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BOTANICAL BRIEFING

Aquaporins and Plant Leaf Movements

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• *Background* Plant leaf movements can be mediated by specialized motor organs, the pulvini, or can be epinastic (i.e. based on different growth velocities of the adaxial and abaxial halves of the leaf). Both processes are associated with diurnally regulated increases in rates of membrane water transport, which in many cases has been shown to be facilitated by aquaporins. Rhythmic leaf movements are known from many plant species, but few papers deal with the involvement of aquaporins in such movements.
• *Scope* Many details of the architecture and function of pulvini were worked out by Ruth Satter and co-workers using *Samanea saman* as a model organism. More recently a contribution of aquaporins to pulvinar movement in *Samanea* was demonstrated. Another model plant to study pulvinus-mediated leaf movements is *Mimosa pudica*. The contribution of both plasma membrane- and tonoplast-localized aquaporins to the seismonastic leaf movements in *Mimosa* was analysed. In tobacco, as an example of epinastic leaf movement, it was shown that a PIP1 aquaporin family member is an important component of the leaf movement mechanism.

Key words: Aquaporins, leaf movement, plasma membrane intrinsic proteins, epinastic, nyctinastic.

AQUAPORINS

Aquaporins are an old family of small (24–30 kDa) pore-forming integral membrane proteins. They belong to the class of major intrinsic proteins (MIPs) and numerous members have been found in all kingdoms from Archaea to animals. Aquaporins provide a proteinaceous pathway for water (Preston *et al*, 1992; Quigley *et al.*, 2001), some small uncharged solutes (Biela *et al.*, 1999; Gerbeau *et al.*, 1999) and even gases (Uehlein *et al.*, 2003; Jahn *et al.*, 2004; Holm *et al.*, 2005; Endeward *et al.*, 2006) across biological membranes. Based on results from sequence analyses and functional characterization, the MIP family was divided into the aquaporins (AQPs), which are strictly water-selective, and the aquaglyceroporins (glycerol facilitator-like proteins, GLPs), which are permeable to small molecules like glycerol and urea in addition to water (Heymann and Engel, 1999; Zardoya, 2005).

To date, 13 different aquaporins are known in vertebrates and correspond to the human AQP0 to AQP12 (Ishibashi *et al.*, 2003; King *et al.*, 2004). Four members (AQP3, 7, 9 and 10) promote glycerol transport and thus belong to the GLP sub-family. Human AQP8 and its homologues diverge more strongly from other AQPs and occur as a single-copy gene in animals, indicating that the diversification and specialization of the other metazoan AQPs occurred after the split of AQP8 (Zardoya, 2005).

Aquaporin homologues are particularly abundant in plants. They show greater functional diversity than the main metazoan paralogues, which has been attributed to a higher degree of compartmentation in plant cells and a greater necessity for finely tuned water control (Johanson *et al.*, 2001) in order to adapt to changing environmental conditions. In the genome of the model plant *Arabidopsis thaliana*, more than 35 different genes coding for aquaporin-like proteins have been found (Johanson *et al.*, 2001; Quigley *et al.*, 2001). The genome of *Zea mays* contains about 33 aquaporin-encoding genes (Chaumont *et al.*, 2001). In contrast to the mammalian aquaporins, plant aquaporins can be sub-divided into four major groups, indicating different possible sub-cellular or plant organ localization: plasma membrane intrinsic proteins (PIPs); tonoplast intrinsic proteins (TIPs); NOD26-like intrinsic proteins (NIPs; where NOD26 is an aquaporin discovered in the peribacteroid membrane of nodulated soy bean roots); and small basic intrinsic proteins (SIPs). Recently the SIP aquaporins were shown to be targeted to the endoplasmic reticulum membrane (Ishikawa *et al.*, 2005).

The PIP sub-family can be further sub-divided into two groups named PIP1 and PIP2, the cytosol-protruding N-terminal domain being longer in the PIP1 aquaporins. Functional analysis in *Xenopus* oocytes showed that production of PIP2 aquaporins increases the water-permeability of the oocyte membrane about 10- to 20-fold. In contrast, production of PIP1 aquaporins alone does not appreciably affect the membrane's water-permeability (Moshelion *et al.*, 2002; Fetter *et al.*, 2004).

