roots under anoxic stress. This aquaporin, which transports lactic acid, may facilitate the leak into the soil of this fermentation product, thereby preventing excessive cell acidification (50).

5. **Diurnal and circadian rhythms**

In relation to diurnal changes in transpiration, many plant species show a peak in \( L_p \), during the day (103, 250). This regulation can be accounted for by an increase in root aqua-
porin transcripts during the early phase of the day (163, 279, 298) and a slightly delayed peak in protein abundance (99, 250). Experiments under constant light and/or in circadian clock mutants have shown that at least in maize and Arabidopsis, these oscillations are under circadian control (39, 279). In maize, the amplitude of these oscillations is dramatically amplified by previous exposure of plants to water-limiting conditions, in the air or in the soil. This climatic control would optimize water uptake in moderate dry soils, by preserving rhizosphere hydration (39). Roots are also able to directly sense light. For instance, the photoreceptor phytochrome A controls the enhancement of At-TIP2;2 expression during adaptation of the Arabidopsis root to darkness (296).

6. Transpirational demand and other shoot-to-root signals

Recent studies in rice and poplar (142, 250) have established a direct role of the transpirational demand in \( L_P \), regulation. This was made possible by independent manipulation of several factors affecting transpiration (i.e., light or relative air humidity) to discard primary effects of these factors on aquaporin functions. In both plant species, transpiration triggered a dramatic increase in \( PIP \) gene expression in roots.

Identifying systemic signals conveyed from the shoots to regulate root hydraulics represents one of most exciting questions in this field. Several studies have identified context to address this topic. For instance, shoot topping in grapevine, soybean, and maize was shown to reduce \( L_P \) and root aquaporin activity and expression in a few tens of minutes (299). Whereas auxin or phloem-borne signals seem to be excluded, hydraulic signals transmitted through the xylem are more likely. In aspen (Populus tremuloides) seedlings, defoliation results in adjustments of both leaf and root hydraulics. The decrease in \( L_P \), observed after 1 day was associated with downregulation of a major \( PIP1 \) isoform (159). In wheat, partial root excision did not alter stomatal conductance nor transpiration but increased after 1.5 h the hydraulic conductivity of the remaining root by severalfold, in relation to enhanced accumulation of ABA in this organ (307).

C. Leaf Water Transport

1. Hydraulic resistances in the leaf

Following root uptake, the soil water enriched in nutrients is transported as sap to the plant shoot through xylem vessels formed of dead cell structures. Except under severe drought (49), xylem transport in the stem is nonlimiting. In contrast, the leaf represents, in addition to the root, a major checkpoint for plant water transport. In fact, the hydraulics of inner leaf tissues is designed for efficient sap delivery through a network of fine xylem vessels down to substomatal chambers, where water evaporates and is transpired through the stomatal pores.

Leaf hydraulics is therefore determined by vascular (xylem) and extravascular resistances, the respective contributions of which vary according to plant species and leaf morphology (233). The extravascular pathway is formed of parenchyma cells packed along the vessels, surrounded by a tight bundle sheath and by the mesophyll, which shows a looser compaction with numerous lacunas (FIGURE 11A). Similar to roots, water can flow in leaves along the apoplastic path or from cell to cell.

The contribution of aquaporins to leaf water transport has been dissected using approaches similar to those used in roots. Expression studies revealed a high aquaporin abundance in the elongating zone of cereal leaves, in the vascular bundles of most plant species and to a lesser extent in the mesophyll (23, 95, 232) (FIGURE 11B). Reverse genetic analysis in Arabidopsis identified three \( PIP \) isoforms that contribute to leaf hydraulics (230, 232). Several lines of evidence indicate that, in Arabidopsis at least, the veins (xylem parenchyma and bundle sheath cells) rather than the mesophyll are hydraulically limiting. First, all PIPs contributing to rosette hydraulic conductivity shared a common expression in the vascular bundles (232). Second, the water permeability of protoplasts purified from the veins but not from the mesophyll showed a regulation by light and ABA that paralleled that of the whole leaf (232, 264). Third, complementation, using vein-specific expression of \( At-PIP2;1 \), of a \( Atpip2;1 \) knockout mutant lacking light-dependent regulation of leaf hydraulics was sufficient to restore a wild-type response (232).

2. Signals

Leaf hydraulics is controlled by numerous environmental or hormonal signals, which most often establish a functional coupling between regulation of inner leaf water transport and stomatal movements. For instance, ABA inhibits inner leaf water transport by downregulating aquaporin activity in bundle sheath cells (264). These effects are physiologically consistent with an additional and direct induction of stomatal closure by the hormone. More generally, a strong correlation between leaf hydraulic and stomatal conductances and expression of a TIP2 homolog was observed in grapevine leaves under both water sufficient and water stress conditions (231).

Besides water stress, the light regime is the other signal that dominates leaf hydraulic regulation. In most plant species, leaf hydraulic conductance is maximal during day time, and a circadian control was established in sunflower (210). In several tree species including walnut (Juglans regia), pedunculate oak (Quercus robur), and common beech (Fagus sylvatica), light-dependent leaf hydraulic conductance showed
a good correlation to PIP gene expression (15, 51). In Arabidopsis, in contrast, quantitative proteomics revealed that the diphosphorylated form of AtPIP2;1 was closely linked to changes in rosette hydraulic conductivity under varying light or dark regimes (232). The ability of a phosphomimetic but not of a phosphodeficient form of AtPIP2;1 to restore the light response of an Atpip2;1 knockout mutant established that phosphorylation of this single isoform is necessary for light-dependent regulation of leaf hydraulics.

