Acta Physiol 2006, **187**, 169–176

REVIEW

Aquaporins in plants

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Received 10 November 2005, accepted 31 January 2006
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Abstract

Although very often exposed to a rapid changing environment, plants are in general unable to evade from unfavourable conditions. Therefore, a fine tuned adaptation of physiology including the water balance appears to be of crucial importance. As a consequence a relatively large number of aquaporin genes are present in plant genomes. So far aquaporins in plants were shown to be involved in root water uptake, reproduction or photosynthesis. Accordingly, plant aquaporin classification as simple water pores has changed corresponding to their molecular function into channels permeable for water, small solutes and/or gases. An adjustment of the respective physiological process could be achieved by regulation mechanisms, which range from post-translational modification, molecular trafficking to heteromerization of aquaporin isoforms. Here the function of the four plant aquaporin family subclasses with regard to substrate specificity, regulation and physiological relevance is described.

Keywords gas transport, plant-aquaporin, water channel.

Water is the most important molecule in any kind of living cells. An efficient regulation of water supply is essential for many biological processes. Aquaporins (AQPs) are a family of small pore forming integral membrane proteins which facilitate the transport of small molecules such as water and glycerol, or volatile substances like CO_2 or NH_3 (Nakhoul *et al.* 1998, 2001, Biela *et al.* 1999, Uehlein *et al.* 2003). They were described in diverse archea (Kozono *et al.* 2003), eubacterial and eukaryotic species (Park & Saier 1996).

In biological membranes aquaporins occur as tetramers and each monomer operates as a separate water channel. The monomer is composed of a characteristic conserved arrangement of six membrane-spanning helices linked by three extra- and two intra-cellular loops. Both the N and C termini facing the cytosol (Borgnia *et al.* 1999a,b). In the pore region a highly conserved amino acid-motive (asparagine-proline-alanine, NPA) appearing twice forms a selective threshold. Hydrophobic regions near the NPA motives are rate-restrictive water barriers and reduce interactions between water molecules. Together with an aromatic/arginine-region, an effective proton filter, and the NPA motives the protein possess a two-stage filter.

The first aquaporin described was the mammalian AQP1. It was found in erythrocytes and renal tubuli facilitating the osmotical driven permeation of water across membranes (Denker *et al.* 1988, Preston & Agre 1991). It was classified into a large superfamily of intrinsic membrane proteins named major intrinsic proteins (MIP) according to the prototype from bovine lens. Because of the discovery of various aquaporin homologues in different organisms and according to their respective function, the MIP superfamily was subdivided into two major protein family clusters: the water selective channels (aquaporins, AQP) and glycerol-transporting homologues (glycerol-uptake facilitator like proteins, GLP) with varying water permeabilities (Heymann & Engel 1999).

A distinctive diversification of the protein family was obtained in vertebrates and higher plants (Fig. 1). By phylogenetic analysis, animal AQPs were classified into nine groups (AQP0-2, 4–6, 8, 11, 12). GLPs were divided in four main groups (AQP3, 7, 9, 10) (Zardoya