AQUAPORIN STRUCTURE

Aquaporins have a characteristic conserved structure with six tilted transmembrane helices linked by three extracellular and two intracellular loops. N- and C-terminal domains protrude into the cytosol and a highly conserved amino-acid motif (asparagine-proline-alanine; NPA) occurs twice in the pore region and is important for aquaporin function. Aquaporins are incorporated into the membranes in a tetrameric arrangement comprising four individual pores.

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The protein operates as a two-stage filter. One is built up by the conserved NPA motifs forming a selectivity-determining region and another is formed by an aromatic/arginine-region functioning as a proton exclusion filter. Hydrophobic regions near the NPA motifs are rate-limiting water barriers and reduce interactions between water molecules. It was shown for human AQP1 that water permeates in a single-file arrangement and that a finely tuned water dipole rotation during passage is essential for water selectivity (Murata *et al.*, 2000; de Groot and Grubmüller, 2001).

AQUAPORIN FUNCTION IN PLANTS

Many observations concerning the physiological role of aquaporins in plants result from analyses of transgenic plants with a modified expression of certain aquaporin genes or from analysis of aquaporin mutant plants. The first evidence for a function in cellular water uptake and whole-plant water transport came from PIP antisense plants. These developed a larger root system than the control plants (Kaldenhoff *et al.*, 1998). In tobacco, the plasma membrane aquaporin NtAQP1 was shown to be important for root hydraulic conductivity and water stress resistance (Siefritz *et al.*, 2002). Studies on plants with impaired production of PIP1 and PIP2 indicated that both of these aquaporins are required for an important role in the recovery from water-deficiency (Martre *et al.*, 2002).

In addition to their ability to transport water, some aquaporins facilitate the passage of gases such as CO_2 and ammonia across membranes (Niemietz and Tyerman, 2000; Holm *et al.*, 2005; Loque *et al.*; 2005; Endeward *et al.*, 2006). Production of the human AQP1 or the plant NtAQP1 in the heterologous *Xenopus* oocyte expression system increases the CO_2 permeability of the oocyte membrane (Nakhoul *et al.*, 1998; Uehlein *et al.*, 2003). The mechanism and the physiological significance of the CO_2 transport facilitated by aquaporins is still a matter of debate (Cooper *et al.*, 2002; Hub and de Groot, 2006). Recently a clear contribution of human AQP1 to CO_2 transport across the erythrocyte membrane was shown (Endeward *et al.*, 2006). Plants with impaired expression of *NtAQP1* showed changes not only in water transport (Siefritz *et al.*, 2002) but also in CO_2-limited processes such as stomatal opening and closing, photosynthesis and leaf growth (Uehlein *et al.*, 2003). In another study it was shown that NtAQP1 increases mesophyll conductance to CO_2 in tobacco (Flexas *et al.*, 2006). Overexpression of *Arabidopsis PIP1b* in tobacco resulted in increased growth rates under optimal irrigation (Aharon *et al.*, 2003), which could be interpreted as the sum of effects on water uptake and photosynthesis. In addition to their function in water management, plant aquaporins play a role during leaf movement, a process requiring high rates of cellular water transport.

PLANT LEAF MOVEMENT

Plant leaf movements can be mediated in two ways: reversible (swelling) or irreversible (growth) changes in cell volume. In contrast to typical growth movements, turgor-regulated movements are reversible. They have been observed in many plants, e.g. *Mimosa*, *Phaseolus*, *Albizzia*, *Desmodium* and *Samanea*, which possess specialized motor organs called pulvini. As the direction of leaf movement is determined by the architecture of the pulvinar joint and not by the direction of the controlling stimulus, this type of movement is referred to as nyctinastic. In other plant species, e.g. tobacco, leaf movements (described as epinastic) occur without the presence of specialized motor organs. In those cases, the movements are the result of periodically different elongation growth velocities of the adaxial and abaxial sides of the leaf blades and petioles.

