Molecular Physiology of Aquaporins in Plants

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In plants, membrane channels of the major intrinsic protein (MIP) super-family exhibit a high diversity with, for instance, 35 homologues in the model species *Arabidopsis thaliana*. As has been found in other organisms, plant MIPs function as membrane channels permeable to water (aquaporins) and in some cases to small nonelectrolytes. The aim of the present article is to integrate into plant physiology what has been recently learned about the molecular and functional properties of aquaporins in plants. Exhaustive compilation of data in the literature shows that the numerous aquaporin isoforms of plants have specific expression patterns throughout plant development and in response to environmental stimuli. The diversity of aquaporin homologues in plants can also be explained in part by their presence in multiple subcellular compartments. In recent years, there have been numerous reports that describe the activity of water channels in purified membrane vesicles, in isolated organelles or protoplasts, and in intact plant cells or even tissues. Altogether, these data suggest that the transport of water and solutes across plant membranes concerns many facets of plant physiology. Because of the high degree of compartmentation of plant cells, aquaporins may play a critical role in cell osmoregulation. Water uptake in roots represents a typical process in which to investigate the role of aquaporins in transcellular water transport, and the mechanisms and regulations involved are discussed.

KEY WORDS: Aquaporin, Major intrinsic protein, Osmoregulation, Permeability, Plant, Plasma membrane, Root, Vacuole, Water transport.

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I. Introduction

Terrestrial plants critically depend on a supply of water for their growth and development and have evolved elaborate strategies to achieve water balance in the most adverse environments. This amazing capacity of plants has long been recognized, and there has been extensive physiological and biophysical research on the water relations of plants over the last decades (Boyer, 1985; Steudle, 1994). Despite this interest, the concept of water channels has not always been popular. After being discussed in the late 1950s (reviewed in Maurel, 1997), the strong case made by Dainty (1963) on artifacts due to unstirred layers convinced plant scientists that water channels can scarcely be detected in plant membranes and that they may be marginal for the physiological needs of plants. Thus the concept of water channels has long remained controversial in plant sciences and used only by a few biophysicists who referred to theoretical aqueous pores in model plant membranes (reviewed in Maurel, 1997).

Recent advances in the molecular characterization of membrane proteins, however, have triggered a very rapid evolution in this area of research. Members of the major intrinsic protein (MIP) super-family have now been found in nearly all living organisms and shown to function as channels permeable to water (aquaporins) and/or certain small nonelectrolytes (Agre *et al.*, 1998). In 1993, the first plant aquaporin was functionally characterized (Maurel *et al.*, 1993). The diversity of MIP proteins in plants, their presence in multiple subcellular compartments and their regulated expression throughout plant development and in response to environmental stimuli, have been the object of many reports. Thus, aquaporins have entered many fields of plant biology and revealed that water transport underlies many facets of plant cell functions (Maurel and Chrispeels, 2001; Santoni *et al.*, 2000). While it is widely admitted that aquaporins play an important role in plant water relations (Johansson *et al.*, 2000; Maurel and Chrispeels, 2001; Tyerman *et al.*, 1999), the central challenge remains, however, to understand the real physiological significance of these proteins.

The aim of the present article is to integrate into plant physiology what has been learned recently about the molecular and functional properties of aquaporins in plants. The expression properties of aquaporins and more generally of MIP homologues (Section II) will be used to guide the reader through some of the typical features of plant cell and tissue organization. For instance, the subcellular localization of MIPs highlights the high degree of compartmentation of plant cells, whereas tissue-specific expression of these proteins sets the stage for the intricate water transport processes which occur in plants. Toward the end of the 1990s, it has become possible to connect these expression studies to functional evidence for water channels in organelles, cells, and tissues (Section III). The link is not yet firmly established, but provides a new basis for addressing the integrated function of aquaporins in plants (Section IV).

II. Expression of Plant Aquaporins

A. Cell Level: Aquaporins and the High Degree of Compartmentation of Plant Cells

1. The High Diversity of MIPs in Plants

During the last few years, the number of *MIP* genes reported in the data base steadily increased, and plant genes largely contributed to this inflow. *MIP* genes have been identified in more than 30 plant species, including monocots and dicots. Plant genomes also appear to characteristically encode a high number of MIPs (Tyerman *et al.*, 1999; Weig *et al.*, 1997). For example, 14 full-length or partial MIP cDNAs have been molecularly characterized in the halophytic species, *Mesembryanthemum crystallinum* (Kirch *et al.*, 2000). The genomic sequence of the model species *Arabidopsis thaliana* has now been completed (Arabidopsis Genome Initiative, 2000), thus allowing for the first time a complete count of *mip* genes in a higher eukaryote. Recent data base searches run in our laboratory indicated at least 34 full-length MIP-like sequences in *Arabidopsis*. Their sequence relationship is presented in the dendrogram of Fig. 1. The encoded proteins fall into three main classes, based on their amino acid sequence: tonoplast intrinsic proteins (TIPs), plasma membrane intrinsic proteins (PIPs), and Nodulin-26-like intrinsic proteins (NIPs) (Weig *et al.*, 1997). An additional class, provisionally named small basic integral proteins (SIPs), has been proposed by Johanson and Kjellbom (2000).

Plant MIPs share 27–97% amino acid sequence identity with homologues in the same plant subfamily, but this homology can drop to ~20% when members of two distinct subfamilies are compared. Typical sequence signatures can also be defined to distinguish between plant MIPs of distinct subfamilies. For instance, Schäffner (1998) showed that the N-terminus of PIPs is more extended than that of TIPs, and that amino acid motifs in the N-terminus and the 2nd and 5th extramembrane loops (B and E) allow unambiguous discrimination between the two subfamilies. The PIPs themselves can be subdivided into the PIP1 and PIP2 subclasses (Kammerloher *et al.*, 1994; Schäffner, 1998) (Fig. 1).

The NIP subfamily of plant MIPs is formed by proteins sharing high homologies with soybean Nodulin-26 (NOD26), hence their name. NOD26 is an aquaglyceroporin, which transports water and glycerol in the peribacteroid membrane of N_2-fixing symbiotic root nodules (Dean *et al.*, 1999; Fortin *et al.*, 1987). An increasing number of NIPs has been identified and, for instance, eight homologues are present in the *Arabidopsis* genome (Fig. 1). The function, cellular localization, and expression pattern of NIPs in species that do not differentiate N_2-fixing symbiotic nodules are totally unknown. The SIP sequences have been identified *in silico*, but the genes and their products have not been experimentally characterized. In

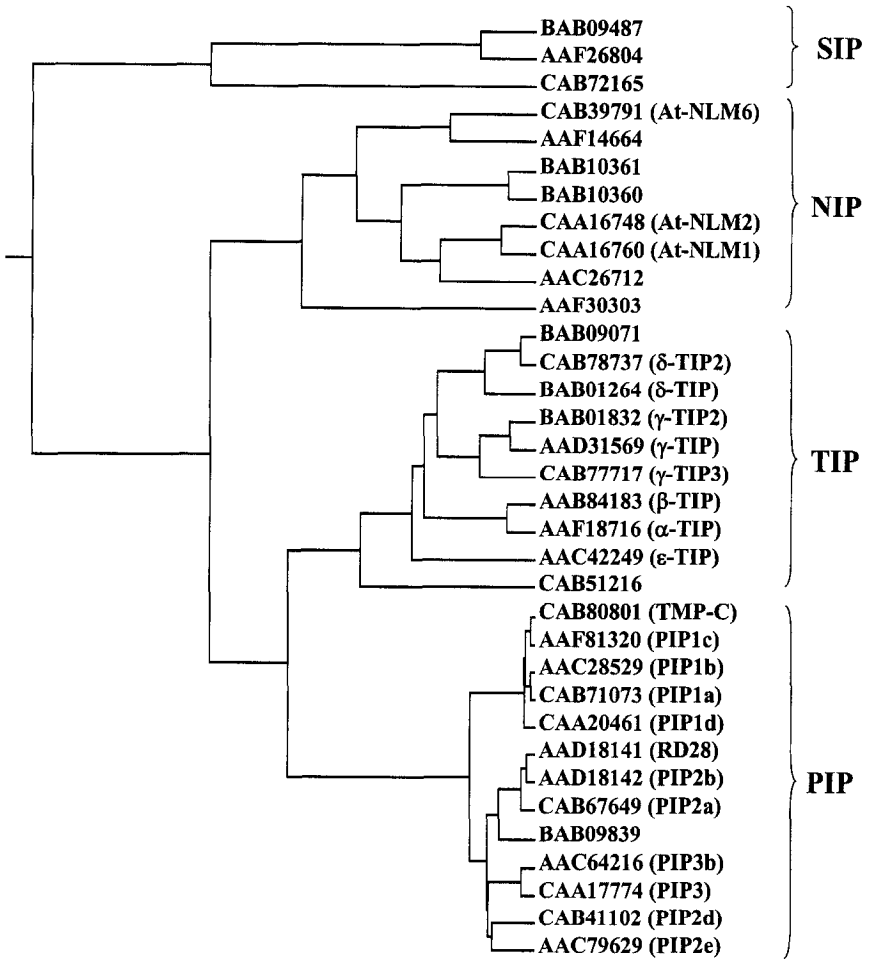

FIG. 1 Phylogenetic tree of Arabidopsis MIPs. MIPs were identified by Blast searches in the *Arabidopsis thaliana* genomic sequence (Arabidopis Sequence Initiative, 2000). Proteins are indicated by their accession number (with their common name in parentheses if the protein or cDNA was already described elsewhere). The tree was generated using the ClustalW software of Thompson *et al.* (1994). Clustering patterns are indicated as discussed in the text. SIP: small basic integral proteins; NIP: Nodulin-26-like intrinsic proteins; TIP: tonoplast intrinsic proteins; PIP: plasma membrane intrinsic proteins.

general, cDNAs of only some of the MIPs identified in *Arabidopsis* (see Table I on pages 113–115.) or other plant species have been described. An even more limited number of them have been characterized as water or solute channels by functional expression in *Xenopus* oocytes or yeast, and the precise allocation of *in planta* functions of the various genes is still awaited.

Plant MIPs possess all typical structural properties of MIPs identified in other organisms with a molecular mass of 25–30 kDa, and an internal homology between the N and C terminal halves, thus defining six putative membrane-spanning domains. The repetition of a Asn-Pro-Ala (NPA) motif in the two halves of the protein has long been taken as an unambiguous signature for MIPs, but exceptions to this rule have now been identified in plant NIPs and SIPs. Despite these variations, the Asn and Pro residues remain perfectly conserved, in agreement with a role proposed for these residues in water permeation and in stabilization of the aqueous pore, respectively (Fu *et al.*, 2000; Murata *et al.*, 2000). Recent molecular structure analyses of two plant aquaporins, bean seed α-TIP and spinach leaf PM28a (Daniels *et al.*, 1999; Fotiadis *et al.*, 2001) confirmed that these proteins have an overall molecular design that resembles that found in MIPs from animals or microorganisms (Fu *et al.*, 2000; Murata *et al.*, 2000; see Engel and Stahlberg, this volume). In particular, projection maps of 2D crystals obtained by cryoelectron microscopy indicated a typical tetrameric structure, with each monomer delineating an individual pore.

2. Differential Expression of TIPs and PIPs in Subcellular Membrane Compartments

a. Vacuolar Localization of TIPs. TIPs were initially identified because of their abundance in the vacuolar membrane, and specific isoforms have been purified and biochemically characterized from bean seeds (Johnson *et al.*, 1989), radish roots (Maeshima, 1992), or spinach leaves (Karlsson *et al.*, 2000). The tonoplast (TP) localization of these and other TIP isoforms in maize, tobacco, *Arabidopsis*, or ice plant has been assessed by immunocytolocalization studies or by immunodetection of the protein in purified subcellular membranes (Daniels *et al.*, 1996; Höfte *et al.*, 1991, 1992; Kirch *et al.*, 2000). Cross-reactivity of an antiserum raised against a TIP-enriched fraction from sugar beet with plasma membranes (PM) of pea cotyledons has been reported, but the specificity of the antibody was not established (Robinson *et al.*, 1996a).