 doi: 10.1111/j.1748-1716.2006.01563.x

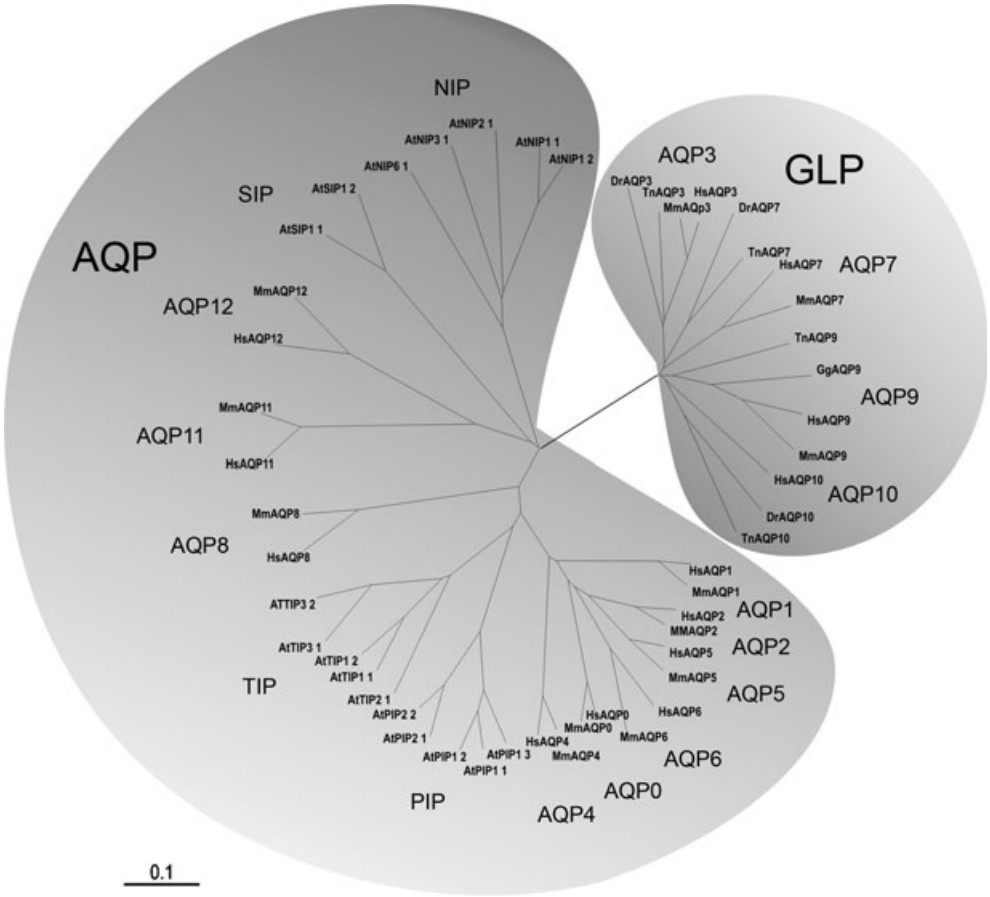

Figure 1 Phylogenetic tree presents the evolutionary relationship of mammalian and plant aquaporins in the aquaporin (AQP) cluster of major intrinsic protein family. Plant AQPs are divided into the four subfamilies plasma membrane intrinsic proteins (PIP), tonoplast intrinsic proteins (TIP), nodulin 26-like intrinsic proteins (NIP) and small intrinsic proteins (SIP). Representatives of the GLP cluster lacking in plants.

2005). The GLP orthologous are not abundant in plants.

However, 35 different AQP genes were identified in *Arabidopsis* (Johanson *et al.* 2001) and in maize 33 MIP like isoforms were identified by transcriptome analysis (Chaumont *et al.* 2001). Higher plant aquaporins are subdivided into four subfamilies, the tonoplast intrinsic proteins (TIPs), nodulin 26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs) (Johanson & Gustavsson 2002) and plasma membrane intrinsic proteins (PIPs). In this review we focus on the properties of the four aquaporin subfamilies of higher plants (Table 1). In particular we will discuss their physiological role in planta and during different regulation mechanisms. Beside the water permeability, the gas conductivity of plant aquaporins is of rising importance and will be discussed on CO_2 and NH_3 permeability. CO_2 permeability as demonstrated for distinct aquaporins implies a function in one of the most important metabolic process in plants, the photosynthesis.

Higher plant aquaporins

Tonoplast intrinsic proteins

The plant vacuole is a cellular storage compartment with functions in turgor regulation, cell signalling and degradation. High flux rates of water and small solutes across the vacuolar membrane (tonoplast) suggested a contribution of aquaporins. In 1999 Gerbeau *et al.* analysed water transport rates in purified tobacco tonoplast vesicles and showed an increased permeability of urea or glycerol compared with plasma membrane vesicles. A cDNA encoding a TIP homologue (NtTIPa) was isolated and the protein was found to be localized in the tonoplast membrane. Functional expression of NtTIPa in *Xenopus* oocytes indicated that this protein was permeable to water, urea and glycerol. Likewise different TIP isoforms were isolated from *Arabidopsis thaliana* and were characterized in the oocyte system as urea permeases (Liu *et al.* 2003). Thus, in addition to their role as water channels, TIPs were assigned to

  doi: 10.1111/j.1748-1716.2006.01563.x

Table 1 Properties of members of higher plant aquaporin subfamilies

Plant aquaporin subfamily	Localization		Physiological functions	Regulation
	Tissue	Subcellular		
TIP	Seed, leaf, root	Vacuolar membranes (tonoplast)	Transport of water, urea, NH_3/NH_4^+	Phosphorylation
NIP	Root nodules	Peribacteroid membrane	Transport of water, glycerol Transport of glycerol	Phosphorylation, protein-protein interaction
SIP	–	Endoplasmic reticulum membrane	–	–
PIP				
	Roots, shoot, leafs, florescence	Plasma membrane	Transport of CO_2, glycerol, water	Hetreromerization of PIP1 and PIP2 monomers, Ca^{2+}, pH, molecular trafficking, phosphorylation

be important for equilibrating urea concentrations between different cellular compartments.

Nodulin 26-like intrinsic proteins

The soybean nodulin 26 was the first described member of the NIP subfamily. It was found to be expressed during the formation of symbiotic nitrogen fixing root nodules (Fortin *et al.* 1987). NIPs are multifunctional transporters and their suggested function is to mediate the bidirectional flux of water, glycerol, NH_3, and other small solutes between plant cytoplasm and symbiotic bacteroids. Homology modelling indicated that the pore selectivity region of the nine NIP genes from *A. thaliana* are organized into two subgroups (Wallace & Roberts 2004). The NIP group I (NIP1;1, NIP1;2, NIP2;1, NIP3;1, NIP4;1, NIP4;2) is similar to nodulin 26 and the proteins function as aquaglyceroporins. Many NIP I exhibit a CDPK phosphorylation site at the carboxyl terminus (Weaver & Roberts 1992). Phosphorylation of soybean nodulin 26 for example is stimulated in response to water deficit, resulting in enhanced transport activity (Guenther *et al.* 2003). Interacting proteins that recognize the carboxy-terminal region of nodulin 26 were identified, suggesting that NIP I proteins may possess a protein binding epitope that might modulate NIP function (Biswas 2004). In the NIP group II (NIP5;1, NIP6;1, NIP7;1), the aromatic Arginine selectivity filter close to the NPA pore region differs to that of NIP I and the proteins do not exhibit the conserved CDPK phosphorylation site. Functional analysis of NIP6;1 confirmed its glyceroporin function and revealed an unusually low water permeability. The amino and carboxyl terminal regions of NIP5;1, 6;1 and 7;1 share consensus phosphorylation sequences for MAP kinases. Taken together, it is evident that the NIP subfamily can be divided into two structurally and functionally distinct groups with different transport selectivity and potentially distinct regulatory properties.