Many data on pulvinus architecture and function were collected on the nyctinastic, leguminous tree *Samanea saman* as a model organism (Satter *et al.*, 1974, 1990; Gorton, 1987). The pulvinus contains two functionally different tissues: the adaxial flexor and the abaxial half referred to as the extensor. Changes in solute content and ion composition, mainly potassium (Satter *et al.*, 1974; Moran *et al.*, 1988), in the motor cells induce osmotic water fluxes. Reversible osmotic swelling of the extensor cells accompanied by shrinkage of the flexor cells induces opening of the pulvinus and thus lifting of the leaves and leaflets. Upon redistribution of potassium ions, extensor cells shrink and flexor cells swell and the pulvinus closes (Satter *et al.*, 1974; Gorton, 1987). The water-permeability of the membranes in both motor tissues is under strict temporal regulation. In pulvini of *Samanea saman*, expression of the genes for plasma membrane-localized aquaporins was shown in both flexor and extensor tissues (Moshelion *et al.*, 2002). The encoded aquaporins were analysed in the heterologous *Xenopus* oocyte expression system and characterized as functional water conducting pores. The gene for one of them, *SsAQP2*, shows a diurnal and circadian rhythm in expression pattern in the pulvinus, coinciding with changing requirements for cellular water permeability over the day. The highest accumulation of specific mRNA was observed in the morning, when the pulvini open and high osmotic water fluxes are expected.

The seismonastic leaf movement of *Mimosa* plants is associated with rapid water transport across cell membranes. In mature motor cells of *Mimosa*, two types of vacuole were identified: small vacuoles containing large amounts of tannins and big aqueous vacuoles. Fast efflux of potassium ions through outward-rectifying ion channels followed by water efflux leads to a rapid turgor loss. A correlation between the resulting pulvinus movement and the presence of a γ-TIP aquaporin was shown by immunogold staining (Fleurat-Lessard *et al.*, 1997) with an antiserum against the water channel protein VM23 from radish vacuolar membranes. The aquaporin protein detected was shown to be located almost exclusively in the tonoplast of the aqueous vacuole, thus probably facilitating fast water efflux.

In another more recent study, transcripts of aquaporin genes (*MpPIP1;1*, *MpPIP2;1*) were detected in young seedlings of *Mimosa pudica*. Functional analysis was performed in the heterologous *Xenopus* oocyte expression system (Temmei *et al.*, 2005). MpPIP1;1 does not confer a significant water conductivity on oocytes whereas expression of

MpPIP2;1 increases the water permeability of the oocyte plasma membrane about 20-fold. Upon co-expression of MpPIP1;1 and MpPIP2;1 a co-operative effect on water channel activity was observed, suggesting heterocomplex formation of MpPIP1;1 and MpPIP2;1. The interaction led to an increase in the water permeability of the oocyte membranes beyond that obtained by expression of MpPIP2;1 alone. Furthermore, phosphorylation of a particular serine residue in MpPIP1;1 increases this co-operative effect on water channel activity. It can be assumed that transport properties and regulation of water channel activity by interaction of the two isoforms and phosphorylation, as analysed in the oocyte system, also apply to the plant system. Taken together, these studies provide a model of regulation of membrane water permeability in *Mimosa*.

Tobacco is an examle of diurnally and circadianly regulated leaf movement occurring without the presence of a specialized motor organ. Tobacco leaves are typically oriented perpendicular to the shoot axis during the day and fold upwards by the end of the light period. Siefritz *et al.* (2004) analysed the contribution of the PIP1 aquaporin NtAQP1 to leaf movements in tobacco. *NtAQP1* promoter activity assays suggested that expression of NtAQP1 is mainly regulated at the level of transcription. The maximum accumulation of specific mRNA was observed in the morning when the leaves returned to their unfolded position. The abundance of *NtAQP1* transcript was regulated diurnally, showing the highest accumulation at the beginning of the light period and decreasing to a basal level at the end of the light period. The presence of NtAQP1 protein was followed by *in situ* immunological studies with an NtAQP1-specific antiserum. It was shown that the abundance of the protein follows the accumulation of the specific mRNA with a delay of approx. 2 h. To address functional changes at the plasma membranes of petiole tissues during changes in aquaporin content, Siefritz *et al.* (2004) performed swelling assays on protoplasts from petioles. It was shown that a high amount of NtAQP1 protein corresponded to an increased cellular permeability to water. This study was followed by a reverse genetic approach involving an analysis of the contribution of NtAQP1 protein to the leaf movements of tobacco. In tobacco plants with a highly impaired expression of NtAQP1, the cellular water permeability of petiole protoplasts was substantially lower than that of controls. Eventually these plants showed highly reduced leaf movement compared with control tobacco plants, indicating that NtAQP1 expression is required for proper epinastic leaf movements.