While early studies showed consistent TP localization of all TIPs examined, even more exquisite subcellular localization of these proteins has been recently accomplished. Antibodies that can discriminate between distinct TIP isoforms have been used in pea root tips (Paris *et al.*, 1996), *Mimosa pudica* motor cells (Fleurat-Lessard *et al.*, 1997), or barley aleurone cells (Swanson *et al.*, 1998) to show the existence of vacuolar subtypes which may fulfill distinct functions within the same plant cell. This view was recently refined in work by Jauh *et al.* (1998, 1999). They showed that combinatorial expression of three basic types of TIP isoforms (i.e., α-TIP, δ-TIP, and γ-TIP) participate in the differentiation of a vast array of vacuolar subtypes. Specific expression of γ-TIP in a compartment containing the protease aleuraine would be typical of large lytic

vacuoles in vegetative tissues. The expression of δ-TIP would rather be associated with vacuoles accumulating vegetative storage proteins, whereas α-TIP would be expressed in protein storage vacuoles (PSVs), when present in conjunction with δ-TIP or δ-TIP and γ-TIP. Expression of α-TIP alone would be typical of autophagic vacuoles (Jauh *et al.*, 1999). It is not clear, however, whether TIPs can only be taken as markers of intracellular membrane differentiation or whether they determine to a large extent the functional specialization of vacuolar subtypes.

The poor specificity of the anti-aquaporin polyclonal antibodies used in some early studies may, however, question some of the proposed interpretations. Paris *et al.* (1996) used a serum raised against a TP fraction purified from beet roots that recognized not only a major TIP isoform in this membrane but also larger molecular weight (MW) products (Jauh *et al.*, 1999). The specificity of anti-aquaporin antibodies has been improved, and monoclonal antibodies and affinity-purified antibodies raised against oligopeptides were used in more recent studies (Jauh *et al.*, 1998, 1999; Kirch *et al.*, 2000). Although the cross-reactivity of the antibody with respect to other homologues was checked, it remains unclear how these tools can differentiate between very close homologues within the same subfamily and, most importantly, what is the relevance of these antibodies for detecting putative homologues in distant plant species. These homologues may have different cellular and subcellular expression patterns (Bethke and Jones, 2000).

b. Localization of PIPs. Initial studies on the localization of PIPs in *Arabidopsis* using membrane fractions purified by aqueous two-phase partitioning suggested that these proteins were mostly expressed in the PM (Daniels *et al.*, 1994; Kaldenhoff *et al.*, 1995; Kammerloher *et al.*, 1994). The subcellular localization of three PIPs (MIP-A, MIP-B, MIP-C) in *M. crystallinum* has recently been investigated by immunodetection of the proteins in membrane fractions separated by sucrose density gradient centrifugation (Barkla *et al.*, 1999; Kirch *et al.*, 2000). Surprisingly, three distinct patterns were revealed using antibodies raised against each of the proteins. In particular, proteins immunoreactive to anti-MIP-A antibodies were totally absent from the PM fraction but abundant in a fraction of intermediate density, distinct from the TP and PM. MIP-C was weakly detected in the PM but strongly in TP. These experiments questioned the general idea that PIPs are mostly expressed in the PM. The presence of PIPs in intracellular membranes may not be surprising and may simply reflect their routing to the PM throughout the secretory system. Barkla *et al.* (1999) and Kirch *et al.* (2000) have hypothesized, however, the existence of a vesicle shuttling mechanism which, like that described for AQP2 in the kidney (Sasaki *et al.*, 1998), would allow a regulated exposure of PIPs at the cell surface.

In another study (Robinson *et al.*, 1996b), antibodies raised against *Arabidopsis* PIP1 allowed the immunolocalization of PIP homologues in convoluted invaginations of *Arabidopsis* leaf PM. These structures called plasmalemmosomes may

favor direct exchanges between vacuole and apoplasm. Alternatively, they may correspond to the cycling of endomembrane vesicles between the cell surface and the cell interior. But here again, dynamic evidence for this model in plants is lacking, and signals that allow a regulated trafficking of aquaporins in plant cells remain to be identified.

In contrast to the use of polyclonal antibodies which possibly cross-react with close aquaporin homologues, expression of aquaporins fused to reporter proteins such as green fluorescent protein (GFP) in transgenic plants provides an unambiguous approach for localization of a well-identified aquaporin isoform. Cutler *et al.* (2000) made random fusions between plant proteins and GFP with the aim of identifying labeling markers of specific subcellular structures. Among proteins that yielded typical labeling of the PM or the TP, a majority (3/5 for the PM; 3/3 for the TP) were isoforms of PIPs and TIPs. GFP fusions have also been used to determine the subcellular localization of two PIP homologues in maize (Chaumont *et al.*, 2000). Expression of the fusion proteins was detected at the cell surface but substantial labeling of a perinuclear compartment, corresponding most probably to the endoplasmic reticulum (ER), was also observed.

c. How Do TIPs and PIPs Reach Their Respective Compartments? Because they form a family of membrane proteins with distinct subcellular localization properties, aquaporins provide interesting models for investigating the molecular bases of membrane protein biogenesis and targeting in plants cells. However, these studies have proved difficult because aquaporins are polytopic membrane proteins, the mutation of which can deeply interfere with insertion and stability within the membrane. Nevertheless, Höfte and Chrispeels (1992) showed that a region containing the sixth transmembrane domain of bean α-TIP with or without the cytoplasmic tail is sufficient to allow localization of a reporter protein in the TP of transformed tobacco cells. Jiang and Rogers (1998) dissected these mechanisms in more detail and studied two TIP isoforms, γ-TIP and αTIP, which are targeted to lytic vacuoles and PSVs, respectively. The cytoplasmic tail of α-TIP was able to redirect a chimeric construct containing the plant vacuolar sorting receptor BP-80 fused to a reporter protein from the lytic vacuole to PSVs, whereas the cytoplasmic tail of γ-TIP was without effect. They concluded the existence of two sorting pathways for plant TP membrane proteins which would contribute to the biogenesis of distinct vacuolar compartment in plant cells. One pathway would direct proteins to lytic vacuoles via the Golgi complex, whereas the second path would be a direct route from the ER to PSVs.

B. Tissue Level

Because they showed a high expression level, a specialized tissue-specific expression pattern, and/or responsiveness to hormonal or environmental factors, many

MIP genes of plants were identified and their expression described, well before the function of their products was understood (Yamaguchi-Shinozaki *et al.*, 1992; Yamamoto *et al.*, 1991). Tables I and II summarize studies performed in *Arabidopsis* and other species, respectively, and show that data on MIP expression in more than 30 plant species are available. It should be stressed, however, that, in most early studies, the existence of an extended multigene *MIP* family had not been suspected and that the specificity of the antibodies and cDNA probes used was not assessed. It is now clear that full-length cDNAs do cross-hybridize with mRNAs of close MIP homologues of the same subfamily. For instance, the five *PIP1* genes in *Arabidopsis* share >75% nucleotidic sequence homology. The 5′ and 3′ untranslated ends of aquaporin genes may not even allow the distinction of very close homologues. The poor specificity of some of the anti-aquaporin antibodies used in plants has been discussed above. Thus, depending on the technique or the probe used for the experiments, the specificity of the expression profile should be considered with caution.

Because of their function in water uptake, roots have been investigated in detail with respect to aquaporin expression. Reporter gene activities (i.e., GFP or GUS) driven by aquaporin promoters, aquaporin mRNAs or proteins have been detected in root tips (TobRB7: Yamamoto *et al.*, 1991), epidermis and root hairs (ZmTIP1: Barrieu *et al.*, 1998a; MipA: Kirch *et al.*, 2000), cortex (MipA: Yamada *et al.*, 1995), endodermis (ZmTIP1: Barrieu *et al.*, 1998a; MipA: Yamada *et al.*, 1995), and in the stele (TobRB7: Yamamoto *et al.*, 1991). The various cell types present in roots have distinct sizes and cytoplasmic density, which makes a comparison of their respective aquaporin expression levels difficult. Yet, studies in *Arabidopsis* and in the ice plant (Schäffner, 1998; Yamada *et al.*, 1995) suggested that PIP homologues would have an expression level higher in the stele than in the cortex. This is consistent with the uptake path of water that converges from the root surface toward the stele for mobilization into the whole plant.

Consistent with this, high expression levels of several PIP and TIP aquaporins in the vascular tissues of roots and shoots have been reported in many plant species. For instance, ZmTIP was expressed in the parenchyma cells that surround the early and late metaxylem of maize roots and stems (Barrieu *et al.*, 1998a), whereas a δ-TIP homologue of sunflower accumulated in root phloem tissues (Sarda *et al.*, 1997, 1999). In *Arabidopsis* leaves, PIP1b (AthH2) was strongly expressed in the parenchyma cells around vascular bundles and within the bundles themselves, in the protoxylem and protophloem (Kaldenhoff *et al.*, 1995). These expression patterns suggest a critical role of transmembrane water transport in loading and unloading phloem and xylem vessels, for continuous circulation between the two types of vessels, and in multidirectional transport of sap throughout the plant.

Although the expression of some aquaporins such as *Arabidopsis* γ-TIP or spinach So-δ-TIP is strictly excluded from dividing cells (Karlsson *et al.*, 2000; Ludevid *et al.*, 1992), specific expression of other isoforms in meristematic cells

TABLE I
Expression Properties of MIP Homologues in *Arabidopsis*

MIP homologue[a]	EST[b]	Expression in tissues	Dependence on stimuli[c]	Technique[d]	Ref.[e]
BAB09487	+				
AAF26804	+				
CAB72165	+				
CAB39791 (At-NLM6)	+				
AAF14664	+				
BAB10361	−				
BAB10360	−				
CAA16748 (At-NLM2)	+				
CAA16760 (At-NLM1)	+	Seedling	*Down:* ABA, NaCl, dark, dehydration; *Stable:* mannitol	QP	(1)
AAC26712	−				
AAF30303	+				
BAB09071	+				
CAB78737 (δ-TIP2)	+	Root		SB (cDNA)	(1)
BAB01264 (δ-TIP)	+	Vascular tissues of shoots		GUS	(2)
		Leaf, bolt	*Down:* mannitol; *Stable:* ABA, NaCl, dark, dehydration	SB (cDNA), QP	(1)
BAB01832 (γ-TIP2)	+	Leaf > root and bolt		SB (cDNA)	(1)
AAD31569 (γ-TIP)	+	Hypocotyl, root, vascular tissues, leaf elongating cells		GUS, IH (cDNA)	(3)
		Root, stem, leaf, flower		SB (cDNA), ID (γ-TIP)	(4)
		Stem, flower > root > leaf	*Up:* GA3	NB (3′ cDNA end)	(5)
		Root, bolt	*Down:* mannitol; *Stable:* ABA, NaCl, dark, dehydration	SB (cDNA), QP	(1)

(continued)

TABLE I (*continued*)