Small intrinsic proteins

The SIP subfamily comprises not only the smallest molecules but is also the smallest family cluster in plants. The SIP family was identified by database mining and phylogenetic analysis (Johanson & Gustavsson 2002). Proteins of this subfamily are highly basic proteins. The main reason for their small size is a very short cytosolic N-terminal region compared with other plant MIPs. The SIP N-terminal region resembles that of AqpZ from *Escherichia coli*.

Current publications report about localization studies with fusions of *Arabidopsis* SIPs to green fluorescent protein (GFP) expressed in suspension cultured cells (Ishikawa *et al.* 2005). Strong fluorescence signals from GFP–SIP fusion were detected in the ER and not in the plasma membrane or tonoplast. By heterologous expression in yeast and vesicle permeability studies, SIP1;1 and SIP1;2 from *Arabidopsis* were characterized as aquaporins, while SIP2;1 showed minor water conductance. Until now, this was the first approach in analysing the SIP physiological function and substrate specificity.

Plasma membrane intrinsic proteins

The plasma membrane intrinsic proteins (PIP) represent the subfamily with the largest number of members. It consists of 13 members in *Arabidopsis* and maize. As the subfamily name indicates, the majority of PIPs is localized in the plasma membrane. The PIP subfamily can be divided into two phylogenetic groups, named PIP1 and PIP2. They differ in length of N- and

 doi: 10.1111/j.1748-1716.2006.01563.x

C-termini and in water permeability characteristics as analysed in different heterologous expression systems. PIP2 aquaporins usually induced comparably higher water permeability than PIP1 in the same assay.

PIP1 subfamily. As indicated by sequence comparison, the amino acid residues at the selectivity filter were similar in PIP1 and PIP2 aquaporin isoforms. However, their permeability and cellular function seems different. Although, in different functional characterization assays PIP1 aquaporins displayed very low water permeability, a participation in plant water relations was indicated by the results of a number of experiments. Decreasing NtAQP1 transcripts in tobacco plants by means of RNA antisense expression resulted in reduced root hydraulic conductivity and lower water stress resistance (Siefritz *et al.* 2002). In studies analysing PIP1 antisense *Arabidopsis* plants, also a decreased hydraulic conductivity in roots and root protoplasts was observed. Moreover, in these plants the hydraulic conductance and transpiration rate recovered slower after drying and rewatering cycles. For protoplasts from tobacco or *Arabidopsis* PIP1 antisense plant root cells a lower water permeability than for protoplasts from controls was obtained, indicating for a significance in root water transport (Martre *et al.* 2002).

In contrast to results in planta, functional studies of heterologous expressed PIP1 isoforms in *Xenopus* oocytes or other expression systems revealed no or very low aquaporin activity (Biela *et al.* 1999, Dean *et al.* 1999, Chaumont *et al.* 2000). None the less, permeability for other small solutes like glycerol, urea or gases like CO_2 or NH_3 was observed. Accordingly, PIP1 aquaporins could be transporters for small solutes and/or gases, or they need to be activated in the plant in order to function as water channels. A regulation mechanism such as phosphorylation was demonstrated for different plant aquaporin isoforms and could be one of the molecular mechanisms modifying plant aquaporin activity (Johansson *et al.* 1998, Tornroth-Horsefield *et al.* 2005).

The PIP1 isoforms in tobacco and maize were localized in nearly all parts of the plant. Roots and leaves differ in morphology and physiological function, e.g. with regard to water or CO_2 conductance. Thus, in planta, cells more permeable for water or gas could be distributed differently and it can be speculated that the PIP1 aquaporin function is modified according to the requirements of the respective tissue or cells. In leaf cells PIP1s might act as transporters for small solutes or gases. In root plasma membranes the same protein type could display water channel activity mediated by modifications or interaction with other aquaporins (see below). Western blots with an NtAQP1 antibody led to signals of different size depending on the origin of proteins, i.e. from roots or leaves. In the former an additional band was observed besides the aquaporin-specific 28 kDa. This slightly larger signal was not abundant in leaf protein preparations and could be interpreted as a result of a post-translational modification in roots.

PIP2 subfamily. In many studies aquaporins of the PIP2 subfamily exhibited more efficient water channel activity than members of the PIP1 cluster, which could be due to the different molecular structure of PIP2s in comparison with PIP1 isoforms. The former have a shorter amino-terminal extension, a longer carboxy-terminal end and in an additional stretch of eight amino acids located in the first extracytosolic loop. In different heterologous expression systems PIP2 aquaporins established five to 20-fold increased water permeability compared with control values (Daniels *et al.* 1994, Weig *et al.* 1997, Johansson *et al.* 1998).

To date members of the PIP2 subfamily were functionally characterized in different species and a role in different physiological processes was assumed. The proteins could be involved in cellular water transport in roots, leaves (Martre *et al.* 2002, Lopez *et al.* 2003), reproductive organs (Bots *et al.* 2005a,b) and seed germination (Schuurmans *et al.* 2003). A member of the PIP2 family with an extraordinary high water channel activity when expressed in *Xenopus* oocytes was found in the leguminous Mimosa tree *Samanea saman* (Moshelion *et al.* 2002). Functional characterization of SsAQP2 induced up to 20-fold increased P_f values compared with oocytes expressing SsAQP1 affiliated to the PIP1 family. The water permeability could be inhibited by $HgCl_2$ and additionally by millimolar concentrations of phloretin, another transport blocker (Dordas *et al.* 2000). In plants, SsAQP2 was found in the pulvini, which are motor organs responsible for the movement of leafs and leaflets.