Crassulacean acid metabolism (CAM) allows some plants to adapt to low-water environment by day/night variation in CO₂ uptake and fixation. In leaves of ice plant, the osmotic water permeability of purified protoplasts and expression of three PIPs and a TIP isoforms peaked at the end of the light period, together with accumulation of CAM cycle metabolites. Thus diurnal regulation of aquaporins may favor cell osmoregulation and leaf water balance throughout the CAM cycle (302).

3. Guard cells and leaf movements

Stomatal aperture is constantly adjusted through reversible changes in guard cell volume and plays a major role in controlling plant transpiration. Whereas a role for aquaporins in stomatal movement has long been hypothesized, supporting evidence has remained scarce, rather suggesting a role in stomatal closure. For instance, overexpression of a Vicia faba PIP1 in Arabidopsis accelerated the stomatal response to dark and ABA (55). In sunflower guard cells, a TIP isoform showed a diurnal peak in expression that coincides with stomatal closure (256). With regard to the plethora of information on hormone signaling and membrane transport regulation in guard cells, getting a better understanding of aquaporin function in these cells is now becoming critical.

Other types of movements in leaves seem to be associated with circadian regulation of aquaporins. In rain tree (Sama nea saman), such regulation contributes to circadian variation of osmotic water permeability in motor cells and as a consequence to diurnal leaf movements (205). Similarly, epinastic movement of tobacco leaves is dependent on circadian expression of a PIP1 homolog (NtAQPI) in the petiole (266).

4. Embolism repair

Long-distance transport of water in vascular plants can be dramatically impeded by the formation of embolisms in xylem vessels, following tissue freezing during winter (249) or under extreme drought, when intense transpiration and limiting water supply result in high tension within the vessels (165). Changes in aquaporin expression in relation to embolism recovery and inhibition of this process by mercury have suggested that aquaporin-facilitated water transport can contribute to embolism refilling (165, 249, 260). Accordingly, transgenic poplar and Arabidopsis with reduced expression of PIPs were more susceptible to drought-induced embolisms and showed reduced recovery response of whole plant water conductance upon rewetting, respectively (182, 259). Yet, the modes of water and solute cotransport to allow embolism refilling in tissues under strong water tensions are still debated (104, 316).
5. Water uptake

In some very specific physiological contexts or species, leaves can substitute for roots in taking up external water. In atmospheric epiphytes for instance, water absorption of air moisture through trichomes can provide a strong adaptation to drought (218). In conifers, leaf absorption of melting snow may support embolism refilling after winter (141). In both cases, a role of aquaporins in water equilibration within leaf tissues was suggested by aquaporin expression patterns or pharmacological inhibition.

D. Perspectives

1. Molecular dissection of plant tissue hydraulics

Despite an expected genetic redundancy within the aquaporin family, a few studies have identified single aquaporin isoforms that, under laboratory conditions, significantly contribute to root or leaf hydraulics (56, 119, 226, 230, 232). These advances will hopefully encourage a more thorough genetic dissection of plant tissue water transport. Also, a special focus should be put onto aquaporin function in cell layers that are thought to be hydraulically limiting, such as the endodermis and exodermis in roots or the bundle sheath cells in leaves. For instance, a crucial role of the aquaporin pathway in suberized root tissues has been hypothesized in most text books but was only investigated in a handful of studies (80, 238). One critical approach in the future may be to use tissue-specific promoters for cell sorting or directed expression of aquaporins (232, 244, 264).

Most importantly, the identification of master regulators of aquaporins in these cell contexts will be critically needed. In the future, quantitative genetics of plant hydraulics in plants under control or stress conditions (275) may provide a privileged access to these regulators. For now, and as outlined below, the dissection of aquaporin regulation networks using the power of transcriptomic and proteomic approaches provides more operational strategies.

2. Transcription factors and transcriptional networks

A myriad of literature reports describe how environmental, developmental, or hormonal signals interfere with water relations in many plant species, and consistently act on regulation of individual aquaporins. It is now required to go beyond these case-by-case studies and access a unified understanding of the mechanisms involved and of their integration in whole plant responses.

With respect to aquaporin gene regulation, significant progress can be expected from genomic studies. Several studies have addressed regulation of the whole aquaporin family (5, 34, 92, 116, 252, 340), and gene coexpression networks may reveal how regulation of individual aquaporins connects with that of other isoforms or great physiological functions (4). Another important direction will be to identify transcription factors that act as master regulators of aquaporin genes. A role for water stress responsive factors belonging to the DREB (237) and ASR1 (240) families was recently established. Regulation of aquaporin expression by additional ethylene (225) or abiotic stress responsive (325, 341) transcription factors is also emerging. In addition, plant aquaporins are predicted targets of miRNAs in cotton (333) and potato (335). The impact of these and other epigenetic regulations on plant water relations has not yet been evaluated.