MIP homologue[a]	EST[b]	Expression in tissues	Dependence on stimuli[c]	Technique[d]	Ref.[e]
CAB77717 (γ-TIP3)	−				
AAB84183 (β-TIP)	+				
AAF18713 (α-TIP)	+	Embryo, seed		GUS, IH and SB (cDNA), ID (TP25 bean)	(3, 4)
AAC42249 (ε-TIP)	+				
CAB51216	−				
CAB80801 (TMP-C)	+	Flower and stem > root and leaf		NB (cDNA); SB (cDNA)	(1, 6)
AAF81320 (PIP1c)	+	Leaf, root	*Up:* heat-shock; *Stable:* dehydration	NB (3′ or 5′ cDNA ends), SB (cDNA)	(1, 7)
AAC28529 (PIP1b)	+	Root, leaf	*Up:* heat-shock; *Stable:* dehydration	NB (3′ or 5′ cDNA ends)	(7)
	+	Elongating and differentiating tissues, vascular tissues, guard cells, stamens, young siliques	*Up:* light (blue >white> red) *Up:* ABA, GA; *Stable:* IAA, heat shock, wounding	NB (cDNA) GUS, IH, ID (Nter PIP1b peptide) SB (cDNA)	(8–10) (1)
		Leaf and root > bolt and flower			
		Pip1b>pip2a>pip1a>pip2b in each organ (light)		RP (3′ end)	(11)
CAB71073 (PIP1a)	+	Root > leaf	Dehydration	NB (3′ or 5′ cDNA ends)	(7)
		Leaf > root		SB (cDNA)	(1)
		Pip1b>pip2a>pip1a>pip2b in each organ (light)		RP (3′ end)	(11)
CAA20461 (PIP1d)	+				
AAD18141 (RD28)	+	Whole plant	*Up:* dehydration; *Stable:* ABA	NB (cDNA)	(12)
		Whole plant except seeds	*Stable:* dehydration	ID (RD28, PIP2A and PIP2B)	(13)
		Pip2b>rd28>pip2a in root	*Down:* mannitol; *Stable:* ABA, NaCl, dark, dehydration	SB (cDNA), QP	(1)

AAD18142 (PIP2b)	+	Root, leaves	*Up:* heat-shock; *Stable:* dehydration	NB (3′ or 5′ cDNA ends)	(7)
		Pip2b>rd28>pip2a in root		SB (cDNA)	(1)
		Pip1b>pip2a>pip1a>pip2b in each organ (light)		RP (3′ end)	(11)
CAB67649 (PIP2a)	+	Root, leaves	*Stable:* heat-shock, dehydration	NB (3′ or 5′ cDNA ends)	(7)
		Pip2b>rd28>pip2a in root		SB (cDNA)	(1)
		Pip1b>pip2a>pip1a>pip2b in each organ (light)		RP (3′ end)	(11)
BAB09839	+				
AAC64216 (PIP3b)	+				
CAA17774 (PIP3)	+	Flower and stem >root and leaf		NB (cDNA)	(6)
		Cell culture, flower and silique > leaf and bolt		SB (cDNA)	(1)
CAB41102 (PIP2d)	+				
AAC79629 (PIP2e)	+	Leaf		SB (cDNA)	(1)

[a] MIP homologues are indicated by their protein accession number (with their common name in parentheses if the protein or cDNA has already been described).

[b] The presence or the absence of EST in data bank is indicated by (+) or (−), respectively.

[c] Stimuli that up-regulate (*Up*), down-regulate (*Down*), or do not alter (*Stable*) MIP expression are indicated.

[d] The techniques used to characterize gene or protein expression were as follows: GUS, promoter: GUS fusion; ID, immunodetection; IH, *in situ* hybridization; NB, northern blot; QP, quantitative PCR; RP, RNAse protection assay; SB, slot-blot. Proteins or peptides used to raise antibodies or DNA probes used in hybridization experiments are indicated in parentheses. Full-length cDNAs are referred to as cDNA and probes with the 3′ or 5′ ends of cDNAs are mentioned.

[e] References: (1) Weig *et al.,* 1997; (2) Daniels *et al.,* 1996; (3) Ludevid *et al.,* 1992; (4) Höfte *et al.,* 1992; (5) Phillips and Huttly, 1994; (6) Utsugi *et al.,* 1996; (7) Kammerloher *et al.,* 1994; (8) Kaldenhoff *et al.,* 1993; (9) Kaldenhoff *et al.,* 1995; (10) Kaldenhoff *et al.,* 1996; (11) Grote *et al.,* 1998; (12) Yamaguchi-Shinozaki *et al.,* 1992; (13) Daniels *et al.,* 1994.

TABLE II

Expression Properties of MIP Homologues in Various Plant Species

Plant species	MIP homologue[a]	Homologue in *Arabidopsis*[b]	Expression in tissues	Dependence on stimuli[c]	Technique[d]	Ref.[e]
Antirrhinum sp.	DIP	δ-TIP2	Cotyledons	*Up:* dark; *Stable:* GA3	QP, IH (cDNA)	(1)
Beta vulgaris	PIP1 and PIP2like	PIP1, PIP2	Storage tissues		ID (Cter PIP *Arabidopsis*)	(2)
	BPM2, BPM3	PIP1	Storage tissues, stems, leaves		NB (cDNA)	(3)
Brassica napus	BnPIP1	PIP2a	Primed seeds, germinating seeds	*Up:* priming with ABA or water	NB (cDNA)	(4)
	Bnγ TIP2	γ-TIP2	Germinating seeds	*Stable:* priming	NB (cDNA)	(4)
Brassica oleracea	MIPa	PIP1b	Stamen, sepals, petals, carpels	*Up:* drought	NB (cDNA: full length and 3′ end)	(5)
	MIPb	PIP1b	Stamen, sepals, petals, carpels, roots	*Stable:* drought	NB (3′ cDNA end)	(5)
	BobTIP26-1	γ-TIP	Meristems, vascular bundles	*Up:* drought	NB (3′ cDNA end), ID (BobTIP26)	(6, 7)
	BobTIP26-2	γ-TIP	Meristems, vascular bundles, elongating tissues	*Up:* drought	NB and IH (3′ cDNA end), ID (BobTIP26)	(6, 7)
Brassica rapa	MOD	PIP1b	Stigma, leaves, anthers	*Stable:* pollination	NB (partial cDNA)	(8)
Craterostigma sp.	CpPIPa2	PIP1	Leaves, roots, calli	*Up:* drought, ABA	NB (5′ and 3′ cDNA ends)	(9)
	CpPIPa6	PIP1	Leaves, roots	*Up:* drought; *Stable:* ABA	NB (5′ and 3′ cDNA ends)	(9)
	CpPIPa7	PIP1	Roots	*Up:* drought; *Stable:* ABA	NB (5′ and 3′ cDNA ends)	(9)
	CpPIPb	PIP1	Leaves, roots, calli	*Stable:* drought, ABA	NB (5′ and 3′ cDNA ends)	(9)
	CpPIPc	PIP3	Leaves, roots, calli	*Up:* drought; *Stable:* ABA	NB (5′ and 3′ cDNA ends)	(9)
	CpTIP	TIP	Leaves, calli	*Down:* drought, ABA		(9)
Cucurbita sp.	VM23 like	γ-TIP1/2	Hypocotyls, cotyledons		ID (VM23)	(10, 11)
	MP23, MP28	α/β-TIP	Seedlings		ID (MP28 and MP23), NB (cDNA)	(12, 13)

Glycine max	TIP	α-TIP	Cotyledons, pro-vascular cells		ID (TP25)	(14, 15)
	NOD26	NLM1/2	Infected cells of root nodules		IP (MPB proteins), EP (5′ cDNA), GUS	(16, 17)
	SPCP1	γ-TIP	Root elongation zone, vegetative tissues		PE (5′ cDNA end)	(17)
	γ-TIP	γ-TIP1/2	Inner cortex and pericycle of roots, transfer cells		ID (VM23)	(18)
	δ-TIPlike	δ-TIP	Leaves	*Up:* depodding	ID (δ-TIP peptide)	(19)
	γ-TIPlike	γ-TIP	Leaves	*Stable:* depodding	ID (γ-TIP peptide)	(19)
Gossypium sp.	δ-TIP	δ-TIP	Etiolated cotyledons, elongating fiber cells		RT-PCR	(20)
Helianthus sp.	SunTIP7	δ-TIP	Leaves, stems, guard cells, phloem, cortical tissues	*Up:* drought, light	NB, IH (3′ cDNA end)	(21, 22)
	SunTIP18	δ-TIP	Roots and stem phloem	Down: drought	NB, IH (3′ cDNA end)	(22)
	SunTIP20	δ-TIP	Leaves, guard cells	*Up:* drought, light	NB, IH (3′ cDNA end)	(21, 22)
	SunRB7	δ-TIP2	Roots	*Stable:* drought	NB (cDNA)	(22)
	Sunγ-TIP	γ-TIP	Vegetative organs	*Stable:* drought	NB (cDNA)	(22)
Lotus sp.	PIP-like	PIP1,/2	Roots	*Up:* light	NB (AtPIP1a, 2b), ID (AthPIP1a)	(23)
	PIP1-like	PIP1	Roots	Down: nitrate starvation	NB (AtPIP1a)	(24)
	LIMP1	γ-TIP1/2	Nodules, roots		NB (partial cDNA)	(25)
	LIMP2	NLM1/2	Nodules		NB (partial cDNA)	(25)
Lycopersicon sp.	TRAMP1	PIP1	Pericarp, leaves	*Up:* ripening, senescence, ethylene	NB (partial cDNA)	(26)
	TRAMP1	PIP1	Roots, stems, leaves, fruits	*Up:* drought; *Stable:* ABA	NB (partial cDNA)	(27)
	δ-TIPlike	δ-TIP	Leaf epidermis, bundle sheath cells, petals		ID (δ-TIP peptide)	(19)
Medicago truncatula	MtAQP1	γ-TIP1/2	Roots	*Up:* mycorrhiza	NB (partial cDNA)	(28)
Mesembryanthemum crystallinum	MIPA	PIP1	Root epidermis (root hair), root and stem vascular tissues	*Stable:* salt stress	NB, IH (cDNA), ID (MIPA peptide)	(29, 30)

(continued)

TABLE II (*continued*)

Plant species	MIP homologue[a]	Homologue in *Arabidopsis*[b]	Expression in tissues	Dependence on stimuli[c]	Technique[d]	Ref.[e]
	MIPB	PIP1	Root cortex, root hairs, stem vascular tissues	*Stable:* salt stress	NB, IH (cDNA), ID (MIPB peptide)	(29, 30)
	MIPC	PIP2	Root elongation zone	*Up:* salt stress	NB (cDNA), ID (MIP C peptide)	(29, 30)
	MIPF	γ-TIP1/2	Roots, mesophyll cells, vascular tissues	*Down:* salt stress	ID (MIP F peptide)	(30)
Mimosa pudica	VM23like	γ-TIP1/2	Pulvini, petioles		ID (VM23)	(31)
Nicotiana excelsior	NeMIP1	PIP1	Roots, flowers	*Up:* salt, drought	NB (3′cDNA end)	(32)
	NeMIP2 and 3	PIP1	Roots, flowers, leaves	*Up:* salt, drought, light	NB (3′cDNA end)	(32)
Nicotiana tabacum	tobRB7	δ-TIP2	Root meristem, immature vascular cylinder, giant cells, leaf		NB and IH (cDNA), *GUS*	(33–35)
	Nt-TIPa	ε-TIP	Suspension cells		cDNA cloning	(36)
	NtAQP1	PIP1	Root apex, vascular tissues, leaf spongy parenchyma		NB and IH (5′ cDNA), ID (N_{ter} NtAQP1)	(37)
Oryza sativa	rTIP1	γ-TIP	Shoots, roots	*Up:* mannitol, NaCl, ABA	NB (cDNA)	(38)
	rMIP1	NLM1/2	Shoots	*Stable:* drought and salt stress	NB (cDNA)	(38)
	OsPIP2a	PIP3/3b	Meristematic, elongating,and differentiating cells–seedlings, leaves	*Up:* GA3; *Down:* ABA, drought, light	NB (3′ cDNA end)	(39)
	OsPIP1a	PIP1	Meristematic, elongating, and differentiating cells–seedlings, leaves	*Up:* GA3; *Down:* ABA, drought	NB (3′ cDNA end)	(39)
	RWC1	PIP1		*Down:* mannitol, NaCl, chilling	NB (cDNA), CP (3′ cDNA end)	(40)
Petroselinum sp.	PcRB7	δ-TIP	Root arbuscular mycorrhiza	*Up:* mycorrhiza, pathogen	NB (partial cDNA)	(41)
Phaseolus vulgaris	TP25	α-TIP	Cotyledons, embryonic axis		ID (TP25)	(14)
Picea abies	TIP	TIP	Root and hypocotyl of seedlings, somatic and zygotic embryos		ID (TP25)	(42)
Pisum sativum	α-TIP	α-TIP	Cotyledons		ID (TP25)	(43)
	γ-TIP	γ-TIP	Cotyledons		ID (TIP-Ma27)	(43)