Sequence comparison of maize EST clones led to the identification of a member of the PIP2 family named ZmPIP2;1 (ZmPIP2b; Chaumont *et al.* 2000). Transient expression in *Xenopus* oocytes increased the P_f of the membranes eightfold above water-injected control cells. $HgCl_2$ reversibly inhibited its water channel activity.

CO_2 permeability of aquaporins

In general, gases such as NH_3 or CO_2 simply cross membranes by diffusion through the membrane lipids. Because some cell membranes exhibit different gas permeability, it was suggested that in the case of an increased gas transport rate, it was mediated by aquaporins (Prasad *et al.* 1998, Terashima & Ono 2002).

  doi: 10.1111/j.1748-1716.2006.01563.x

CO_2 transport capability was initially demonstrated for the human AQP1 (Nakhoul *et al.* 1998) and could be associated to a gas channel function. Effects of the membrane lipid composition or expression pattern of intrinsic genes that could modify oocyte CO_2 permeability were excluded (Cooper & Boron 1998). However, physiological consequences of AQP1 facilitated CO_2 transport are still a matter of debate (Cooper *et al.* 2002). As AQP1 knock-out mice were similar with regard to CO_2 exchange rates (Sun *et al.* 2001, Fang *et al.* 2002) a significant role of aquaporins in animal CO_2 transport was challenged. On the other hand, results obtained on human erythrocytes at low chemical CO_2 gradients, demonstrated that nearly the entire CO_2 transport across the membrane was mediated by AQP1 and the $HCO_3^-Cl^-$ transporter (Blank & Ehmke 2003). In conclusion it was hypothesized that some aquaporins may function as high affinity CO_2 transporters in the erythrocyte membrane. Taken together, the situation in animals appears rather controversy because diverse tissues, cells and membranes were analysed and different experimental setups were applied.

Physiological studies in plants provided evidences for relevance of an aquaporin mediated CO_2 transport. Tobacco plants with an increased or decreased intrinsic aquaporin expression were changed in attributes towards water transport as well as CO_2 dependent processes like photosynthesis (Siefritz *et al.* 2002). When *Vicia faba* or *Phaseolous vulgaris* leaf discs were treated with minimum concentrations of $HgCl_2$, the hydraulic permeability of the plasma membrane was decreased by 70–80%. In a similar matter, photosynthetic CO_2 fixation and conductance of CO_2 from the intercellular spaces to the chloroplast stroma were restricted by mercury treatment. Although, the application of heavy metals should be considered with the same carefulness as in experiments investigating water conductivity, it was assumed that the photosynthetic CO_2 uptake across the plasma membrane of the mesophyll cells was facilitated by $HgCl_2$ sensitive aquaporins (Terashima & Ono 2002). Under favourable growth conditions *Arabidopsis* AtPIP1;2 (AtPIP1b) overexpressing tobacco plants revealed significant increased transpiration rate, higher stomatal density and a greater photosynthetic efficiency (Aharon *et al.* 2003). Nevertheless, the authors did not relate the effects to an increase in CO_2 transport rate, but to facilitated water transport.

Functional characterization of tobacco NtAQP1 in *Xenopus* oocytes indicated for a high conductance to CO_2 (Uehlein *et al.* 2003). The oocytes were injected with NtAQP1 cRNA and carbonic anhydrase, which accelerates the conversion of CO_2 to HCO_3^-. In this experimental set up a decrease of intra-cellular pH indicates the transport of CO_2 into the oocyte. CO_2 membrane transport is rate limiting for HCO_3^- accumulation rather than the conversion reaction to HCO_3^-. For NtAQP1 it was found that CO_2 uptake was 45% higher compared with control oocytes injected with water.

CO_2 transport was suggested for PIP2 aquaporins, too. The barley aquaporin HvPIP2;1 was overexpressed in rice in order to examine if members of the PIP2 subfamily contribute to facilitated CO_2 transport (Hanba *et al.* 2004). The internal conductance for CO_2 diffusion (g_i) and CO_2 assimilation rate was determined on intact leaves by concurrent measurements of gas exchange and carbon isotope ratio. It was found that g_i strongly related to rate of HvPIP2;1 expression and the results were interpreted in a way that HvPIP2;1 has a role in CO_2 diffusion in rice leaves. However, it remained to be determined whether the correlation between aquaporin expression and CO_2 permeability increase was just a side-effect or causative to HvPIP2;1 expression.