Similar advances are foreseen at the protein level. Protein interaction network comprising aquaporins (53, 123) should reveal as yet unknown functional interactions and help identify the interplay of aquaporins with novel cellular functions.

3. General role of phosphorylation

Maintaining the plant water status under ever changing light, temperature, or water availability requires constant cell and tissue hydraulic adjustments. Aquaporin phosphorylation provides a crucial means for such rapid and reversible regulation, without the costs associated with protein synthesis and degradation. Quantitative proteomics in Arabidopsis roots has revealed a general correlation across abiotic and nutritional stimuli between Lp, and aquaporin phosphorylation (62). This type of approach (200, 232, 234) will have to be refined for identifying the most relevant signals and aquaporin isoforms involved in roots and other organs. Most importantly, the putative regulations will have to be functionally validated by expression in transgenic plants of phosphomimetic and phosphodeficient forms of the aquaporin isoform involved (232).

Exploring upstream signaling events also represents an important challenge for future studies. A pioneering work recently identified SIRK1, a protein kinase involved in plant responses to sugar and targeting PIPs (318). Hypothetical phosphorylation cascades triggered by ethylene and negatively regulating aquaporin phosphorylation have recently been modeled (326). Yet, tremendous work remains for identifying the numerous protein kinases and protein phosphatases that likely regulate aquaporins. ABA-dependent phosphorylation may be of central importance (135), but we still ignore the signaling components involved.

VII. PLANT GROWTH AND DEVELOPMENT

A. Hydraulics of Plant Growth

1. Fundamental principles and role of aquaporins

Plants show continuous apical growth of their roots and shoots, through active cell multiplication in meristems and
subsequent cell expansion. These growth processes are remarkably plastic, providing efficient means for long-term adaptation of plants to their environment. The plant water and nutrient status in particular exert multiple controls on these processes.

Plant cell expansion has been formalized in the so-called Lockhart model. It is primarily driven by the intracellular positive pressure (turgor) and mostly restricted by the cell wall that yields beyond a turgor threshold (54). Since cell expansion requires a steady water intake, the model also predicts that the rate of tissue growth can be restricted due to a drop in turgor, when water delivery, through transcellular transport from the vasculature, encounters significant hydraulic resistances. These ideas have been validated by measuring growth-associated water potential gradients (36). They provide a frame for understanding the role of aquaporins in plant tissue growth. Accordingly, aquaporins were shown to be strongly expressed in tissues that can be hydraulically limiting during growth, in barley or maize leaves or hypocotyls of Arabidopsis or castor oil plants (Ricinus communis) (68, 95, 166, 317). Aquaporin expression in expanding tissues of barley leaves was also associated with a high cell hydraulic conductivity (306).

A direct assessment of the role of aquaporins in tissue growth is experimentally challenging. It was attempted using mercury, which reversibly inhibited root tip growth in maize and embryo expansion in germinating broad bean seeds (110, 215). A hydraulic control of growth can also be revealed at the whole plant level, when water supply from roots becomes limiting for leaf growth. A role of aquaporins in this control was established by pharmacological inhibition of root water transport using a panel of treatments (mercury, acid loads, H$_2$O$_2$) with distinct modes of action (39). These findings indicate that water availability in the soil and improve plant performance appreciably, thereby favoring plant water transport and preventing stomatal closure.

Whereas PIPs can play an obvious role in transcellular water transport towards expanding tissues, vacuolar aquaporins (TIPs) also seem critical for plant growth. In Arabidopsis for instance, expression of a specific TIP isoform (AtTIP1;1) was associated with cell expansion (166) and regulated by the growth-promoting hormone gibberellic acid (GA$_3$) (228). Overexpression in the same plant of a ginseng TIP ortholog enhanced overall plant growth through increased leaf cell size (154). It has been suggested that TIPs critically contribute to osmoregulation and vacuolar differentiation in expanding cells. Yet, the enhancement of tobacco protoplast division after TIP overexpression has remained unexplained (219).

### 2. Particular developmental processes in shoots and roots

Auxin was recently found to potently downregulate the tissue-specific expression and function of most root aquaporins. This regulation shed light onto a new role of aquaporins in root growth, to favor the emergence of secondary roots (226). In brief, physiological analyses in plants with various genetic alterations in PIP function were coupled to mathematical modeling to show that a fine spatial and temporal control of PIP expression favors water entry in the lateral root primordium. This flow is made at the expense of overlaying cells, thereby reducing their mechanical resistance and facilitating lateral root emergence. These results are crucial since they reveal for the first time a link between the capacity of the root to transport water and its ability to grow and potentially adjust its architecture to water availability.

A role for aquaporins in local stimulation of growth has also been proposed in gravistimulated rice leaves. Accumulation of GA$_3$ on the abaxial side was shown to promote expression of a PIP gene (OsRWC3) thereby favoring local growth and leaf bending (108).