Raphanus sativus	γ-VM23	γ-TIP1/2	Root, hypocotyl, cotyledon, immature tap root, petiole, leaf	*Down:*light; *Up:* light	ID (VM23), NB (5′ cDNA end)	(10, 47)
	δ-VM23	δ-TIP	Hypocotyls, immature tap root, petiole, leaf (vein)	*Down:* light	NB (5′ cDNA end)	(47)
Spinacia oleracea	PM28a	PIP3	Roots, petiole, leaf veins		NB (partial cDNA)	(48)
	PM28b	PIP1	Leaves		cDNA cloning	(48)
	δ-TIP	δ-TIP	Leaves, petiole, roots		ID (So-δ-TIP peptide)	(49)
Tulipa gesneriana	TIP1	γ-TIP	Vascular tissues, elongationg stalk		QP	(50)
Zea mays	ZmPIP1a	PIP1	Root tip	*Stable:* glucose starvation	NB (cDNA)	(51)
	CHEM8	γ-TIP	Leaves	*Up:* salt stress, heat shock	NB (cDNA)	(52)
	ZmTIP1	γ-TIP	Root meristem, primordia, vascular bundles, epidermis, endodermis, nucellus, pedicels		NB, IH (3′ cDNA end)	(53, 54)
	ZmPIP1a	PIP1	Shoots, roots, leaves, tassels		NB (3′ cDNA end)	(55)
	ZmPIP1b	PIP1	Whole plant, especially developing tassel		NB (3′ cDNA end)	(55)
	ZmPIP2a	PIP2a	Roots		NB (3′ cDNA end)	(55)

[a]Name of MIP homologue as indicated in the original reference.

[b]Closest MIP homologue in *Arabidopsis* or name of the corresponding subfamily.

[c]Stimuli that up-regulate (*Up*), down-regulate (*Down*), or do not alter (*Stable*) MIP expression are indicated.

[d]The techniques used to characterize gene or protein expression were as follows: CP, competitive PCR; GUS, promoter: GUS fusion; ID, immunodetection; IH, *in situ* hybridization; IP, immunoprecipitation; NB, northern blot; PE, primer extension analysis; QP, quantitative PCR; RO, run-on in isolated nuclei. Proteins or peptides used to raise antibodies or DNA probes used in hybridization experiments are indicated in parentheses. Full-length cDNAs are referred to as cDNA and probes with the 3′ or 5′ ends of cDNAs are mentioned.

[e] References: (1) Culianez-Macia and Martin, 1993; (2) Qi *et al.*, 1995; (3) Barone *et al.*, 1998; (4) Gao *et al.*, 1999; (5) Ruiter *et al.*, 1997; (6) Barrieu *et al.*, 1998b; (7) Barrieu *et al.*, 1999; (8) Ikeda *et al.*, 1997; (9) Mariaux *et al.*, 1998; (10) Maeshima, 1992; (11) Maeshima *et al.*, 1994; (12) Inoue *et al.*, 1995a; (13) Inoue *et al.*, 1995b; (14) Johnson *et al.*, 1989; (15) Melroy and Herman, 1991; (16) Fortin *et al.*, 1987; (17) Miao *et al.*, 1992; (18) Serraj *et al.*, 1998; (19) Jauh *et al.*, 1998; (20) Ferguson *et al.*, 1997; (21) Sarda *et al.*, 1997; (22) Sarda *et al.*, 1999; (23) Henzler *et al.*, 1999; (24) Clarkson *et al.*, 2000; (25) Guenther and Roberts, 2000; (26) Davies and Grierson, 1989; (27) Fray *et al.*, 1994; (28) Krajinski *et al.*, 2000; (29) Yamada *et al.*, 1995; (30) Kirch *et al.*, 2000; (31) Fleurat-Lessard *et al.*, 1997; (32) Yamada *et al.*, 1997; (33) Conkling *et al.*, 1990; (34) Yamamoto *et al.*, 1991; (35) Opperman *et al.*, 1994; (36) Gerbeau *et al.*, 1999; (37) Otto and Kaldenhoff, 2000; (38) Liu *et al.*, 1994; (39) Malz and Sauter, 1999; (40) Li *et al.*, 2000; (41) Roussel *et al.*, 1997; (42) Oliviusson and Hakman, 1995; (43) Hoh *et al.*, 1995; (44) Guerrero *et al.*, 1990; (45) Guerrero and Crossland, 1993; (46) Jones and Mullet, 1995; (47) Higuchi *et al.*, 1998; (48) Johansson *et al.*, 1996; (49) Karlsson *et al.*, 2000; (50) Balk and de Boer, 1999; (51) Chevalier *et al.*, 1995; (52) Didierjean *et al.*, 1996; (53) Chaumont *et al.*, 1998; (54) Barrieu *et al.*, 1998a; (55) Chaumont *et al.*, 2000.

has been observed (Barrieu *et al.*, 1998b; Chaumont *et al.*, 1998). In these cells, vacuolar aquaporins may participate in the biogenesis of the vacuolar apparatus and contribute to the adjustments of vacuolar surface and volume that are required for differentiation of a large central vacuole.

Expression of MIPs in most aerial parts of plants, such as hypocotyls (Higuchi *et al.*, 1998), stems and petioles (Otto and Kaldenhoff, 2000), and cotyledons and leaves (Johansson *et al.*, 1996), has also been reported, with a preferential expression in vascular tissues and in cells that undergo rapid elongation and differentiation (Chaumont *et al.*, 1998; Higuchi *et al.*, 1998; Kaldenhoff *et al.*, 1995; Ludevid *et al.*, 1992). Highly specialized cells such as trichomes (Jones and Mullet, 1995), motor cells of *Mimosa pudica* pulvini (Fleurat-Lessard *et al.*, 1997), and guard cells (Kaldenhoff *et al.*, 1995; Sarda *et al.*, 1997) also express specific aquaporin isoforms. Water transport in the two latter cell types plays a crucial role in cell movements.

Flowers exhibit exquisite patterns of aquaporin expression (Jones and Mullet, 1995; Ludevid *et al.*, 1992; Yamada *et al.*, 1997). Plant reproduction requires subtle water exchanges to achieve pollen grain desiccation (maturation) (Ruiter *et al.*, 1997), imbibition on the stigma surface of pollinated flowers, and dramatic growth of the pollen tube. Flower expansion and blooming itself also require accurate control of water relations in sepals and petals. Fruit and seeds also express specific aquaporin isoforms (Fray *et al.*, 1994; Johnson *et al.*, 1989).

C. Whole Plants: Aquaporins in a Changing Environment

Because of their lack of mobility, plants have to adapt constantly to subtle or drastic changes of their environment. For this, they have developed an amazing capacity to perceive the nature and intensity of a large variety of environmental signals. Many of these changes can interfere with plant water relations and, accordingly, with expression of aquaporins.

1. Abiotic Factors

Among all abiotic factors that potentially interfere with aquaporin expression, water and salt stresses are those which have been most investigated. For instance, thorough studies in the model halophytic plant *M. crystallinum* revealed a coordinated and transient decrease in the expression of three aquaporin genes in the 24–48 hr following salt exposure (Yamada *et al.*, 1995). Aquaporin genes that are up- or down-regulated by drought stress and osmotic stress have been identified in numerous species such as *Arabidopsis*, sunflower, cauliflower, and rice (Barrieu *et al.*, 1999; Liu *et al.*, 1994; Sarda *et al.*, 1999; Yamaguchi-Shinozaki *et al.*, 1992). These regulations may allow efficient mobilization of water in tissues whose metabolism is critical for the plant, or they may accompany the deep

alteration of plant cell and tissue morphology observed upon prolonged stress (Barrieu *et al.*, 1999; Kirch *et al.*, 2000). The role of aquaporins under extreme desiccation conditions can typically be explored in resurrection plants such as *Craterostigma plantagineum* (Mariaux *et al.*, 1998).

Cold stress results in a water deficit, and a role of a rice PIP1 homologue (RWC1) in chilling tolerance has been proposed (Li *et al.*, 2000), based on the observation that both an osmotic challenge (0.5 M mannitol) and a cold stress (4°C) decreased, independently and synergistically, the amount of RWC1 mRNA. Aquaporins may also be involved in the response of plants to heat shock, since a 38–42°C treatment dramatically enhanced the expression of the CHEM8 aquaporin in maize (Didierjean *et al.*, 1996).

The expression of many plant aquaporins can also be determined by the light conditions during growth. Expression of mRNAs encoding PIP1 homologues in *Lotus japonicus* roots (Henzler *et al.*, 1999) and a δ-TIP homologue in sunflower guard cells (Sarda *et al.*, 1997) showed a marked increase during daytime. These regulations may be related to diurnal variations in root hydraulic conductivity and stomatal aperture in these plants. The *pip1b* gene of *Arabidopsis* is specifically induced by blue light, a signal for floral induction (Kaldenhoff *et al.*, 1993). In contrast, other aquaporins are up-regulated in etiolated tissues, a pattern which may be related to the preferential expression of these aquaporins in elongating cells (Higuchi *et al.*, 1998; Ludevid *et al.*, 1992).

2. Hormones and Biotic Factors

Aquaporin expression is controlled by endogenous hormonal factors. For instance, the stress hormone abscisic acid (ABA), which plays a crucial role in the response of plants to drought or in the maturation of seeds, is known to regulate aquaporins in various species (Gao *et al.*, 1999; Kaldenhoff *et al.*, 1993; Mariaux *et al.*, 1998). Note, however, that some aquaporin genes are induced by drought through an ABA-independent pathway (Fray *et al.*, 1994; Mariaux *et al.*, 1998). Induction of *Arabidopsis* γ-TIP by gibberellic acid, a hormone that promotes cell expansion, is consistent with the expression pattern of this aquaporin, specifically in the elongating zones of roots and stems (Phillips and Huttly, 1994).

Interaction of plants with symbiotic or pathogenic organisms is mediated by biotic signals, many of which remain to be discovered. Arbuscular miccorhiza or N_2-fixing nodules in legumes illustrate some of the deep morphological alterations that plant roots can undergo to accommodate the presence of symbionts. Plant-encoded aquaporins are specifically expressed in these organs and have been localized in the highly compartmented vacuole of arbuscule cells (Roussel *et al.*, 1997) and in the symbiosome membrane that surrounds N_2-fixing bacteroids (Fortin *et al.*, 1987). These aquaporins probably optimize nutrient and water exchange between the two symbiotic partners. They may also permit efficient osmoregulation of the highly compartmented root cells. Plant infection by root-knot nematodes results

in a dramatic increase in size for a few root cells that form a feeding site for the parasite. Nutrient and water uptake by the parasite from these reservoirs seems to be facilitated by induction of aquaporin TobRB7 (Opperman *et al.,* 1994). A promoter region of the *tobRB7* gene that is specifically responsive to the pathogen has been identified.

In conclusion, aquaporin expression has been found in virtually all plant tissues examined. Strong aquaporin expression typically occurs in the root cortex, vascular bundles of shoots and roots, and elongating tissues, consistent with the idea that these tissues have special needs for intense water exchange. The diversity of aquaporin expression patterns and regulation in other tissues has shown, however, that very fine adjustments of water transport are needed in more physiological situations than was initially suspected.