NH_3 permeability of aquaporins

Besides the debate about CO_2 conductivity there is also a lively discussion on ammonia (NH_3) and ammonium (NH_4^+) permeability of aquaporins and recent studies led to novel insights to this issue. Plant ammonium uptake at low extracellular concentration is catalysed by members of the ammonium transporter/methylammonium permease (AMT/Mep) family (Ninnemann *et al.* 1994), but there is also evidence from inhibitor studies in plants in favour of an NH_3 permeability by aquaporins (Niemietz & Tyerman 2000). Using functional complementation of yeast ammonium transport mutant (Dmep1–3), three wheat (*Triticum aestivum*) TIP2 aquaporins were characterized, which complement the effect of the deletion mutations on growth medium with reduced ammonium supply (2 mM). When expressed in oocytes an additional conductivity for the NH_4^+ analogues methylammonium and formamide was registered. Homology modelling of the TIP2 combined with data from site directed mutagenesis and electrically measurements suggested that NH_3 enters the pore, is protonated and released as NH_4^+ (Jahn *et al.* 2004). The specific TIP2 seems to fulfil the requirements for the predicted low affinity NH_4^+ transporter. In a recent study the mammalian aquaporins AQP8, AQP9, AQP3, AQP1 and the plant aquaporin TaTIP2;1 (see above) were expressed in *Xenopus* oocytes to analyse the transport of NH_3 and NH_4^+ in a solution-exchange chamber or voltage clamp conditions (Holm *et al.* 2005). In order to investigate NH_3 conductivity, the aquaporin expressing oocytes were placed in a well stirred bathing medium with low buffer capacity. NH_3 transport into the oocytes was accompanied by an

 doi: 10.1111/j.1748-1716.2006.01563.x

acidification of the bathing solution. By employment of this technique AQP8, AQP9, AQP3 and TaTIP2;1 could be identified as ammonia transporters. NH_4^+ transport was measured via voltage clamp techniques and confirmed NH_4^+ conductance for AQP8, AQP9, AQP3 and TaTIP2;1. Physiological relevance was suggested for the mammalian aquaporins. AQP8 can be found in mitochondria of hepatocytes (Ferri *et al.* 2003), suggesting involvement in NH_4^+ uptake to supply the urea cycle. In heptatocyte plasma membrane AQP9 may play a role in NH_4^+ transport from the blood into the periportal heptatocyte. AQP3 is proposed to be involved in acid secretion by NH_4^+ across the collecting duct epithelium in the kidney. The suggested functions were supported by phenotypes of knock out mice, but still have to be further confirmed to asses a function of aquaporin mediated NH_4^+ transport in animal physiology. A physiological function of the plant aquaporin TaTIP2;1 has not been circumstantiated to date.

Regulation of aquaporins

On view to the high diversity of plant aquaporins several regulation mechanisms could be conceivable. Post-transcriptional modification like phosphorylation was shown for AtTIP3;1 (α-TIP) from *Arabidopsis* (Maurel *et al.* 1995) and the soybean nodulin 26 as mentioned above. In recent studies the crystal structure of the tetrameric protein of *Spinacia oleracea* PIP2;1 [PM28A, (Johansson *et al.* 1996)] could be obtained (Kukulski *et al.* 2005). The 3D structure of the tetramer suggests a novel mechanism for the regulation of distinct aquaporins. In oocyte expression assays PIP2;1 was shown to be activated by phosphorylation at two serine residues. Moreover, phosphorylation of one of these residues by a plasma membrane-associated Ca^{2+}-dependent protein kinase was demonstrated in response to a high water potential gradient (Johansson *et al.* 1998). Besides the suggested phosphorylation at several serine residues a highly conserved cystein (Cys) at the C terminus of loop A is proposed to be involved in regulation of the channel. Together with three Cys residues at the N-terminal part of helix 2, the conserved Cys may stabilize the SoPIP2;1 monomer by hydrogen bonds or complexing a metal ion initiating an opening or closure of the protein.

The plasma membrane permeability appears also to be influenced by divalent cations and the pH (Gerbeau *et al.* 2002). Cell hydraulic conductivity (L_p) was measured on *Arabidopsis* suspension cells using a cell pressure probe and varying bathing solutions. Ca^{2+} added to the pipette and bathing solution reduced L_p fourfold. The results were confirmed on purified plasma membrane vesicles and stopped flow spectrophotometer measurements. Furthermore H^+ was shown to reversible decrease water channel activity. This led to the assumption that divalent cations and the pH influences directly membrane permeability and may allow coupling of water transport to cell signalling and metabolism.

Intra-cellular acidification through anoxic stress led to a decrease in water permeability of root cell membranes in *Arabidopsis* (Tournaire-Roux *et al.* 2003). In experiments expressing *Arabidopsis* aquaporins in *Xenopus* oocytes a drop of intra-cellular pH resulted in a diminishment of water conductance implicating a closure of aquaporins by protons. A histidin (His) residue at position 197 in Loop D of AtPIP2;2 was identified to be the major pH-sensing site under physiological conditions (Tournaire-Roux *et al.* 2003, Chaumont *et al.* 2005). In a structural model of AtPIP2;2 with protonated His^{197}, Loop D is folded over the pore and caused the closure of the protein.

Molecular trafficking is also an optional regulation mechanism of aquaporins. The regulation and redistribution of a TIP (McTIP1;2) from *Mesymbrytanthemum crystallinum* (ice plant) was investigated by sucrose density gradient separation and immunofluorescence microscopy (Vera-Estrella *et al.* 2004). Osmotic stress induces a delocalization of the protein from the tonoplast to other membrane structures and could be blocked by inhibitors of vesicle trafficking-related processes. The early effects of osmotic stress on aquaporin expression and regulation were further analysed by exposure of *Arabidopsis* roots to salt (Boursiac *et al.* 2005). A decrease of AQP mRNA could be recognized after 2–4 h after application of salt. The protein abundance decrease was different within the aquaporin subfamilies. The amount of PIP1 protein decreased within 30 min, whereas members of PIP2 and TIP subfamily showed reduction after 6 h of treatment. Results from fusion with GFP indicated for relocation of TIP and PIP into intra-cellular spherical structures after 45 min and 2 h, respectively. The trafficking of the mammalian AQP2 is well ascertained in apical membranes of kidney collecting duct epithelia. AQP2 containing vesicles were routed to the membrane in response to a hormonal stimulus (Brown 2003).