CONCLUSIONS

Current literature presents a few contributions dealing with the involvement of aquaporin expression and regulation to instantaneous or diurnally regulated plant leaf movements. The studies were performed on *Mimosa pudica* and *Samanea saman* regarding pulvinus-mediated leaf movements and on tobacco with regard to epinastic leaf movements. Fleurat-Lessard *et al.* (1997) showed the contribution of a tonoplast intrinsic aquaporin to the fast and instantaneous movements of *Mimosa pudica* leaves and leaflets. The work presented by Temmei *et al.* (2005) presents an interaction model of two PIP aquaporin isoforms which in combination with phosphorylation of certain amino acid residues account for rapid regulation of aquaporin activity in the plasma membrane of *Mimosa*. Siefritz *et al.* (2004) and Moshelion *et al.* (2002) showed a connection between temporarily adapted expression of plasma membrane-localized aquaporins and diurnally regulated leaf movement. Swelling assays of protoplasts isolated from petiole tissues at different times of day connected the expression data with the proposed function at the cellular level. Furthermore, a direct contribution of specific aquaporins to properly regulated leaf movement has been analysed through a comparison of genetically modified tobacco plants with an impaired aquaporin expression and the controls. The slow epinastic leaf movement of the tobacco plants is highly affected by the genetic modification.

For an analysis of the contribution of aquaporins to fast, pulvinus-mediated leaf movements it could be helpful to analyse genetically modified plants, for example in *Mimosa*, with an altered expression of the respective aquaporin regarding their ability to exhibit leaf movement. The involvement of aquaporins in growth movements suggests that aquaporins may also play an equally prominent role in overall growth regulation of leaves, stems and roots. Tobacco, and particularly *Arabidopsis*, where collections of T-DNA insertional mutants exist, could serve as model systems to study the importance of single aquaporins for regulation of plant organ growth. However, pleiotropic effects as well as functional compensation by closely related homologues have to be considered during attempts to manipulate the expression of single aquaporin isoforms in plants.

LITERATURE CITED

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. *The 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. *The Plant Journal* **18**: 565–570.

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

Cooper GJ, Zhou Y, Bouyer P, Grichtchenko II, Boron WF. 2002. Transport of volatile solutes through AQP1. *Journal of Physiology* **542**: 17–29.

Endeward V, Musa-Aziz R, Cooper GJ, Chen LM, Pelletier MF, Virkki LV, *et al.* 2006. Evidence that aquaporin 1 is a major pathway for CO_2 transport across the human erythrocyte membrane. *FASEB Journal* **20**: 1974–1981.

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

Fleurat-Lessard P, Frangne N, Maeshima M, Ratajczak R, Bonnemain JL, Martinoia E. 1997. Increased expression of vacuolar aquaporin and H^+-ATPase related to motor cell function in *Mimosa pudica* L. *Plant Physiology* **114**: 827–834.

Flexas J, Ribas-Carbo M, Hanson DT, Bota J, Otto B, Cifre J, *et al.* 2006. Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO_2 *in vivo*. *The Plant Journal* **48**: 427–439.

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

Gorton HL. 1987. Water relations in pulvini from *Samanea saman*. I. Intact pulvini. *Plant Physiology* **83**: 945–950.

de Groot BL, Grubmüller H. 2001. Water permeation across biological membranes: mechanism and dynamics of aquaporin-1 and GlpF. *Science* **294**: 2353–2357.

Heymann JB, Engel A. 1999. Aquaporins: phylogeny, structure and physiology of water channels. *News in Physiological Sciences* **14**: 187–193.

Holm LM, Jahn TP, Moller AL, Schjoerring JK, Ferri D, Klaerke DA, *et al*. 2005. NH_3 and NH_4^+ permeability in aquaporin-expressing *Xenopus* oocytes. *Pfluegers Archiv* **450**: 415–428.