Despite the wealth of data collected over recent years, we are far from a complete picture of aquaporin expression properties in plants. This is due mostly to the very high diversity of aquaporin genes in plants. Extensive characterization of all aquaporins expressed in a given plant cell type is lacking, and coexpression of several aquaporin isoforms in the same cell type seems to be common, which makes expression patterns even more difficult to decipher. Basic quantitative questions also remain difficult to answer unambiguously. Which aquaporin isoforms are the most strongly expressed? Do some organs, tissues, and cell types have a higher aquaporin content than others?

The use of a restricted number of models such as *Arabidopsis* or maize will now allow coordinated efforts by several laboratories. For instance, the analysis of ESTs in *Arabidopsis* suggests that certain MIPs such as PIP2d (CAB41102) are specifically expressed in siliques, whereas others such as γ-TIP (BAB01832) or PIP1b (AAF81320) are strongly expressed throughout the plant (Javot *et al.,* unpublished results). The latter results confirm expression studies using RNAse protection assays (Grote *et al.,* 1998) or plants containing a promoter:GUS fusion construct (Ludevid *et al.,* 1992). Because the sequence of all the MIPs in *Arabidopsis* is now accessible, expression studies using macro- or microarrays will become extremely useful, and it will be possible to estimate the cross-reactivity of probes with very close homologues.

However, studies in model plants may be difficult to generalize, since the expression profile of an aquaporin cannot be deduced from data obtained on a close homologue in another plant species. α-TIP homologues in bean, *Arabidopsis,* and Norway spruce all showed abundant expression in seeds (Höfte *et al.,* 1992; Johnson *et al.,* 1989; Ludevid *et al.,* 1992; Oliviusson and Hakman, 1995). In contrast, *Arabidopsis* γ-TIP showed preferential expression in elongating tissues (Ludevid *et al.,* 1992), whereas a close homologue in cauliflower was strongly expressed in meristematic tissues (Barrieu *et al.,* 1998b), and a homologue in maize showed a broader expression pattern, with preferential expression in root vascular tissues (Barrieu *et al.,* 1998a). Thus, expression properties of aquaporins cannot be predicted based on their amino acid sequence identity.

III. Functional Evidence for Plant Aquaporins

As in other organisms, the presence of active water channels in plant membranes can be assessed based on the conjunction of the following parameters:

i. A high osmotic water permeability ($P_f > 50$–$100\ \mu m\ s^{-1}$)
ii. A sensitivity of water transport to mercury inhibition and reversion of mercury effects by reducing agents
iii. A low dependency on temperature, reflecting a low Arrhenius activation energy (E_a)
iv. A ratio of osmotic to diffusional water permeability (P_f/P_d) greater than unity.

Before the molecular identification of plant aquaporins, these parameters had been separately characterized in plant membranes but, with the exception of a study in *Chara* cells by Wayne and Tazawa (1990), had not been assembled as evidence for water channels in plants. This gap has been filled over the last few years, and numerous reports now describe the activity of water channels in plant membranes, in intact cells, and even in tissues. Although interpretations in the two latter systems may not be straightforward, these approaches open new perspectives to address the specific function of aquaporins in plants. The possibility that plant aquaporins transport small neutral solutes has also led several laboratories to revisit the solute transport properties of plant membranes.

Tables III and IV summarize some of the water transport measurements that have been reported in different plant species over the last 4 years, with emphasis on those studies where mercury inhibition of water transport was investigated. The techniques used are also mentioned but will not be detailed here since they have been described in recent reviews (Maurel, 1997; Steudle, 1993; Verkman, 2000).

A. Subcellular Membranes

1. Intracellular Membranes

a. Water Transport Measurements in Isolated Vesicles or Organelles. The discovery of aquaporins in the intracellular membranes of plant cells has led several laboratories to investigate in closer detail the water transport properties of subcellular membrane compartments. Initial attempts were done by cell membrane fractionation and stopped-flow measurements on isolated membrane vesicles (Maurel *et al.,* 1997b; Niemietz and Tyerman, 1997; Rivers *et al.,* 1997). Extremely high water permeability ($P_f \geq 500\ \mu m\ s^{-1}$) and strong inhibition by mercury ($\geq$80%), and/or $P_f/P_d \geq 7$ have been reported both in the peribacteroid membrane of soybean N_2-fixing root nodules (Rivers *et al.,* 1997) and in the TP of tobacco suspension

TABLE III

Characteristics of Water Transport in Isolated Membrane Vesicles, Vacuoles, Protoplasts, and Intact Cells

Plant material[a]	Mean P_F (μm/s)	Technique[b]	E_a (kcal/mol)	Inhibition by $HgCl_2$				References
				Dose (mM)	Treatment (min)	Inhibition (%)	Reversion[c]	
Membrane vesicles								
Wheat root PM	12.5	SF	11.4					Niemietz and Tyerman (1997)
Tobacco PM	6.1	SF	13.5					Maurel *et al.* (1997b)
Tobacco TP	690	SF	2.5	1	5	80	+	Maurel *et al.* (1997b)
Wheat root IM	86	SF	5.5	0.1		70		Niemietz and Tyerman (1997)
Soybean PBM	500	SF	3.3	0.1		92		Rivers *et al.* (1997)
Isolated vacuoles								
Rape leaf	623	PMM-TC						Morillon and Lassalles (1999)
Petunia leaf	955	PMM-TC						Morillon and Lassalles (1999)
Onion leaf	184	PMM-TC		1	10	70	+	Morillon and Lassalles (1999)
Rape hypocotyl	1100	PMM-TC						Morillon and Lassalles (1999)
Rape root	656	PMM-TC						Morillon and Lassalles (1999)
Red beet root	270	PMM-TC		1	10	85	+	Morillon and Lassalles (1999)
Isolated protoplasts								
Onion leaf	9	PMM-TC	15.5					Ramahaleo *et al.* (1999)
Rape hypocotyl	370	PMM-TC						Ramahaleo *et al.* (1999)
Rape root	305	PMM-TC	0.3	0.2		98		Ramahaleo *et al.* (1999)
Wheat root	2.5	PMM-TC	11.7					Ramahaleo *et al.* (1999)
Melon root	11	PMM						Martinez-Ballesta *et al.* (2000)
Intact cells								
Chara (low CO_2)	353	TCO		1	60	63		Wayne *et al.* (1994)
Chara (high CO_2)	244	TCO		1	60	81		Wayne *et al.* (1994)
Chara	260	TCO		0.1	2	90	+	Tazawa *et al.* (1996)
Onion root	257	CPB		0.05	20	26		Barrowclough *et al.* (2000)
Wheat	76	CPB		0.1	60	75	+	Zhang and Tyerman (1999)

[a] Abbreviations for plant materials are as follows: PM, plasma membrane; TP, tonoplast; IM, intracellular membranes; PBM, peribacteroid membrane.

[b] The P_f value for onion roots is from a root segment with mature endodermis and exodermis. P_f values were measured using the following methods:

TABLE IV

Characteristics of Water Transport in Root Tissues

Plant species	Hydraulic conductivity (L_p)	Technique[a]	Inhibition by $HgCl_2$				References
			Dose (mM)	Treatment (min)	Inhibition (%)	Reversion[b]	
Tomato	4.6×10^{-7} m s^{-1} MPa^{-1}	HPC	0.5	1	57	+	Maggio and Joly (1995)
Wheat	760 mg g^{-1} h^{-1} MPa^{-1}	EM	0.05	5	64	+	Carvajal *et al.* (1996)
Barley	0.29 pm s^{-1} Pa^{-1}	TRO	0.1	20	85	−	Tazawa *et al.* (1997)
Sugar beet	0.145[c] μL min^{-1} cm^{-2}	HPC	1		80	+	Amodeo *et al.* (1999)
Paprika	800 mg g^{-1} h^{-1} MPa^{-1}	EM	0.05	5	85	+	Carvajal *et al.* (1999)
Sunflower	756 μL g^{-1} h^{-1} MPa^{-1}	EM	1	160	21	n.d.	Quintero *et al.* (1999)
Aspen	9.7×10^{-8} m s^{-1} MPa^{-1}	HPC	0.1	30	47	+	Wan and Zwiazek (1999)
Onion[d]	2×10^{-6} m s^{-1} MPa^{-1}	P	0.05	20	72	+	Barrowclough *et al.* (2000)
Melon	645 mg g^{-1} h^{-1} MPa^{-1}	EM	0.05	5	68	+	Martinez-Ballesta *et al.* (2000)

[a] Water transport measurements were made using the following methods: HPC, hydrostatic pressure chamber; EM, exudate measurement; TRO, trans-root osmosis; P, potometer.

[b] Reversion of $HgCl_2$ inhibition by a reducing agent (β-mercaptoethanol, DTT), or a lack of reversion, is indicated by (+) or (−), respectively. n.d.: not determined.

[c] Value refers to a flux not a hydraulic conductivity.

[d] Measurements on a root segment with mature endodermis and exodermis.

and wheat root cells (Maurel *et al.*, 1997b; Niemietz and Tyerman, 1997). This provided unambiguous evidence for active water channels in these two classes of endomembranes. In complement to measurements on purified TP vesicles, water transport properties have also been characterized in intact vacuoles isolated from protoplasts of various origin (Morillon and Lassalles, 1999). These studies confirmed the idea that the TP of most plant cells exhibits a high P_f ($\geq$200 μm s^{-1}). The significance of a high water permeability for plant endomembranes will be discussed further in Section IV of the present review.

b. Solute Transport Properties. TP-enriched vesicles purified from tobacco cells also exhibited a high permeability to small neutral solutes such as glycerol and urea ($P_{urea} = 74.9 \pm 9.6 \times 10^{-6}$ cm s^{-1}) with features of a facilitated (channel-mediated) transport (Gerbeau *et al.*, 1999). Functional expression of NtTIPa, a novel aquaporin expressed in the TP of these cells, showed this aquaporin to transport urea and to a lesser extent glycerol, thus paralleling the solute transport properties of native membranes (Gerbeau *et al.*, 1999). It was thus proposed that Nt-TIPa can account for most of the high permeability of the tobacco TP to small nonelectrolytes.

The contribution of NOD26 to solute transport in the peribacteroid membrane has been investigated in even greater detail. Early experiments in which NOD26 was reconstituted in artificial membranes revealed an ion channel activity for the protein (Weaver *et al.*, 1994). Accordingly, it has been suggested that NOD26 could mediate the export of organic acids from the bacteroid toward the plant cell (Ouyang *et al.*, 1991). Serious doubt about this hypothesis has been cast by the failure of NOD26 (and of its mammalian homologue MIP/AQP0) to elicit any ion conductance after expression in *Xenopus* oocytes (Dean *et al.*, 1999). In contrast, NOD26 exhibited a good capacity to transport glycerol but not urea, both in oocytes and in artificial membranes (Dean *et al.*, 1999). This agrees with the high and low permeability of the symbiosome membrane to glycerol and urea, respectively (Rivers *et al.*, 1997). Two *Arabidopsis* homologues of NOD26, NLM1 and NLM2, also transport glycerol (Weig and Jakob, 2000), but *Arabidopsis* does not establish any N_2-fixing symbiosis, and the membrane to which NLM1 and NLM2 confer this property is unknown.

NH_3 may be another substrate for plant aquaporins and may be more physiologically relevant than small polyols or urea in the particular case of NOD26. NH_3 entry into peribacteroid membrane vesicles was recently shown to be partially protein-mediated (Niemietz and Tyerman, 2000) based upon the low activation energy of NH_3 uptake and its inhibition by mercury. NOD26 is regulated by phosphorylation (Lee *et al.*, 1995), and the participation of this protein in NH_3 permeation was evaluated by preincubating vesicles with ATP: ATP addition increased water transport on the one hand and inhibited NH_3 transport on the other. This suggested either that two transport mechanisms for water and NH_3 coexist inside NOD26 or that another protein is responsible for NH_3 transport.