Above and beyond the regulation by mechanisms mentioned above, a multimerization of membrane proteins can regulate their activity and function (Veenhoff *et al.* 2002). Coexpression of maize ZmPIP1;2 (PIP1) and different PIP2 isoforms (ZmPIP2;1/2;3/2;4/2;5) in *Xenopus* oocytes resulted in an increase in P_f compared with individual expression of the PIP2 isoform (Fetter *et al.* 2004). The authors assume, that the increased P_f indicates that heteromerization of PIP1 and PIP2 isoforms is required for both to act as functional water channels. Heteromerization was also demonstrated for *Mimosa pudica* aquaporins PIP1;1 and PIP2;1 (Temmei *et al.* 2005).

  doi: 10.1111/j.1748-1716.2006.01563.x

References

Aharon, R., Shahak, Y., Wininger, S., Bendov, R., Kapulnik, Y. & Galili, G. 2003. Overexpression of a plasma membrane aquaporin in transgenic tobacco improves plant vigor under favorable growth conditions but not under drought or salt stress. *Plant Cell* **15**, 439–447.

Biela, A., Grote, K., Otto, B., Hoth, S., Hedrich, R. & Kaldenhoff, R. 1999. The *Nicotiana tabacum* plasma membrane aquaporin NtAQP1 is mercury-insensitive and permeable for glycerol. *Plant J* **18**, 565–570.

Biswas, S. 2004. Functional properties of soybean nodulin 26 from a comparative three-dimensional model. *FEBS Lett* **558**, 39–44.

Blank, M.E. & Ehmke, H. 2003. Aquaporin-1 and HCO3(−)-Cl− transporter-mediated transport of CO2 across the human erythrocyte membrane. *J Physiol* **550**, 419–429.

Borgnia, M., Nielsen, S., Engel, A. & Agre, P. 1999a. Cellular and molecular biology of the aquaporin water channels. *Annu Rev Biochem* **68**, 425–458.

Borgnia, M.J., Kozono, D., Calamita, G., Maloney, P.C. & Agre, P. 1999b. Functional reconstitution and characterization of AqpZ, the E. coli water channel protein. *J Mol Biol* **291**, 1169–1179.

Bots, M., Feron, R., Uehlein, N., Weterings, K., Kaldenhoff, R. & Mariani, T. 2005a. PIP1 and PIP2 aquaporins are differentially expressed during tobacco anther and stigma development. *J Exp Bot* **56**, 113–121.

Bots, M., Vergeldt, F., Wolters-Arts, M., Weterings, K., Van As, H. & Mariani, C. 2005b. Aquaporins of the PIP2 class are required for efficient anther dehiscence in tobacco. *Plant Physiol* **137**, 1049–1056.

Boursiac, Y., Chen, S., Luu, D.T., Sorieul, M., Van Den Dries, N. & Maurel, C. 2005. Early effects of salinity on water transport in *Arabidopsis* roots. Molecular and cellular features of aquaporin expression. *Plant Physiol* **139**, 790–805.

Brown, D. 2003. The ins and outs of aquaporin-2 trafficking. *Am J Physiol Renal Physiol* **284**, F893–F901.

Chaumont, F., Barrieu, F., Jung, R. & Chrispeels, M.J. 2000. Plasma membrane intrinsic proteins from maize cluster in two sequence subgroups with differential aquaporin activity. *Plant Physiol* **122**, 1025–1034.

Chaumont, F., Barrieu, F., Wojcik, E., Chrispeels, M.J. & Jung, R. 2001. Aquaporins constitute a large and highly divergent protein family in maize. *Plant Physiol* **125**, 1206–1215.

Chaumont, F., Moshelion, M. & Daniels, M.J. 2005. Regulation of plant aquaporin activity. *Biol Cell* **97**, 749–764.

Cooper, G.J. & Boron, W.F. 1998. Effect of PCMBS on CO2 permeability of *Xenopus* oocytes expressing aquaporin 1 or its C189S mutant. *Am J Physiol* **275**, C1481–C1486.

Cooper, G.J., Zhou, Y., Bouyer, P., Grichtchenko, I.I. & Boron, W.F. 2002. Transport of volatile solutes through AQP1. *J Physiol* **542**, 17–29.

Daniels, M.J., Mirkov, T.E. & Chrispeels, M.J. 1994. The plasma membrane of *Arabidopsis thaliana* contains a mercury-insensitive aquaporin that is a homolog of the tonoplast water channel protein TIP. *Plant Physiol* **106**, 1325–1333.

Dean, R.M., Rivers, R.L., Zeidel, M.L. & Roberts, D.M. 1999. Purification and functional reconstitution of soybean nodulin 26. An aquaporin with water and glycerol transport properties. *Biochemistry* **38**, 347–353.

Denker, B.M., Smith, B.L., Kuhajda, F.P. & Agre, P. 1988. Identification, purification, and partial characterization of a novel Mr 28,000 integral membrane protein from erythrocytes and renal tubules. *J Biol Chem* **263**, 15634–15642.

Dordas, C., Chrispeels, M.J. & Brown, P.H. 2000. Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. *Plant Physiol* **124**, 1349–1362.

Fang, X., Yang, B., Matthay, M.A. & Verkman, A.S. 2002. Evidence against aquaporin-1-dependent CO2 permeability in lung and kidney. *J Physiol* **542**, 63–69.