### 3. Growth of plants under water deficit

Water deficit usually results in leaf growth arrest, in part because downregulation of root or leaf hydraulic conductance induces hydraulic limitations for growth (67, 223). Yet, there is a large array of responses to water deficit across all plant species. For instance, some cultivated plants such as maize or tomato exhibit an ABA-induced aquaporin upregulation, to enhance soil water uptake and whole plant conductance and maintain plant growth under mild water deficit (39, 223). Under water-limiting conditions, the so-called isohydric plants protect the leaf water status at midday through a tight stomatal regulation. In contrast, anisohydric plants have a less conservative strategy and favor gas exchange and photosynthesis at the expenses of water consumption. As long as water resources are not sharply limiting, the latter plants can exhibit better growth performance than the former. Transgenic tomato plants ectopically expressing a TIP gene or grapevine cultivars differing in drought tolerance (246, 298) have revealed that enhanced aquaporin activity typically confers an anisohydric behavior by favoring plant water transport and preventing stomatal closure.
matal closure. These phenomena certainly involve the general link found in many species, between root water transport \((L_{P_w})\) and transpiration (142) (see sect. VIB6).

**B. CO₂ Fixation**

1. **Mesophyll conductance to CO₂**

Strikingly enough, the plant photosynthetic capacity, and therefore plant growth, are primarily limited by CO₂ delivery from the atmosphere to the sites of carboxylation in the chloroplasts (314). Whereas stomatal conductance \((g_s)\) accounts for gas diffusion through the stomatal pore, the mesophyll conductance to CO₂ \((g_m)\) corresponds to the subsequent transport of CO₂, from the substomatal chamber to the chloroplast stroma (74, 314). Whereas stomatal conductance \((g_s)\) can differ by more than 10-fold between species. It also varies according to physiological conditions such as water availability or atmospheric CO₂ (69). Several molecular and cellular entities such as cell walls, carbonic anhydrases, or aquaporins have been proposed to contribute to \(g_m\) (69). Each of these components may therefore underlie the genetic and physiological variations of \(g_m\) in plants.

2. **Role of aquaporins**

Several lines of converging evidence indicate that aquaporins significantly contribute to \(g_m\). For instance, a 60–70% inhibition of \(g_m\) by millimolar concentrations of mercuric chloride was reported in broad bean \((Vicia faba)\) and French bean \((Phaseolus vulgaris)\) leaves (283). Genetic studies in transgenic tobacco (75, 293, 295), rice (100), or Arabidopsis (101) with enhanced or reduced PIP functions have revealed parallel variations in \(g_m\). Finally, it was proposed that the dependency of \(g_m\) to atmospheric CO₂ reflects a contribution of PIPs to \(g_m\). High CO₂ would result in cell acidification, which in turn would reduce \(g_m\) through pH-dependent gating of PIPs (73).

3. **Open questions**

Although a physiological role of PIPs in leaf CO₂ transport is emerging, this idea is not fully settled yet and requires a few notes of caution. First, the methodology for measuring \(g_m\) and its CO₂ dependency, in particular, is still disputed (73). Second, the \(g_m\) variations observed after pharmacological or genetic manipulation of PIP functions are larger than those anticipated for the membrane path (69). Finally, some genetic studies are difficult to interpret because of confounding effects on stomatal gas exchanges \((g_s)\) that would indirectly alter \(g_m\). In these respects, ABA has recently been identified as a dual regulator of \(g_s\) and \(g_m\) (196), but its mode of action on \(g_m\) is as yet unknown. Thus studies where PIP genetic manipulation allows to uncouple effects on \(g_m\) and \(g_s\) are more reliable (261).

In any case, the role of aquaporins in CO₂ transport certainly represents a very exciting and significant field of research. Some biological membranes seem to have lower than expected permeability to CO₂, which would call for a significant contribution of membrane channels (125). More specifically, aerial plants have to achieve a critical tradeoff between water conservation and carbon fixation, and aquaporins might be at the center of these mechanisms. Future studies on this topic will surely shed light onto the molecular bases of \(g_m\) and of its physiological regulations.

**C. Nutrient Allocation and Toxicity**

1. **Boron**

Boron \((B)\) is necessary for plant growth. It is taken up from the soil and deposited in tissues to reinforce cell walls. Arabidopsis plants strongly react to B limiting conditions by decreased root length, burst in root hair development, and strong induction of the AtNIP5;1 gene (278) (FIGURE 12). Reverse genetic studies have shown that this aquaporin is central for root B uptake and plant growth under B limiting conditions. Maize tsl1 plants mutated in a probable AtNIP5;1 ortholog also show growth defects in these conditions, with altered root growth and reduced size of the shoot apical meristem (65). In Arabidopsis, the AtNIP6;1 and AtNIP7;1 homologs also serve in B transport and mediate its allocation to shoots and pollen, respectively (148, 281). Additional aquaporins may be involved in B transport and, for instance, a role of XIPs in deposition of B within growing tissues has been proposed in tobacco (26).

Whereas B is an essential micronutrient, an excess in the soil can be toxic for the plant. Thus the activity of some NIPs can become detrimental for plant growth. For instance, a quantitative trait locus for B toxicity in barley was associated with elevated expression of a NIP homolog in roots of the parental line showing the highest sensitivity to high B (257).