2. Plasma Membrane

Whereas a high water and solute permeability has been consistently found in both the TP and the peribacteroid membrane, measurements with isolated PM vesicles have pointed to a much reduced permeability. In two initial studies where tobacco suspension cells or wheat roots were used, a low membrane P_f associated with a high E_a for water transport suggested a lack of active water channel in purified PMs (Maurel *et al.,* 1997b; Niemietz and Tyerman, 1997). PM vesicles isolated from tobacco also showed a reduced permeability to urea and to a series of small linear polyols (Gerbeau *et al.,* 1999), with no facilitated (channel-mediated) transport for these molecules. Recently, Nt-AQP1, a PIP homologue from *Nicotiana tabacum,* was reported to be permeable to water and glycerol upon expression in *Xenopus* oocytes (Biela *et al.,* 1999). This aquaporin is probably targeted to the PM, but the activity of solute transporting aquaporins in this membrane remains to be demonstrated.

The respective water transport properties of the vacuolar and plasma membranes have also been recently investigated in yeast cells (see Hohmann, this volume). Surprisingly, no active water channel activity could be detected in purified membrane vesicles (Coury *et al.,* 1999), and the reason for these results is not yet clear. Because of the restricted number of MIPs in yeast and because of the power of genetic tools developed in this organism, future studies on water transport in yeast will nicely complement studies on the function of aquaporins in plant cells.

3. Posttranscriptional Regulation of Plant Aquaporins

There is to date no definite molecular explanation of the dramatic difference in water channel activity in vesicles isolated from various subcellular compartments. Clearly, this difference cannot be simply accounted for by differences in gene and/or protein expression level, since aquaporins seem to be abundant both in the PM and in the endomembranes of plants. Studies in animals showed that aquaporins can exhibit a large range of unitary water conductance that span up to two orders of magnitude (AQP0: $p_f = 0.25 \times 10^{-14}$ cm^3 s^{-1}; AQP4: $p_f = 24 \times 10^{-14}$ cm^3 s^{-1}) (Yang and Verkman, 1997). These parameters will have to be investigated and compared in plant TP and PM aquaporins.

Observations by several laboratories also point to regulatory mechanisms that may target plant aquaporins. For instance, Niemietz and Tyerman (1997) failed to detect active water channels in PM vesicles purified from wheat roots, whereas cortex cells from the same material had a L_p which was inhibited by mercury (Zhang and Tyerman, 1999). Mercury inhibition appears to take place both at the PM and at the TP of wheat cells (Zhang and Tyerman, 1999), suggesting that inactivation of PM water channels may have occurred during membrane purification. Along these lines, we recently discovered that active water channels can be detected in membrane vesicles purified from *Arabidopsis* suspension cells, provided

that chelators of divalent cations are used during cell homogenization to prevent water channel down-regulation by these cations (Gerbeau *et al.*, submitted). The molecular bases of this inhibition are not yet clearly understood. Other clues may arise from the failure of several laboratories to functionally express homologues of the PIP1 family in *Xenopus* oocytes. At least in the case of maize ZmPIP1 (Chaumont *et al.*, 2000), the aquaporin was correctly expressed and targeted to the oocyte PM, suggesting the requirement of posttranslational modifications for water channel activity.

Reversible phosphorylation of plant aquaporins has been described in various subcellular membranes and may represent such a kind of regulatory mechanism. For instance, α-TIP can be phosphorylated in PSVs of bean seeds or in transgenic tobacco by a TP-bound calcium-dependent protein kinase (CDPK) (Johnson and Chrispeels, 1992). CDPKs are also responsible for the phosphorylation of NOD26 and PM28a, in the symbiosome membrane of soybean root nodules and in the PM of spinach leaves, respectively (Johansson *et al.*, 1996; Weaver *et al.*, 1991). Interestingly, phosphorylation of PM28a is also dependent on extracellular water potential and could be reduced by external addition of mannitol to leaf tissues (Johansson *et al.*, 1996). The functional significance of plant aquaporin phosphorylation has been addressed after expression in *Xenopus* oocytes (α-TIP, PM28a) (Johansson *et al.*, 1998; Maurel *et al.*, 1995) or after reconstitution of the protein in artificial membranes (NOD26) (Lee *et al.*, 1995). *Xenopus* oocytes proved to be a very convenient expression system since aquaporin phosphorylation could be modulated by agonists and antagonists of endogenous protein kinases and phosphatases. The water channel activity of both α-TIP and PM28a was enhanced by phosphorylation at sites that are recognized by plant CDPKs, but the role of subsidiary sites, whose occurrence remains to be demonstrated *in planta,* was also uncovered (Johansson *et al.*, 1998; Maurel *et al.*, 1995). In another study where purified NOD26 was reconstituted in planar lipid bilayers, phosphorylation by a recombinant CDPK from *Arabidopsis* altered the ion channel behavior of NOD26 (Lee *et al.*, 1995). From all these reports, it has been hypothesized that, in contrast to the phosphorylation of mammalian AQP2 which provides a signal for AQP2 targeting to the cell surface (Fushimi *et al.*, 1997; Katsura *et al.*, 1997), the phosphorylation of plant aquaporins may provide a means for rapid and reversible *in situ* gating. This property remains, however, to be established in native plant membranes.

B. Cell Level

The observation that plant cells from different species, tissues, or even developmental stages can exhibit a large range of water permeability has been a long-standing one (Maurel, 1997; Steudle, 1989) and can now be taken as an indication of membrane water transport being the object of a strict control in which aquaporins may play a crucial role. For instance, the differential regulation of

aquaporins may explain why the mean water permeability of intact wheat root cells was 76 μm s^{-1}, whereas a value of 2.5 μm s^{-1} was measured in protoplasts from the same origin (Ramahaleo *et al.*, 1999; Zhang and Tyerman, 1999). The recent work by Ramahaleo *et al.* (1999) also emphasizes the large variability of P_f values (over more than two orders of magnitude) that can be found in isolated protoplasts from the same preparation. These authors showed that, despite this very high variability, the averaged P_f of root protoplasts from maize, rape, and wheat all increased >10-fold with root age, suggesting a modulation of water channel activity during root development (Ramahaleo *et al.*, 1999).

1. Water Transport Measurements in Intact Cells Have Established the Presence of Active Water Channels in Algae and in Higher Plants

Because of their size, the internodal cells of charophytes are a convenient model for water transport studies and provided the first evidence for water channels in plant cells (Wayne and Tazawa, 1990). A high hydraulic conductivity, low E_a, and inhibition by sulfhydryl reagents have been reported by several authors using either transcellular osmosis or, more recently, pressure probe measurements (Henzler and Steudle, 1995; Schütz and Tyerman, 1997; Tazawa *et al.*, 1996; Wayne and Tazawa, 1990). Evidence for water channels in intact cells of higher plants has proved more difficult to obtain, but recent work by Zhang and Tyerman (1999) also demonstrated inhibition of L_p by mercury in cortex cells of wheat roots.

There have been concerns about the general effects that mercury may exert on cell functions. In *Chara* and wheat cells, for instance, mercury rapidly depolarized the membrane (without altering the membrane electrical conductance in *Chara*) with a dose-dependence that was similar to that of L_p inhibition (Schütz and Tyerman, 1997; Tazawa *et al.*, 1996; Zhang and Tyerman, 1999). It was also found that mercury inhibits cell respiration and blocks cell cyclosis (Schütz and Tyerman, 1997; Zhang and Tyerman, 1999). Thus, the effects of $HgCl_2$ on cell water transport may be mediated by direct blockage of water channels or by a general alteration of cell metabolism that would in turn influence water channel activity.

2. Genetic Evidence for the Activity of Aquaporins in Plant Membranes

Reverse genetics provides more solid evidence for aquaporin function in the plant PM. Transgenic *Arabidopsis* plants that contained a *PIP1B* antisense construct displayed an overall reduction in the level of mRNA and proteins for several PIP1 homologues, consistent with the very high sequence homology that exists between members of the PIP1 subfamily in *Arabidopsis* (Kaldenhoff *et al.*, 1998). The P_f of protoplasts prepared from transgenic leaves was 3- to 4-fold lower than the P_f

of wild-type protoplasts and was reduced to the level of the latter treated with $HgCl_2$. Although the P_f values reported in this work are strikingly low, within a range typical for membranes deprived of water channels, these data demonstrate that PIP aquaporins significantly contribute to water transport at the PM level.

3. Channel-Mediated Solute and Gas Transport in Intact Plant Cells

In *Chara* cells the effects of mercury and temperature on the permeability and reflection coefficient of lipophilic molecules, such as small size alcohols, formamide, or acetone, have been taken to demonstrate the capacity of plant water channels to transport solutes (Henzler and Steudle, 1995; Hertel and Steudle, 1997; Schütz and Tyerman, 1997). The contribution of the water channel path remained minor as compared to transport through lipid membranes. Yet these studies questioned for the first time the notion that plant water channels function as highly selective channels. In particular, extremely low reflection coefficient values of the water channel path suggested that strong friction between water and solutes may occur within the aqueous pore (Schütz and Tyerman, 1997; Steudle and Henzler, 1995).

In a previous study, Wayne *et al.* (1994) established a link between the permeability of *Chara* cells to water and CO_2 availability. Whereas transport of H_2O and HCO_3^- showed distinct pharmacological properties, the correlation between H_2O and CO_2 permeability suggested either that the PM lipid composition was modified by CO_2 in a way affecting water transport or that proteins that are induced by CO_2 mediate the transport of both CO_2 and water. The second idea conforms with recent evidence showing that mammalian AQP1 can transport CO_2 (Nakhoul *et al.*, 1998; Prasad *et al.*, 1998).

4. The Water Permeability of the PM and the TP Can Be Deduced from Measurements Made in Cellular Systems

The water transport properties of wheat roots cells have been investigated both by stopped-flow measurements on purified membrane vesicles (Niemietz and Tyerman, 1997) and by means of a cell pressure probe (Zhang and Tyerman, 1999). Water transport simulations in a model cell revealed, however, that the behavior of intact cells cannot be consistently explained by the water permeability values determined in isolated vesicles (Zhang and Tyerman, 1999). To fit their cell pressure probe data, Zhang and Tyerman (1999) had to assume that the PM water permeability of living cells was higher by a factor of 1.2 to 10 than the value recorded in isolated PM vesicles. To interpret the effects of mercury on the L_p of root cells, they also had to assume that mercury blocked water transport both at the PM and at the TP, suggesting that water channels were active in these two membranes (see above). It was concluded, however, that in all cases, the water transport properties of the PM dominated the whole cell behavior.

Water transport measurements made in protoplasts and in vacuoles isolated from the same materials can also provide hints at the respective permeabilities of the TP and the PM (Morillon and Lassalles, 1999; Ramahaleo *et al.*, 1999; Url, 1971). A rough estimate of PM water permeability can be obtained by assuming that the TP and the PM act as two hydraulic resistances in series ($1/L_{p\ cell} \sim 1/L_{p\ PM} + 1/L_{p\ TP}$). In certain plant materials such as the hypocotyls and roots from rape (Morillon and Lassalles, 1999; Ramahaleo *et al.*, 1999), the PM must be nearly as permeable as the TP. In contrast, most of the hydraulic resistance of onion bulb protoplasts resides in the PM (Url, 1971). In the giant cells of *Chara* internodes, the TP can be disrupted by intracellular perfusion of an EGTA solution. Measurements using this approach suggested that the TP does not significantly contribute to the overall hydraulic resistance of the cell (Kiyosawa and Tazawa, 1977) and that mercury exerts its most significant inhibitory effects at the PM level (Tazawa *et al.*, 1996).

C. Tissue Level

With the exception of one study on sunflower hypocotyls (Hejnowicz and Sievers, 1996), the search for water channel activity in plant tissues has exclusively focused on roots. In this organ, the radial transport of water occurs via two parallel routes, either across cell walls (apoplastic path) or from cell to cell. Cell-to-cell flow is itself a blend of transcellular and symplastic flow (Steudle and Peterson, 1998). Using traditional biophysical analyses, it has not been possible to experimentally distinguish between the symplastic and transcellular contributions to root water transport. The barrier that membranes may present to water flow has thus remained uncertain.