Ferri, D., Mazzone, A., Liquori, G.E., Cassano, G., Svelto, M. & Calamita, G. 2003. Ontogeny, distribution, and possible functional implications of an unusual aquaporin, AQP8, in mouse liver. *Hepatology* **38**, 947–957.

Fetter, K., Van Wilder, V., Moshelion, M. & Chaumont, F. 2004. Interactions between plasma membrane aquaporins modulate their water channel activity. *Plant Cell* **16**, 215–228.

Fortin, M.G., Morrison, N.A. & Verma, D.P. 1987. Nodulin-26, a peribacteroid membrane nodulin is expressed independently of the development of the peribacteroid compartment. *Nucleic Acids Res* **15**, 813–824.

Gerbeau, P., Güçlü, J., Ripoche, P. & Maurel, C. 1999. Aquaporin Nt-TIPa can account for the high permeability of tobacco cell vacuolar membrane to small neutral solutes. *Plant J* **18**, 577–587.

Gerbeau, P., Amodeo, G., Henzler, T., Santoni, V., Ripoche, P. & Maurel, C. 2002. The water permeability of *Arabidopsis* plasma membrane is regulated by divalent cations and pH. *Plant J* **30**, 71–81.

Guenther, J.F., Chanmanivone, N., Galetovic, M.P., Wallace, I.S., Cobb, J.A. & Roberts, D.M. 2003. Phosphorylation of soybean nodulin 26 on serine 262 enhances water permeability and is regulated developmentally and by osmotic signals. *Plant Cell* **15**, 981–991.

Hanba, Y.T., Shibasaka, M., Hayashi, Y., Hayakawa, T., Kasamo, K., Terashima, I. & Katsuhara, M. 2004. Overexpression of the barley aquaporin HvPIP2;1 increases internal CO(2) conductance and CO(2) assimilation in the leaves of transgenic rice plants. *Plant Cell Physiol* **45**, 521–529.

Heymann, J.B. & Engel, A. 1999. Aquaporins: phylogeny, structure, and physiology of water channels. *News Physiol Sci* **14**, 187–193.

Holm, L.M., Jahn, T.P., Moller, A.L., Schjoerring, J.K., Ferri, D., Klaerke, D.A. & Zeuthen, T. 2005. NH(3) and NH(4)(+) permeability in aquaporin-expressing *Xenopus* oocytes. *Pflugers Arch* **450**, 415–428.

Ishikawa, F., Suga, S., Uemura, T., Sato, M.H. & Maeshima, M. 2005. Novel type aquaporin SIPs are mainly localized to the ER membrane and show cell-specific expression in *Arabidopsis thaliana*. *FEBS Lett* **579**, 5814–5820.

Jahn, T.P., Moller, A.L., Zeuthen, T., Holm, L.M., Klaerke, D.A., Mohsin, B., Kuhlbrandt, W. & Schjoerring, J.K. 2004. Aquaporin homologues in plants and mammals transport ammonia. *FEBS Lett* **574**, 31–36.

Johanson, U. & Gustavsson, S. 2002. A new subfamily of major intrinsic proteins in plants. *Mol Biol Evol* **19**, 456–461.

 doi: 10.1111/j.1748-1716.2006.01563.x

Johanson, U., Karlsson, M., Johansson, I., Gustavsson, S., Sjövall, S., Fraysse, L., Weig, A.R. & Kjellbom, P. 2001. The complete set of genes encoding major intrinsic proteins in *Arabidopsis* provides a framework for a new nomenclature for major intrinsic proteins in plants. *Plant Physiol* **126**, 1358–1369.

Johansson, I., Larsson, C., Ek, B. & Kjellbom, P. 1996. The major integral proteins of spinach leaf plasma membranes are putative aquaporins and are phosphorylated in response to Ca2+ and apoplastic water potential. *Plant Cell* **8**, 1181–1191.

Johansson, I., Karlsson, M., Shukla, V.K., Chrispeels, M.J., Larsson, C. & Kjellbom, P. 1998. Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. *Plant Cell* **10**, 451–459.

Kozono, D., Ding, X., Iwasaki, I., Meng, X., Kamagata, Y., Agre, P. & Kitagawa, Y. 2003. Functional expression and characterization of an archaeal aquaporin. AqpM from *Methanothermobacter marburgensis*. *J Biol Chem* **278**, 10649–10656.

Kukulski, W., Schenk, A.D., Johanson, U., Braun, T., de Groot, B.L., Fotiadis, D., Kjellbom, P. & Engel, A. 2005. The 5A structure of heterologously expressed plant aquaporin SoPIP2; 1. *J Mol Biol* **350**, 611–616.

Liu, L.H., Ludewig, U., Gassert, B., Frommer, W.B. & Von Wiren, N. 2003. Urea transport by nitrogen-regulated tonoplast intrinsic proteins in *Arabidopsis*. *Plant Physiol* **133**, 1220–1228.

Lopez, F., Bousser, A., Sissoeff, I., Gaspar, M., Lachaise, B., Hoarau, J., Mahe, A. 2003. Diurnal regulation of water transport and aquaporin gene expression in maize roots: contribution of PIP2 proteins. *Plant Cell Physiol* **44**, 1384–1395.

Martre, P., Morillon, R., Barrieu, F., North, G.B., Nobel, P.S. & Chrispeels, M.J. 2002. Plasma membrane aquaporins play a significant role during recovery from water deficit. *Plant Physiol* **130**, 2101–2110.