Hub JS, de Groot BL. 2006. Does CO_2 permeate through aquaporin-1? *Biophysical Journal* **91**: 842–848.

Ishibashi K, Kuwahara M, Kageyama Y, Sasaki S, Suzuki M, Imai M. 2003. Molecular cloning of a new aquaporin superfamily in mammals. In: Hohmann S, Nielsen S, eds. *Molecular biology and physiology of water and solute transport*. New York, NY: Kluwer Academic/Plenum Publishers, 123–126.

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

Jahn TP, Moller AL, Zeuthen T, Holm LM, Klaerke DA, Mohsin B *et al*. 2004. Aquaporin homologues in plants and mammals transport ammonia. *FEBS Letters* **574**: 31–36.

Johanson U, Karlsson M, Johansson I, Gustavsson S, Sjovall S, Fraysse L, *et al*. 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 Physiology* **126**: 1358–1369.

Kaldenhoff R, Grote K, Zhu JJ, Zimmermann U. 1998. Significance of plasmalemma aquaporins for water-transport in *Arabidopsis thaliana*. *The Plant Journal* **14**: 121–128.

King LS, Kozono D, Agre P. 2004. From structure to disease: the evolving tale of aquaporin biology. *Nature Reviews Molecular Cell Biology* **5**: 687–698.

Loque D, Ludewig U, Yuan L, von Wiren N. 2005. Tonoplast intrinsic proteins AtTIP2;1 and AtTIP2;3 facilitate NH_3 transport into the vacuole. *Plant Physiology* **137**: 671–680.

Martre P, Morillon R, Barrieu F, North GB, Nobel PS, Chrispeels MJ. 2002. Plasma membrane aquaporins play a significant role during recovery from water deficit. *Plant Physiology* **130**: 2101–2110.

Moran N, Ehrenstein G, Iwasa K, Mischke C, Bare C, Satter RL. 1988. Potassium channels in motor cells of *Samanea saman*: a patch-clamp study. *Plant Physiology* **88**: 643–648.

Moshelion M, Becker D, Biela A, Uehlein N, Hedrich R, Otto B, *et al*. 2002. Plasma membrane aquaporins in the motor cells of *Samanea saman*: diurnal and circadian regulation. *The Plant Cell* **14**: 727–739.

Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, *et al*. 2000. Structural determinants of water permeation through aquaporin-1. *Nature* **407**: 599–605.

Nakhoul NL, Davis BA, Romero MF, Boron WF. 1998. Effect of expressing the water channel aquaporin-1 on the CO_2 permeability of *Xenopus* oocytes. *American Journal of Physiology* **274**: C543–C548.

Niemietz CM, Tyerman SD. 2000. Channel-mediated permeation of ammonia gas through the peribacteroid membrane of soybean nodules. *FEBS Letters* **465**: 110–114.

Preston GM, Carroll TP, Guggino WB, Agre P. 1992. Appearance of water channels in *Xenopus oocytes* expressing red cell CHIP28 protein. *Science* **256**: 385–387.

Quigley F, Rosenberg JM, Shachar-Hill Y, Bohnert HJ. 2001. From genome to function: the *Arabidopsis* aquaporins. *Genome Biology* **3**: 1–17

Satter RL, Geballe GT, Applewhite PB, Galston AW. 1974. Potassium flux and leaf movement in *Samanea saman*. I. Rhythmic movement. *Journal of General Physiology* **64**: 413–430.

Satter R, Gorton H, Vogelmann T. 1990. *The pulvinus: motor organ for leaf movement*. Rockville, MD: American Society of Plant Physiology.

Siefritz F, Otto B, Bienert GP, van der Krol A, Kaldenhoff R. 2004. The plasma membrane aquaporin NtAQP1 is a key component of the leaf unfolding mechanism in tobacco. *The Plant Journal* **37**: 147–155.

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

Temmei Y, Uchida S, Hoshino D, Kanzawa N, Kuwahara M, Sasaki S, *et al*. 2005. Water channel activities of *Mimosa pudica* plasma membrane intrinsic proteins are regulated by direct interaction and phosphorylation. *FEBS Letters* **579**: 4417–4422.

Uehlein N, Lovisolo C, Siefritz F, Kaldenhoff R. 2003. The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. *Nature* **425**: 734–737.

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