2. **Silicon**

Silicon \((Si)\), an abundant element of the soil, can accumulate in some plants (mostly cereals), to promote their growth and resistance to abiotic and biotic stresses. In particular, Si was shown to alleviate drought stress in sorghum, by enhancing root water uptake and aquaporin activity (161). Elegant genetic studies in rice have revealed a role for several NIP homologs \((OsNIP2;1/Lsi1) and OsNIP2;2/Lsi6) in Si uptake and allocation to shoots (169, 323). As for B, Si transporting NIPs function as efflux channel. They mediate Si transcellular transport by coexpression with secondary active transporters involved in Si import. Similar Si transport equipment exists in maize (195).
3. Other metalloids

Other metalloids than B and Si, such as arsenic (As), antimony (Sb), or selenium (Se), naturally occur in soils and are absorbed by plants. When entering the food chain, through contamination of crops or drinking water, As and Sb are highly toxic for humans. In contrast, Se is beneficial for human health. At high levels, As and Sb compounds are also toxic for the plants. Consistent with the ability of NIPs to transport arsenous acid in heterologous expression assays, direct genetic studies have revealed a central role for At-NIP1;1 in arsenite sensitivity of Arabidopsis (127). Accumulation of As in rice seeds was shown to be mediated by Si transporters including OsNIP2;1 (Lsi1) (170). Interestingly, this toxic accumulation could be counteracted by an excess of Si.

OsNIP2;1 also mediates selenous acid uptake in rice (337). Due to these multiple roles, members of the NIP subfamily seem to play a crucial role in plant health and food quality. Analyzing their selectivity profile and tissue specific expression is therefore crucial to understand how accumulation of toxic compounds can be avoided, especially in edible parts of crops.

D. Plant Reproduction

Two desiccated forms of plant life, pollen and seeds, play a central role in the life cycle of higher plants and in their dissemination. In contrast to other plant organs, pollen and seeds express specific aquaporin isoforms of the TIP5 and TIP3 subclasses, respectively (271, 301, 320) (FIGURE 13). This specificity may be due to the highly specialized growth and germination processes observed in these organs. More generally, plant reproduction offers striking examples of specialized cell water transport.

1. Flowers

The sophisticated beauty and function of flowers owe much to aquaporins. For instance, the blue sepal color of hydrangea (Hydrangea macrophylla) grown in acidic soil is due to a vacuolar accumulation of aluminum (Al) complexed with anthocyanins. To reach the vacuole, Al is transported as Al(OH)₃ through the plasma and vacuolar membrane, by a NIP and a TIP aquaporin, respectively (211). It is as yet unclear whether and how TIPs and NIPs work in concert with other Al transporters of the NRAMP family to mediate Al homeostasis in other plant organs (321).

Flowers are also remarkable by their blooming and diurnal movements. These processes require highly controlled cell expansion processes. Accordingly, PIP1s and PIP2s were found to play an interacting role during ethylene-dependent expansion of rose petals (47, 171). In addition, the opening and closing of tulip sepals induced at normal (20°C) and low (5°C) temperature, respectively, were associated with reversible phosphorylation of PIP homologs (13).
In flowers, the reproductive function itself requires a tight control of tissue desiccation, involving aquaporins at various stages. For instance, anther dehydration is necessary for dehiscence and release of mature pollen and was hampered in tobacco plants with reduced expression of PIP2s (32). The formation of pollen grains is accompanied by a progressive dehydration, whereas its imbibition triggers germination through dramatic growth of the pollen tube. Arabidopsis plants invalidated for two pollen TIPs specific of the vegetative and sperms cells, respectively, showed reduced fertility under limited water or nutrient supply (320). However, the modes of water transport from the receptive papilla into dry pollen and was hampered in tobacco plants with reduced expression of PIP2s (32). The formation of pollen grains is accompanied by a progressive dehydration, whereas its imbibition triggers germination through dramatic growth of the pollen tube. Arabidopsis plants invalidated for two pollen TIPs specific of the vegetative and sperms cells, respectively, showed reduced fertility under limited water or nutrient supply (320). However, the modes of water transport from the receptive papilla into dry pollen and the possible role of plasma membrane aquaporins during pollen tube growth remain as yet unknown (178, 269). The numerous reproductive development defects of the maize B transport mutant tsl1 (65) indicate that, in addition to water, micronutrients such as B also are critical for proper growth and function of flowers.

2. Seeds

Seed maturation is accompanied by a profound reorganization of the vacuolar apparatus, with formation of numerous protein storage vacuoles. Although genetic evidence is lacking, the expression of seed-specific TIPS is thought to be crucial during this process (82, 276, 301). Studies in developing bean seeds also revealed a specific aquaporin equipment, for phloem-mediated import of water and nutrients into developing coat, and for water recycling in the xylem and its delivery to cotyledons (339).

Seed germination is triggered by a rapid imbibition of desiccated tissues and shows a second phase of water uptake associated with embryo growth. Mercury inhibition experiments have indicated that aquaporins may be limiting during the first phase of water uptake in species such as pea (305), whereas in Arabidopsis or broad bean, they would contribute to embryo growth (215, 301). The transition from protein storage vacuoles to a large central vacuole, with a shift from TIP3 to TIP1 expression, also seems crucial during this process.

Despite the presumed importance of aquaporins in seeds, we note that, so far, genetic evidence is only available in rice. In this species, the knockout and/or overexpression of OsPIP1;1 and OsPIP1;3 was shown to alter both the rate and speed of germination (155, 158). Interestingly, the two genes are under the control of nitric oxide. The control of seed aquaporin function by osmotic or hormonal signals will deserve more efforts in future research.
VIII. BIOTIC INTERACTIONS

A. Rhizobium-Legume Symbiosis

Besides plant responses to abiotic stimuli, aquaporins also serve in plant biotic interactions. The best described examples are root symbiosis.