Maurel, C., Kado, R.T., Guern, J. & Chrispeels, M.J. 1995. Phosphorylation regulates the water channel activity of the seed-specific aquaporin alpha-TIP. *Embo J* **14**, 3028–3035.

Moshelion, M., Becker, D., Biela, A., Hehlein, N., Hedrich, R., Otto, B., Levi, H., Moran, N. & Kaldenhoff, R. 2002. Plasma membrane aquaporins in the motor cells of *Samanea saman*: diurnal and circadian regulation. *Plant Cell* **14**, 727–739.

Nakhoul, N.L., Davis, B.A., Romero, M.F. & Boron, W.F. 1998. Effect of expressing the water channel aquaporin-1 on the CO2 permeability of *Xenopus* oocytes. *Am J Physiol* **274**, C543–C548.

Nakhoul, N.L., Hering-Smith, K.S., Abdulnour-Nakhoul, S.M. & Hamm, L.L. 2001. Transport of NH(3)/NH in oocytes expressing aquaporin-1. *Am J Physiol Renal Physiol* **281**, F255–F263.

Niemietz, C.M. & Tyerman, S.D. 2000. Channel-mediated permeation of ammonia gas through the peribacteroid membrane of soybean nodules. *FEBS Lett* **465**, 110–114.

Ninnemann, O., Jauniaux, J.C. & Frommer, W.B. 1994. Identification of a high affinity NH4+ transporter from plants. *Embo J* **13**, 3464–3471.

Park, J.H. & Saier, M.H. Jr. 1996. Phylogenetic characterization of the MIP family of transmembrane channel proteins. *J Membr Biol* **153**, 171–180.

Prasad, G.V., Coury, L.A., Finn, F. & Zeidel, M.L. 1998. Reconstituted aquaporin 1 water channels transport CO2 across membranes. *J Biol Chem* **273**, 33123–33126.

Preston, G.M. & Agre, P. 1991. Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: member of an ancient channel family. *Proc Natl Acad Sci USA* **88**, 11110–11114.

Schuurmans, J.A., Van Dongen, J.T., Rutjens, B.P., Boonman, A., Pieterse, C.M. & Borstlap, A.C. 2003. Members of the aquaporin family in the developing pea seed coat include representatives of the PIP, TIP, and NIP subfamilies. *Plant Mol Biol* **53**, 633–645.

Siefritz, F., Tyree, M.T., Lovisolo, C., Schubert, A. & Kaldenhoff, R. 2002. PIP1 plasma membrane aquaporins in tobacco: from cellular effects to function in plants. *Plant Cell* **14**, 869–876.

Sun, X.C., Allen, K.T., Xie, Q., Stamer, W.D. & Bonanno, J.A. 2001. Effect of AQP1 expression level on Co(2) permeability in bovine corneal endothelium. *Invest Ophthalmol Vis Sci* **42**, 417–423.

Temmei, Y., Uchida, S., Hoshino, D., Kanzava, N., Kuvahara, M., Sasaki, S. & Tsuchiya, T. 2005. Water channel activities of *Mimosa pudica* plasma membrane intrinsic proteins are regulated by direct interaction and phosphorylation. *FEBS Lett* **579**, 4417–4422.

Terashima, I. & Ono, K. 2002. Effects of HgCl(2) on CO(2) dependence of leaf photosynthesis: evidence indicating involvement of aquaporins in CO(2) diffusion across the plasma membrane. *Plant Cell Physiol* **43**, 70–78.

Tornroth-Horsefield, S., Wang, Y., Hedfalk, K., Johansson, U. Karlsson, M., Tajkhorshid, E., Neutze, R. & Kjellbom, P. 2005. Structural mechanism of plant aquaporin gating. *Nature* **439**, 688–694.

Tournaire-Roux, C., Sutka, M., Javot, H. *et al.* 2003. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* **425**, 393–397.

Uehlein, N., Lovisolo, C., Siefritz, F. & Kaldenhoff, R. 2003. The tobacco aquaporin NtAQP1 is a membrane CO2 pore with physiological functions. *Nature* **425**, 734–737.

Veenhoff, L.M., Heuberger, E.H. & Poolman, B. 2002. Quaternary structure and function of transport proteins. *Trends Biochem Sci* **27**, 242–249.

Vera-Estrella, R., Barkla, B.J., Bohnert, H.J. & Pantoja, O. 2004. Novel regulation of aquaporins during osmotic stress. *Plant Physiol* **135**, 2318–2329.

Wallace, I.S. & Roberts, D.M. 2004. Homology modeling of representative subfamilies of *Arabidopsis* major intrinsic proteins. Classification based on the aromatic/arginine selectivity filter. *Plant Physiol* **135**, 1059–1068.

Weaver, C.D. & Roberts, D.M. 1992. Determination of the site of phosphorylation of nodulin 26 by the calcium-dependent protein kinase from soybean nodules. *Biochemistry* **31**, 8954–8959.

Weig, A., Deswarte, C. & Chrispeels, M.J. 1997. The major intrinsic protein family of *Arabidopsis* has 23 members that form three distinct groups with functional aquaporins in each group. *Plant Physiol* **114**, 1347–1357.

Zardoya, R. 2005. Phylogeny and evolution of the major intrinsic protein family. *Biol Cell* **97**, 397–414.

  doi: 10.1111/j.1748-1716.2006.01563.x