Inhibition of root L_p by mercuric chloride ($HgCl_2$) has been reported in several species including tomato (Maggio and Joly, 1995), wheat (Carvajal *et al.*, 1996), barley (Tazawa *et al.*, 1997), aspen (Wan and Zwiazek, 1999), sugar beet (Amodeo *et al.*, 1999), sunflower (Quintero *et al.*, 1999), paprika (Carvajal *et al.*, 1999), and melon (Martinez-Ballesta *et al.*, 2000) (Table IV). Note, however, that depending on the studies, high (0.5 mM) or much lower (50 μM) doses of $HgCl_2$ were used. Nevertheless, mercury inhibition has been interpreted in all cases as a direct blockage of water channels and supports, for the first time, the idea that cell membranes can represent a major penetration resistance for water in roots. Similar to results obtained in cells, mercury treatment has strong effects on whole-organ physiology and reduced both root respiration and stomatal conductance in aspen seedlings (Wan and Zwiazek, 1999). Because of this general toxicity, the reversal of mercury effects by scavenging agents such as β-mercaptoethanol or dithiothreitol is mandatory when water channel functions are investigated, to ensure that no irreversible damage has been made to roots. Some authors have also checked that ion uptake in roots was not altered, by measuring the ionic composition of the

xylem sap (Maggio and Joly, 1995; Wan and Zwiazek, 1999). The dependence on temperature of water transport in controls and in mercury-treated aspen roots has recently been investigated by Wan and Zwiazek (1999). In contrast to what is commonly observed in isolated membranes or cells, E_a was decreased after mercury treatment, and this observation is awaiting explanation.

To dissect mercury effects even further, Barrowclough *et al.* (2000) have adapted mini-potometers to measure water influx into well-defined anatomical zones of onion roots. They probed the effects of mercury in each of these zones and found that water channel contribution is most important in the oldest part of the root where both an exodermis and endodermis were differentiated, and had developed Casparian bands and suberin lamellae. The penetration of mercury in roots was investigated by precipitation by hydrogen sulfide vapor and microscopic observation of the precipitate in root sections. Surprisingly, mercury did not noticeably enter the root beyond the outer cell layers (exodermis) (Barrowclough *et al.*, 2000).

IV. Putative Functions for Plant Aquaporins

A. Cell Osmoregulation

1. What Is the Physiological Significance of Distinct Water Transport Properties at the PM and the TP?

a. Single-Cell Osmoregulation. It is commonly acknowledged that the PM must represent the limiting barrier that regulates water exchange between the cell and its bathing medium. This is consistent with estimations of PM and TP water permeability derived from measurements on purified membrane vesicles or most intact cells (see above). Theoretical analyses of model plant cells have been performed to evaluate the significance of these parameters in single-cell osmotic regulation. Water transport simulations showed that, if the water permeability of the PM remains low, an additional resistance at the TP will marginally determine the rate of cell equilibration (Maurel *et al,* unpublished results; Tyerman *et al.*, 1999). However, additional constraints for cell osmoregulation are imposed by the compartmentation of plant cells into cytosol and vacuole. The cytosol is the most critical compartment for most cell metabolic functions and requires very fine regulation of its volume and osmotic potential. Because it also has to mediate water exchange between the vacuole and the cell exterior, the cytosol is subject to possible volume fluctuations if water mobilization into and from the vacuole becomes limiting. A swelling or shrinking can then be expected in case of a sudden water influx or efflux, respectively (Fig. 2A). Although these processes are only transient, they can represent very rapid and drastic changes well beyond those that can be felt at the whole-cell or vacuole level. For instance, sudden exposure to hypotonicity

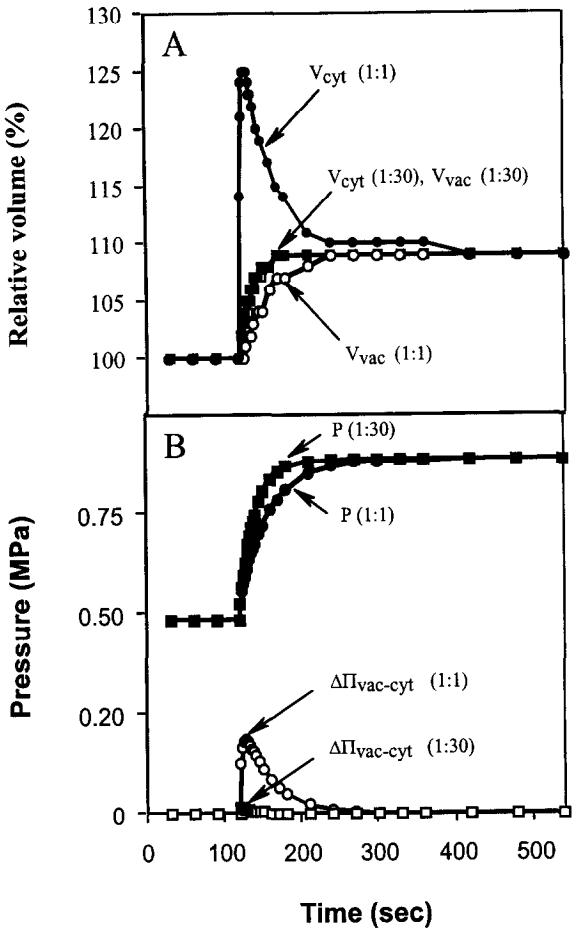

FIG. 2 Response of a model plant cell to hypotonicity. Water transport simulations were done essentially as described in Tyerman *et al.* (1999), considering a spherical cell with a spherical vacuole, and surrounded by a cell wall. The P_f of the PM was set to a constant value ($P_{f-PM} = 6\ \mu m\ sec^{-1}$; see Maurel *et al.*, 1997b) and two distinct P_f values for the TP (i.e., two cell configurations) were investigated: $P_{f-TP} = 6\ \mu m\ sec^{-1}$ (configuration 1:1) and $P_{f-TP} = 180\ \mu m\ sec^{-1}$ (configuration 1:30). For details on the equations used to model the cell, see Fig. 3 of Tyerman *et al.* (1999). (A) Variations of the relative volumes of the cytosol (V_{cyt}) and of the vacuole (V_{vac}) in response to an instantaneous change in external osmotic pressure, at time $t = 120$ sec, from 200 to 0 mOsmol kg^{-1} H_2O. (B) Variations in turgor pressure (P) and osmotic pressure gradient between the vacuole and cytosol ($\Delta\Pi_{vac-cyt}$) in response to the same hypotonic shock as in (A). Initial conditions were as follows: cell compartment volumes, $V_{cyt} = 0.05 \times V_{cell} = 1.68 \times 10^{-15}\ m^3$ and $V_{vac} = 0.95 \times V_{cell} = 3.18 \times 10^{-14}$; osmotic pressures, $\Pi_{ext} = 0.49$ MPa and $\Pi_{cyt} = \Pi_{vac} = 0.97$ MPa; cell volumetric elastic modulus, $\varepsilon = 4$ MPa; temperature, $T = 293$ K.

of a hypothetical tobacco cell with a low P_f (6 μm s^{-1}) both at the PM and at the TP can result in a + 15–25 % increase in cytosolic volume in <30 sec (Fig. 2A) (Tyerman *et al.*, 1999). In contrast, a cell with a higher P_f at the TP (180–600 μm s^{-1}) exhibited an increase in cytosolic volume of much reduced amplitude (+ 5–9 %) which developed over >150 sec (Fig. 2A) (Tyerman *et al.*, 1999). The water permeability of the plant PM *in vivo* may not be always minimal as in this model. This can be inferred from measurements in intact cells (see above) and from the possible up-regulation of PM aquaporins by phosphorylation. Simulation studies show, however, that a very efficient cytosol osmoregulation can be achieved as long as the TP has a ≥5-fold higher P_f than the PM (Maurel *et al.*, unpublished results).

Another view of the osmotic regulation properties needed by plant cells involves considering the dissipation of water potential (osmotic) gradients across the TP rather than volume equilibration of the cytoplasm (Fig. 2B). In fact, these two processes are linked since the amount of water that is exchanged between the vacuole and the cytoplasm is proportional to the osmotic gradient across the TP and contributes to its dissipation. Thus, simulations similar to those discussed above show that a high P_f at the TP allows quasi-isoosmotic water transport whatever the rate of water transport in and out of the cell and the vacuole (Fig. 2B). Therefore, physical constraints on the TP of plant cells, and activation of mechano- and osmo-sensitive ion channels, that have been described in this membrane (Alexandre and Lassalles, 1991), could be damped by the water transport properties of the TP.

b. Transcellular Water Transport. Under optimal growing conditions, enormous amounts of water (i.e., the equivalent of the plant fresh weight in a couple of hours) are taken up from the soil, flow across the living tissues of roots, the xylem vessels, the mesophyll, and are lost by transpiration. The differential regulation of water transport at the TP and PM of plant cells can also be physiologically relevant in this context.

Although the contribution of the transcellular path to the overall flow may be critical in certain tissues only, it is still assumed that most of the flow control resides at the PM. Because the plant vacuole can be bypassed by water flow across cytoplasmic strands, it is indeed not clear whether the vacuole can create a significant resistance to transcellular water flow. Thus, the water permeability of vacuoles will hardly determine the overall flow rate of water across tissues. However, it can be critical that the cytosol and vacuole remain in perfect osmotic equilibrium at all times, and this can be achieved if the TP has a high P_f. Transtissue water transport possibly fluctuates in response to environmental changes and concomitant modulation of stomatal aperture. As detailed above, the resistance of the vacuolar membranes is critical in determining the kinetic changes in water potential on both sides of the membrane. If the vacuolar membrane poses significant hydraulic resistance, any fluctuation in transcellular water flow would generate a

strong osmotic gradient, perturbate vacuolar volume, and result in a potential drag of the vacuole within the cell.

Thus, a unified model would propose that the plant TP be maintained in a state of high water permeability, whereas water transport at the PM can show marked regulation and determine the overall exchange of water between the cell and its exterior. It is assumed that this property can be maintained under various physiological conditions and plays an important role for single cell osmoregulation or under conditions where plant cells mediate an important transcellular water flow.

2. Solute Transport across the PM and Peribacteroid Membrane

The physiological significance of solute transport by aquaporins is uncertain, but may be relevant to their role in plant cell osmoregulation. Gerbeau *et al.* (1999) have proposed that the controlled transport of compatible solutes by TP aquaporins, such as tobacco NtTIPa, may provide a mechanism for long-term regulation of the respective volumes of the cytosol and the vacuole. Soybean NOD26 (Dean *et al.*, 1999) and a close homologue in *Lotus* (Guenther and Roberts, 2000) are other intracellular aquaporins that transport small solutes. They may contribute to dissipating osmotic gradients across the symbiosome membrane. Solute transporting aquaporins at the plant PM, similar to yeast Fps1 (Luyten *et al.*, 1995), may mediate the efflux of compatible solutes, thus allowing the down-regulation of turgor under hypoosmotic conditions. For either membrane, however, the physiological relevance of such transport remains speculative because none of the small molecules involved has clear physiological relevance.

3. Under Which Conditions Do Rapid and Significant Changes in Water Potential Occur?

The models for cell osmoregulation presented above imply that plant cells can experience rapid changes in water potential and/or volume. Under normal conditions, the volume of plant cells can be altered, but the processes involved occur over several tens of minutes (stomatal guard cell movements) or several hours or even days (cell elongation). There are, however, several types of environmental or developmental constraints where the models discussed above may apply.

Firstly, the transpiration regime of plants is determined in part by the water demand exerted onto the aerial parts. Rapid alterations in this demand, due to variations in wind regime, temperature, or light intensity, may occur and surpass the rapidity of stomatal regulations. These alterations are propagated through changes in tension in xylem vessels (Schneider *et al.*, 1997). Even more dramatic changes in tension also occur locally following vessel cavitation. *In situ* measurements using a cell pressure probe have shown that changes in xylem tension result in immediate and large amplitude alterations in the turgor of cells adjacent to the xylem vessels (Wegner and Zimmermann, 1998).