Nitrogen is, together with potassium and phosphorus, a major macronutrient of plants. It is most often limiting for plant growth, hence the intensive use of nitrogen fertilizers in agriculture. Legumes have acquired the remarkable capacity to develop symbiotic root nodules able to fix atmospheric nitrogen (N$_2$). For this, specific soil bacteria (Rhizobiaceae) infect plant root cells and differentiate into intracellular bacteroids, each surrounded by a plant symbiosome membrane (292). During root nodule differentiation in barrel clover (Medicago truncatula), a TIP1 homolog was shown to be transiently retargeted from the tonoplast to the symbiosome membrane, this process being necessary for functional nodule formation (84). The fully differentiated symbiosome membrane then expresses NIPs which transport both water and NH$_3$ and likely play a dual role in cell osmoregulation and nitrogen assimilation. One of these NIP homologs, named nodulin-26 and present in soybean root nodule, was actually the first aquaporin identified in plants. Nodulin-26 molecularly interacts with cytosolic glutamine synthase (184). Upon import of NH$_4^+$/NH$_3$ into the plant cytosol by paths that still need to be unambiguously identified, this molecular interaction may optimize fixation of NH$_4^+$ onto glutamate, to prevent a feedback leak of NH$_4^+$/NH$_3$ to the peribacteroid space through nodulin-26.

B. Mycorrhizae

Mycorrhizae represent a very common symbiotic interaction between soil fungi and plant roots. Depending on the two partners, the plant interface with the fungus is either intracellular (arbuscular mycorrhizae) or extracellular (ecto-mycorrhizae). In all cases, the mycelia optimize soil exploration and nutrient capture, whereas the plant root provides carbon metabolites for nutrient assimilation. Besides plant mineral nutrition, mycorrhizae seem to ameliorate plant water relations and stress responses. For instance, numerous reports indicate that, both in crops (maize, bean, soybean, lettuce) and trees (poplar), mycorrhizae can enhance plant tolerance to drought, flooding, cold, or salinity stress (10, 17, 41, 179, 229). However, the molecular and physiological bases of these effects are not fully elucidated yet. For instance, root water transport and overall aquaporin expression were enhanced in poplar, whereas an opposite response was seen in soybean and lettuce (179, 229). In the latter two species, arbuscular mycorrhizae may help the plant anticipate root responses to stress. In maize, inter-active effects of mycorrhizae with plant ABA enhance root aquaporin inhibition and water conservation under drought (243).

The role of plant aquaporin-mediated solute transport in arbuscular mycorrhizae was recently examined (17). Gene profiling indicated that, in symbiotic roots, glycerol export from the plant to the microbe, and NH$_4^+$/NH$_3$ import into the plant, for storage and detoxification, may be enhanced through activation of proper plant aquaporin genes. In contrast, the transport of B and Si would be downregulated. Indeed, the B released by the fungus can be toxic for the plant, and Si is known to antagonize mycorrhizal infection.

A current challenge is now to characterize the aquaporin equipment of fungi, a task that was accelerated through fungal genome sequencing (63, 149, 322). Similar to their plant counterparts, aquaporins of arbuscular or ecto-mycorrhizal fungi such as Glomus intraradices or Laccaria bicolor, respectively, appear to play a crucial role in both water and nutrient transport. An ultimate challenge will be to understand the molecular dialog between plant and fungal cells, and how this coordinates expression of aquaporins and complementary transport systems between the two partners (9). Even though the underlying mechanism are not known, it was recently shown that genetic manipulation of aquaporin expression in Laccaria bicolor alters expression of the plant host aquaporins (322).

C. New Directions: Investigating Beneficial or Pathogenic Interactions

Recent studies on plant root symbiosis have revealed how aquaporins can contribute to a functional dialog between transport proteins of distinct organisms. Yet, many other biotic interactions remain to be explored to ultimately apprehend the full ecological integration of aquaporin functions.

For instance, the plant-growth-promoting rhizobacterium Bacillus megatorium exerts ameliorative effects on plant salt tolerance. Accordingly, it enhances Lp, in maize and modifies aquaporin expression in both control and salt stress conditions (183). Yet, the precise modes of aquaporin regulation and the role of bacterial signals, possibly auxin, remain unknown. The plant hormone methyl jasmonate also represents an important signal in plant defense. It was shown to enhance aquaporin activity in roots of French bean, tomato, and Arabidopsis through interaction with calcium- and/or ABA-dependent signaling pathways (253).

The effects of pathogenic infection on aquaporin expression and their significance in plant disease also deserve specific attention. One of most striking example is the induction of a tobacco TIP1 expression in giant root cells induced upon nematode infection (220). This induction may favor the
osmoregulation of these cells that serve as feeding sites for the pathogen. Infection of soybean leaves by *Pseudomonas syringae* resulted after 8 h downregulation in 24 of 32 aquaporin genes (342), but the significance of this regulation was not assessed.

**IX. AQUAPORINS AND PLANT GENETIC IMPROVEMENT**

**A. Success Stories and Failures**

Water relations and mineral nutrition represent major traits in crop improvement. Aquaporins, which provide genetic and molecular tools to act on these traits, are therefore attractive targets for plant molecular breeders (180). Genetic manipulation of PIPs, and NIPs has now been explored in many plant species, with varying rates of success.