Secondly, root cells at the plant/soil interface can be exposed to drastic changes in water potential, upon sudden flooding of the soil after, for instance, prolonged drought. In certain plant species such as tobacco, root cells have a very low cell wall elastic modulus. This means that significant changes in root cell volume can be observed in response to a sudden decrease in soil solution osmotic potential (Tyerman *et al.*, 1989). A down-regulation of turgor was experimentally measured in this situation, suggesting that a release of ions or neutral solutes was triggered in response to hypotonicity (see above). Resurrection plants can lose more than 90% of their cell water upon exposure to drought (Ingram and Bartels, 1996). Subsequent tissue rehydration imposes severe osmotic constraints that may be overcome by the presence of aquaporins (Mariaux *et al.*, 1998).

The normal developmental cycle of plants also implies drastic desiccation and hydration processes. Seed germination is one such process. It is triggered by the imbibition of seed tissues and involves the hydrolysis of products stored in PSVs, their release in the cytosol, and a complete reorganization of the fragmented vacuolar apparatus to yield a large central vacuole. The water and possibly solute transport activity of seed-specific TP aquaporins may be critical for cytoplasm osmoregulation during these processes (Maurel *et al.*, 1997a). A role for PM aquaporins (PIPs) in the early stages of seed germination has also been proposed (Gao *et al.*, 1999). Priming is a treatment of plant seeds that can substantially improve their subsequent germination efficiency. For this, seeds are hydrated in the presence of polyethyleneglycol (PEG) or ABA and further desiccated prior to their conservation. Gao *et al.* (1999) investigated various priming treatments in rape seeds and found a strong correlation between the subsequent seed germination index and mRNA levels for a PIP homologue. It was suggested that strong expression of this aquaporin may facilitate tissue imbibition and/or counteract osmotic perturbations linked to this process (Gao *et al.*, 1999).

Pollen germination is yet another process which is critically dependent on water transport (Sarker *et al.*, 1988). A role for aquaporins in the self-incompatibility response of *Brassica* has been proposed, based on genetic evidence (Ikeda *et al.*, 1997). However, the finding that loss of self-incompatibility was associated with mutation of a PIP homologue has not received any clear physiological explanation.

B. Control of Transcellular Water Flow: The Example of Root Water Transport

1. General Mechanisms of Water Transport

One of the most interesting generalizations which can be made about roots is that water flow into roots can be modeled as though there were only a single membrane that water must cross on entry. In herbaceous species, the hydraulic

conductivity (L_p) of the whole root, as determined by osmotic experiments, is in the range of 10^{-7} m s^{-1} MPa^{-1} and is of the same order as the L_p of a single cell (Steudle, 2000; Steudle and Peterson, 1998). This may be surprising because roots are formed by several radial cell layers which potentially oppose a resistance to water flow between the soil solution and the xylem vessels. Singular properties of the root indicate, however, that this organ cannot be modeled by a single membrane barrier. The reflection coefficient for ionic compounds is far below unity, indicating that the root is not as selective as a single membrane barrier. The hydraulic conductance is dependent on the type of force applied, whether osmotic or hydrostatic, and increases with the amplitude of these forces (Steudle and Peterson, 1998).

When water moves radially into a root, there can be both apoplastic and cell-to-cell flow. In the apoplast, water movement is purely hydraulic; in the cell-to-cell route, which involves passage across cell membranes, there will be an osmotic and hydraulic component to the flow. There is a constant and rapid local water flow between compartments along the path, but the net driving force for water entry is a water potential gradient between the xylem vessels and the medium in which the roots are growing. At high rates of transpiration, there is a strong gradient of water potential, and the apoplastic route will predominate. At lower rates of flow (closed stomata, times of water shortage), interactions between water and solute flow should be more important (Steudle and Peterson, 1998).

Longitudinal (axial) flow along the xylem can be described by the Hagen–Poiseuille equation, which gives great importance to vessel diameter. Axial flow is generally regarded as having a much lower hydraulic resistance than radial flow (1–2 orders of magnitude for maize roots) and therefore does not contribute a significant resistance to total flow (Steudle and Peterson, 1998).

2. Anatomical Dissection of Root Water Transport

In root tips and young roots of certain species such as maize and onion (Frensch *et al.*, 1996; Peterson and Steudle, 1993), water flow can be described by a "uniform resistance" model, that is, the root L_p is constant along the specified region of the root, and cell L_p is not highly variable across the cortex. In more mature root tissue, an alternate "nonuniform resistance" model of water flow applies. Mature roots are characterized by the development of significant barriers to radial apoplastic water flow, such as a mature exodermis, and stelar tissues that surround the phloem and xylem vessels (Steudle and Peterson, 1998).

To understand the cellular mechanisms of root water transport, a major issue has been to map the resistance barriers for radial and axial water flow. Different approaches have been pursued to experimentally explore this idea and to identify which cell layer can be limiting for radial water transport. Zimmermann and

Steudle (1998), using maize plants grown in hydroponic or aeroponic conditions, found that plants grown aeroponically had reduced hydraulic conductivity. This change in root L_p was associated to the differentiation of a root exodermis, with Casparian bands which impose water-tight wall barriers.

Some workers have used apoplastic dyes to directly measure the extent of apoplastic water flow (e.g., rhodamine B; Skinner and Radin, 1994). These data need to be used with caution, since the dyes are generally large and do not move as freely as water through the apoplast. A good example of their use was the approach of Wan and Zwiazek (1999), who showed that the *proportion* of water flowing through the apoplast was increased after mercury treatment; they took this as evidence that the mercury effect genuinely reduced cell-to-cell flow rather than caused, some nonspecific effect on all paths of water movement.

In onion roots, cell hydraulic conductivities were similar in all root regions. Root hydraulic conductivity was, however, greater in the middle region (which had a mature exodermis with both Casparian bands and suberin lamellae), and in older root regions (in which the endodermis also had developed suberin lamellae), as compared with the root tips (which had Casparian bands in the endodermis and an immature exodermis (Barrowclough *et al.*, 2000)). Mercury effectively inhibited cell L_p in the youngest regions of onion roots, but not root L_p. In contrast, it did inhibit L_p in older regions of the root. This suggested that apoplastic flow predominated in the root tips. In older regions of the root, the mature exodermis provided a significant resistance to apoplastic water uptake, leaving only the mercury-sensitive cell-to-cell route across that cell layer (Barrowclough *et al.*, 2000).

3. Regulations with Aquaporins Involved?

Root water transport is regulated by various environmental factors, and the contribution of aquaporins to these regulations has been inferred, based mostly on their sensitivity to mercury inhibition.

In many plant species including maize, melon, tomato, and paprika, salinization with 50–100 mM NaCl reduces root L_p by >65% after 2–4 days (Azaizeh and Steudle, 1991; Carvajal *et al.*, 1999; Martinez-Ballesta *et al.*, 2000; Peyrano *et al.*, 1997) and, in maize and melon, this effect can be counteracted with the addition of 10 mM calcium chloride. More detailed measurements of cellular L_p in maize roots showed the same effects, but these were even more dramatical than on whole roots (Azaizeh *et al.*, 1992). In melon, the P_f of root protoplasts was reduced by 70–90% after 2 days of salt treatment, and these effects were antagonized by calcium (Carvajal *et al.*, 2000; Martinez-Ballesta *et al.*, 2000). These data suggested that a major effect of salinity was on transcellular water transport. The role of aquaporins has been further proposed based on the observations that, under salinity conditions, the reduced L_p of roots and of isolated protoplasts became independent of mercury inhibition. These results are consistent with the transient down-regulation of the

water channel message observed in *M. crystallinum* (Yamada *et al.*, 1995), but other regulatory mechanisms such as calcium-dependent phosphorylation of aquaporins may be involved (Carvajal *et al.*, 2000; Johansson *et al.*, 1998; Martinez-Ballesta *et al.*, 2000). More generally, it is assumed that down-regulation of root L_p under water stress conditions may allow the plant to limit a backflow of water into the soil when the driving force for water uptake is reduced because of limited transpiration (Steudle, 2000).

Anaerobiosis is another stress condition experienced by roots. It is associated with flooding or with mechanical compression of the soil and results in a decrease in root L_p (Birner and Steudle, 1993; Zhang and Tyerman, 1991, and references cited therein). Cell pressure probe measurements demonstrated that part of the control occurs at the cell membrane level (Zhang and Tyerman, 1991). These effects could be mimicked by NaN_3 treatment. Both anaerobiosis and NaN_3 induced a decrease in the apparent osmotic volume of the cells, as estimated with a cell pressure probe (Zhang and Tyerman, 1991). From this, it was deduced that the reduction in cell L_p may follow from a closure of plasmodesmata. In more recent studies (Zhang and Tyerman, 1999), it was shown that hypoxic or NaN_3 treated cells became insensitive to mercury inhibition, and the idea that water channels are down-regulated under these conditions seems to be favored at present.

Nutrient deficiency is yet another condition that induces a down-regulation of root L_p (Clarkson *et al.*, 2000). In wheat, deprivation of nitrogen or phosphorus resulted in a reversible 80–85% reduction in root L_p, similar in amplitude to the inhibition observed after mercury treatment (Carvajal *et al.*, 1996). In addition, the L_p of nutrient-deprived roots was insensitive to mercury inhibition, further suggesting that water channel down-regulation was occurring under these conditions. The mechanisms that allow plant roots to sense nutrient deficiency before any metabolic inhibition occurs have been recently discussed by Clarkson *et al.* (2000) but remain unknown.

Diurnal fluctuation of root L_p has also been observed in numerous plant species (Carvajal *et al.*, 1996; Clarkson *et al.*, 2000; Henzler *et al.*, 1999). In *Lotus corniculatus*, these variations were paralleled by a diurnal fluctuation in the expression of mRNAs homologous to an *Arabidopsis pip1a* probe (Clarkson *et al.*, 2000; Henzler *et al.*, 1999). This provides, besides mercury inhibition (Carvajal *et al.*, 1996), a different line of argument to link aquaporin expression and/or activity with the regulation of root water uptake.

Despite the numerous stress conditions that have been supposed to involve water channel regulation, it should be borne in mind that root anatomy is relatively plastic according to the growing conditions of the plant. For instance, water deficit induces the differentiation of an exodermis and promotes root growth as compared to that of shoot (Steudle, 2000). Thus, it is necessary to carefully evaluate to what extent anatomical changes may be affecting water flow, distinct from aquaporin-mediated effects. In general, a time course may help to distinguish between short-term physiological responses and longer-term anatomical changes.

C. Conclusion

Root water transport clearly represents a paradigm with which to investigate the role of plant aquaporins, but the amazing diversity of aquaporins and their expression throughout the plant can allow us to speculate that these proteins play a critical role in many other physiological processes. Functional genomics now provides new tools to address gene functions in large multigene families (Gura, 2000). Original functions for aquaporins, such as facilitating CO_2 diffusion in leaf tissues (Tyerman *et al.*, 1999), or embolism repair in xylem vessels (Holbrook and Zwieniecki, 1999), have also been proposed and will have to be experimentally explored. The role of water uptake in cell expansion has been largely controversial (Cosgrove, 1993), but the intense expression of aquaporins in elongating tissues provides a strong reason to revisit this question. Thus, there is still a critical need to combine molecular biology, genetics, and biophysics to develop an integrated concept of aquaporin function in whole-plant physiology. This effort should test our presumption that aquaporins are relevant targets for plant improvement and biotechnology.

Note added in proof. A description of 35 full-length *MIP* genes in *Arabidopsis* has recently appeared (Johanson *et al.*, 2001, *Plant Physiol.* **126,** 1358–1369). A new nomenclature was proposed and the *Arabidopsis* MIPs mentioned in the present review will have to be renamed accordingly.

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