Transgenic strategies aimed at altering aquaporin function have first been developed in herbaceous species, leading to contrasting effects on plant growth and stress response. Ectopic expression of an aquaporin in a heterologous plant species seems hazardous, since the fine regulations that govern each individual isoform in the native plant may not work in a distinct transgenic species. Yet, overexpression of a *Vicia faba* PIP1 (VvPIP1) or a wheat PIP2 (TaAQP7) in *Arabidopsis* and tobacco, respectively, enhanced plant drought resistance (55, 338). Identification of stress-regulated aquaporin isoforms and subsequent genetic manipulation in the same species seems to be a more solid approach. For instance, constitutive expression of a stress-responsive TIP2 of tomato (STIP2;2) enhanced the growth performance of transgenic tomato plants under both normal and water stress conditions by favoring their anisohydic behavior (246). OsPIP1;3 (RWC3) was initially identified as stress-induced in a upland rice cultivar. Its expression in a lowland rice cultivar, using an engineered stress-induced promoter, conferred on transgenic plants drought avoidance properties (such as maintenance of leaf water potential) reminiscent of those observed in the upland cultivar (153). In rice again, tolerance of root and leaf growth to salt stress and ameliorated germinative properties of seeds were obtained upon constitutive expression of OsPIP1;1, provided that the transgene was expressed at a moderate level (155).

In addition to these few success stories, the literature abounds with more mitigated reports. These works are informative with respect to aquaporin physiology but represent clear failures in terms of biotechnology. For instance, tobacco plants overexpressing *Arabidopsis* ATPIP1;2 grew better than control plants in normal conditions but became dramatically more sensitive to water deprivation (3), probably because of a stomatal deregulation. Expression of cucumber and figleaf gourd aquaporins in *Arabidopsis* had either beneficial or detrimental effects on plant survival and seed germination, whether plant where subjected to a dehydration (mimicked by PEG application) or a salt (NaCl) stress (117).

By comparison to herbaceous crops, the genetic improvement of woody plants poses special difficulties due to slower growth and longer reproduction cycles. Yet, with the emergence of genomic tools and reverse genetics, aquaporin functions and the potentialities of their genetic alteration are now being explored in these species. For instance, overexpression of a root PIP (VvPIP2;4N) in transgenic grapevine (227) enhanced root hydraulics, transpiration, and shoot growth, and therefore water consumption, under control conditions, whereas the plant exhibited a normal water conservation response under water stress. In poplar, RNAi was recently used as an efficient means for downregulating expression of several PIP1 homologs. Whereas no major phenotype was observed in transgenic trees with fast growth under no stress conditions, trees under water stress unfortunately showed severe defects in recovery from embolism (259, 261). *Eucalyptus* is another forest tree of great use in the paper industry. Aquaporin function was explored in the species using transgenesis (288). Finally, targeting ethylene-regulated aquaporins in *Hevea* may help improve latex production (289).

**B. Mechanisms**

The phenotype of plants with genetically altered aquaporins is usually complex to decipher, as it integrates far more than direct effects of the manipulated aquaporin on tissue hydraulics or carbon fixation. For instance, several studies have revealed that ectopic expression of a foreign aquaporin gene can perturb the expression pattern of endogenous aquaporin genes, thereby resulting in unpredictable beneficial or detrimental effects on plant growth and stress responses (117, 288). Heteromerization of an artificially expressed aquaporin with endogenous ones may also result in dominant negative effects on plant water transport (270). In addition, altered water relations can influence other physiological responses to stress, which in turn contribute to most of plant phenotype. For instance, PIP overexpression in grapevine may have disturbed water stress sensing in root, thereby enhancing ABA synthesis, specifically under water stress (227). These hormonal effects likely explain why water conservation was improved in these conditions (227). At high concentrations, sodium (Na⁺) is toxic for plants. Controlling its accumulation and compartmentalization is therefore critical in plants under salt stress. In *Arabidopsis*, overexpression of a wheat NIP homolog (81) or a TIP from the halophyte *Thelungiella salsuginea* (312) reduced salt loading or favored its vacuolar accumulation, respectively. In both cases, plant stress tolerance was improved. ROS metabolism, which is central to plant stress responses, can also be altered after aquaporin genetic manipulation. Overexpression of a wheat PIP2 homolog (TaAQP7) in tobacco resulted in enhanced superoxide dismu-
tase and catalase activities through an unknown mechanism (338), thereby contributing to drought tolerance. Conversely, an *Arabidopsis* line inactivated for *TIP1;1* and *TIP1;2* showed a reduced catalase activity and higher anthocyanin content that somewhat mimicked a response to high light. This phenotype was tentatively associated with the capacity of the two TIP homologs to channel H$_2$O$_2$ (258). In most studies, information on root and shoot hydraulics, but also leaf morphology, stomatal density, and shoot/root ratio is lacking to fully interpret how aquaporin manipulation can interfere with plant growth properties. Yet, elegant grafting experiments using wild-type and transgenic tobacco showed that overexpression of *NtAQP1* in roots was sufficient to sustain a high transpiration in wild-type shoots. In contrast, the beneficial effects of *NtAQP1* on stomatal conductance and photosynthesis required its expression in shoots (245).

**C. Targeting Micronutrients**

Besides water relations and growth under drought or salt stress, the plant micronutrient status represents another, easier target for aquaporin genetic manipulations, which, however, has barely been explored. Thus enhanced expression of *Ar*NIP5;1 and *Ar*TIP5;1 in *Arabidopsis* improved plant tolerance to low B and high B, respectively (129, 222). In the latter case, the effects were likely due to facilitated storage of B in the vacuole. Reduced expression of NIPs that transport B at the plasma membrane should also enhance plant tolerance to high B toxicity (257). We note however that, as for water transport, ectopic expression of aquaporins transporting B and Si can lead to detrimental effects on growth. Thus organ- or tissue-specific expression seems to be crucial, to prevent mislocalization of these micronutrients (129, 201).

**X. CONCLUSIONS AND PERSPECTIVES**

**A. Conclusions**

Two decades after their discovery, it is now established that aquaporins play a broad role in plant physiology. Genetic and physiological studies, in support of gene expression data and functional characterization in heterologous systems, have revealed that, despite their multiplicity, plant aquaporins can individually fulfill multiple functions. For instance, some PIP1s seem to play a dual role in water and CO$_2$ transport in *Arabidopsis* and tobacco (75, 230, 264, 267). In the former species, a single PIP2 isoform (*At*PIP2;1) is involved in root water uptake, lateral root formation, and water transport in leaf veins (226, 232). Therefore, a next challenge will be to dissect, at least for a few representative isoforms, the cell specificity of aquaporin functions and regulations. One direction would be targeted proteomics, to access cell-specific aquaporin phosphorylation profiles and interacting partners. Transgenic plants expressing cell-type specific reporters (232, 264) and aquaporin knockout plants with cell-specific complementation (232, 244) will be crucial in these studies. Beyond these generic approaches, we believe that a few, more specific topics will deserve a specific attention.

**B. Emerging Research Directions**

1. **Aquaporins and plant cell signaling: interplay with ROS and hormones**

Through their multiple transport functions and regulations, aquaporins emerge as critical nodes that integrate cell metabolism and signaling into whole plant responses to environmental and hormonal signals. The interplay of aquaporins with ROS appears as a particularly central topic. On the one hand, ROS mediate the effects of salt, salicylic acid, and cold (33, 145) on water transport and aquaporin regulation in roots. Yet, we do not understand why ROS have contrasting effects in bean (21) and *Arabidopsis* roots (35). On the other hand, the capacity of plant aquaporins to transport H$_2$O$_2$ points to emerging roles in cell signaling and ROS detoxification. The former has been established in animals (192), and the latter is supported by recent studies in transgenic plants. The regulation of aquaporins by hormones in plants under environmental stress will also deserve more attention. For instance, ethylene may play a crucial role for water transport regulation during anoxia (126) or potassium starvation. Finally, the circadian regulation of aquaporins and the significance of hydraulics as an input or output signal of the clock will represent an exciting challenge in coming years (39, 279). Mutants such as *early flowering 3* will allow investigating how aquaporin function is coupled to the circadian clock (279). In addition, understanding the synchronizing role of light but also ABA on oscillating aquaporin functions may provide clues on how plant growth is optimized according to soil water ability and transpiration demand (39, 279).

2. **Role of intracellular aquaporins**

Whereas it definitely pertains to original plant specific functions, the role of intracellular aquaporins has remained a kind of black box. Since reverse genetics of TIPs and SIPs has failed to reveal macroscopic phenotypes (19, 174, 258), cellular phenotypes will have to be inspected in closer detail. In particular, an accurate monitoring of compartmental volumes in osmotically challenged cells will be required to establish the putative role of these aquaporins in cell osmoregulation. Also, the actual substrates of these aquaporins are as yet undetermined. It was suggested that SIPs may transport ethylene across the ER membrane (174) but, to our knowledge, this hypothesis has not been further explored. Finally, the role of PIPs in CO$_2$ transport across the chloroplast envelope is indicated by a single study in tobacco (295) and definitely deserves more attention.
3. Exploring more diverse plant systems

While initially performed in a few plant model species (Arabidopsis, tobacco) or crops (maize, rice), studies on aquaporins are now expanding to more and more plant species. Plant biodiversity is definitely worth being further explored. In particular, extremophile plants such as resurrection plants or halophytes (177, 300, 312) may reveal novel physiological regulations. In ice plant, aquaporin function is dramatically changed during the salt-induced switch from C3 to CAM metabolism (134, 302). Original information is also expected from unicellular photosynthetic organisms such as Synecocystis (12) or Chlamydomonas reinhardtii (138), from mosses (154) or representative species of early evolved plants (8). In particular, recent studies in horsetail (Equisetum arvense), a primitive vascular plant that was identified for its remarkably high Si content, allowed identification of a new subfamily of aquaporin Si channels (89).

By delineating ever-expanding fields in plant integrative biology, aquaporins have been fascinating research objects over the last two decades. This is quite not finished: we have outlined here a few research directions which, we believe, are particularly relevant and have been somewhat neglected. We are confident that these and other directions will provide exciting discoveries and establish further the crucial role of aquaporins in plants.

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