doi:10.1093/plphys/kiab373 PLANT PHYSIOLOGY 2021: 187: 2056–2070

Plant Physiology®

Hormonal and environmental signaling pathways target membrane water transport

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Update

All authors contributed to the literature survey. C.M. wrote the article which was revised by all other authors. C.T.R., L.V., and V.S. designed the figures and table.

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Abstract

Plant water transport and its molecular components including aquaporins are responsive, across diverse time scales, to an extremely wide array of environmental and hormonal signals. These include water deficit and abscisic acid (ABA) but also more recently identified stimuli such as peptide hormones or bacterial elicitors. The present review makes an inventory of corresponding signalling pathways. It identifies some main principles, such as the central signalling role of ROS, with a dual function of aquaporins in water and hydrogen peroxide transport, the importance of aquaporin phosphorylation that is targeted by multiple classes of protein kinases, and the emerging role of lipid signalling. More studies including systems biology approaches are now needed to comprehend how plant water transport can be adjusted in response to combined stresses.

Introduction

As elaborate terrestrial living organisms, vascular plants have to maintain their water status as tightly as possible and throughout their life cycle (Steudle, 1992; Davies and Bennett, 2015). Particularly challenging processes include the expansion/growth of underground and aerial parts and the desiccation, imbibition, and germination of seeds and pollen. Most often, these processes occur under variable environmental conditions, including changes to water availability and temperature in the soil and atmosphere, which have marked impacts on the plant water status. The latter is primarily maintained through tight stomatal regulation controlling transpirational water flow (Tardieu et al., 2015). Osmotic adjustment and regulation of water permeability (hydraulics), which both contribute to water potential gradients and water movement intensity, provide a complementary and finely tuned regulation of plant water relations at the cell and tissue levels. In support of these general principles, a large body of studies has shown that plant water transport and its molecular components are responsive, across diverse time scales, to an extremely wide array of environmental and hormonal signals (Chaumont et al., 1998; Maathuis et al., 2003; Aroca et al., 2012; Maurel et al., 2015). Thus, plant water relations are under constant adjustment virtually in any physiological context.

Plant aquaporins, which are present in the plasma and intracellular membranes of most plant cells, play a central role in these processes by ensuring cell-to-cell water transport and to a lesser extent, single-cell osmotic regulation (Tyerman et al., 1999; Chaumont and Tyerman, 2014; Maurel et al., 2015). More generally, aquaporins have emerged as central membrane targets of environmental and hormonal signaling pathways acting on plant water relations. In addition, aquaporins are highly abundant proteins

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Received March 09, 2021. Accepted July 13, 2021. Advance access publication August 26, 2021

ADVANCES

- ABA-dependent and independent signaling pathways act on aquaporins in plants under water deficit.
- Aquaporin-mediated water transport is targeted by multiple abiotic, biotic, or endogenous stimuli; recently identified stimuli include peptide hormones and PAMPs.
- ROS play a central signaling role in regulating water transport in plants by acting on aquaporin subcellular dynamics, localization, and/or phosphorylation.
- Plasma membrane aquaporins exhibit a dual water- and H_2O_2-transport function, acting as players in and targets of signaling processes.
- Phosphorylation of aquaporins, which interferes with their gating, trafficking, and stability, is a major target of plant signaling cascades, and multiple classes of protein kinases that act on distinct phosphorylation sites have been identified.

amenable to biochemical (proteomic) and cell biological approaches. Besides studies in the plant, their function and regulation can be easily studied after functional reconstitution (proteoliposomes) or heterologous expression (yeast, *Xenopus* oocytes). Thus, aquaporins are particularly relevant and convenient entry points for bottom-up dissections of plant signaling cascades.

Due to the central position of aquaporins in multiple hormonal and environmental responses determining the plant water status, the first aim of the present review was to inventory corresponding signaling pathways. Beyond the diversity of these pathways, a knowledge network with common or converging paths is now emerging. Conversely, the dedicated study of signaling pathways per se seems to reveal regulation states/contexts that escaped classical physiological analyses of plant water relations. A particularly challenging question is to understand how plants are able to perceive and respond to combined stresses.

Water deficit, abscisic acid, and related signals

Drought

Abscisic acid-dependent pathways

Whereas physiological links among drought stress, abscisic acid (ABA), and plant water transport have been identified for some time, it is only recently that the signaling mechanisms involved have started to be elucidated. Studies on epidermal peels from wild-type and aquaporin knockout Arabidopsis (*Arabidopsis thaliana*) plants have revealed that the plasma membrane aquaporin Plasma membrane Intrinsic Protein (PIP) 2;1 (*At*PIP2;1) is required for ABA-induced stomatal closure, but dispensable during light- and CO_2-induced stomatal movements (Grondin et al., 2015). Consistent with this, isolated guard cell protoplasts responded to exogenous ABA by a two-fold increase in osmotic water permeability that was strictly dependent on the functionality of *At*PIP2;1. This aquaporin, which is known to be permeable to hydrogen peroxide (H_2O_2; Dynowski et al., 2008), was also required for ABA-induced accumulation of H_2O_2 in the guard cell interior (Rodrigues et al., 2017). These studies establish a dual water- and H_2O_2-transport function for guard cell plasma membrane aquaporins as players and targets of signaling processes triggering stomatal closure (Grondin et al., 2015; Rodrigues et al., 2017). More specifically, in vitro phosphorylation assays and transgenic plant analysis led to a model whereby Snf1-related protein kinase 2.6 (SnRK2.6)/Open stomata 1 (OST1), a protein kinase that plays a pivotal role in ABA signaling, is able to phosphorylate *At*PIP2;1 at a specific cytosolic Ser residue (Ser121), with this phosphorylation being necessary for proper ABA-induced stomatal closure. SnRK2.6/OST1 also activates plasma membrane NADPH oxidases, which feed superoxide dismutases for production of apoplastic H_2O_2 (Maurel et al., 2016; Figure 1A). While the above-mentioned studies were performed on epidermal peels of wild-type and *pip2;1* plants, we are concerned that intact leaves of a quadruple *pip1;1 pip1;2 pip2;1 pip2;2* mutant showed normal responses to ABA (Ceciliato et al., 2019). We hypothesize that integration of guard cells within whole leaf physiology can compensate for the defects captured in isolated guard cells or protoplasts. We also note that, in contrast to stomata, ABA can downregulate aquaporin activity in bundle-sheaths (Shatil-Cohen et al., 2011) and roots (Rosales et al., 2019). Whereas the canonical ABA signaling machinery formed by RCAR/PYR/PYL receptors, protein Phosphatase 2C co-receptors acting on Class 2 Sucrose-NonFermenting Protein kinases (SnRK2s) is likely to be involved, in roots in particular (Rosales et al., 2019), the exact components providing these hydraulic regulations remain as yet unknown. Aquaporins and water transport can also be under ABA control through more indirect pathways. In Arabidopsis, ABA induced the expression of the membrane protein Tryptophan-rich sensory protein/translocator (TPSO), which physically interacts with *At*PIP2;7 in the early secretory pathway, to trigger its degradation through the autophagic pathway, thereby reducing its water transport activity at the plasma membrane (Hachez et al., 2014b). In rice (*Oryza sativa*), the gene encoding a leucine-rich repeat receptor-like kinase (RLK) named Leaf Panicle 2 (LP2) is downregulated by drought and ABA, and acts as a negative regulator of drought response (Wu et al., 2015). Its action involves a molecular interaction at the plasma membrane with the drought-responsive aquaporins *Os*PIP1;1, *Os*PIP1;3, and *Os*PIP2;3.

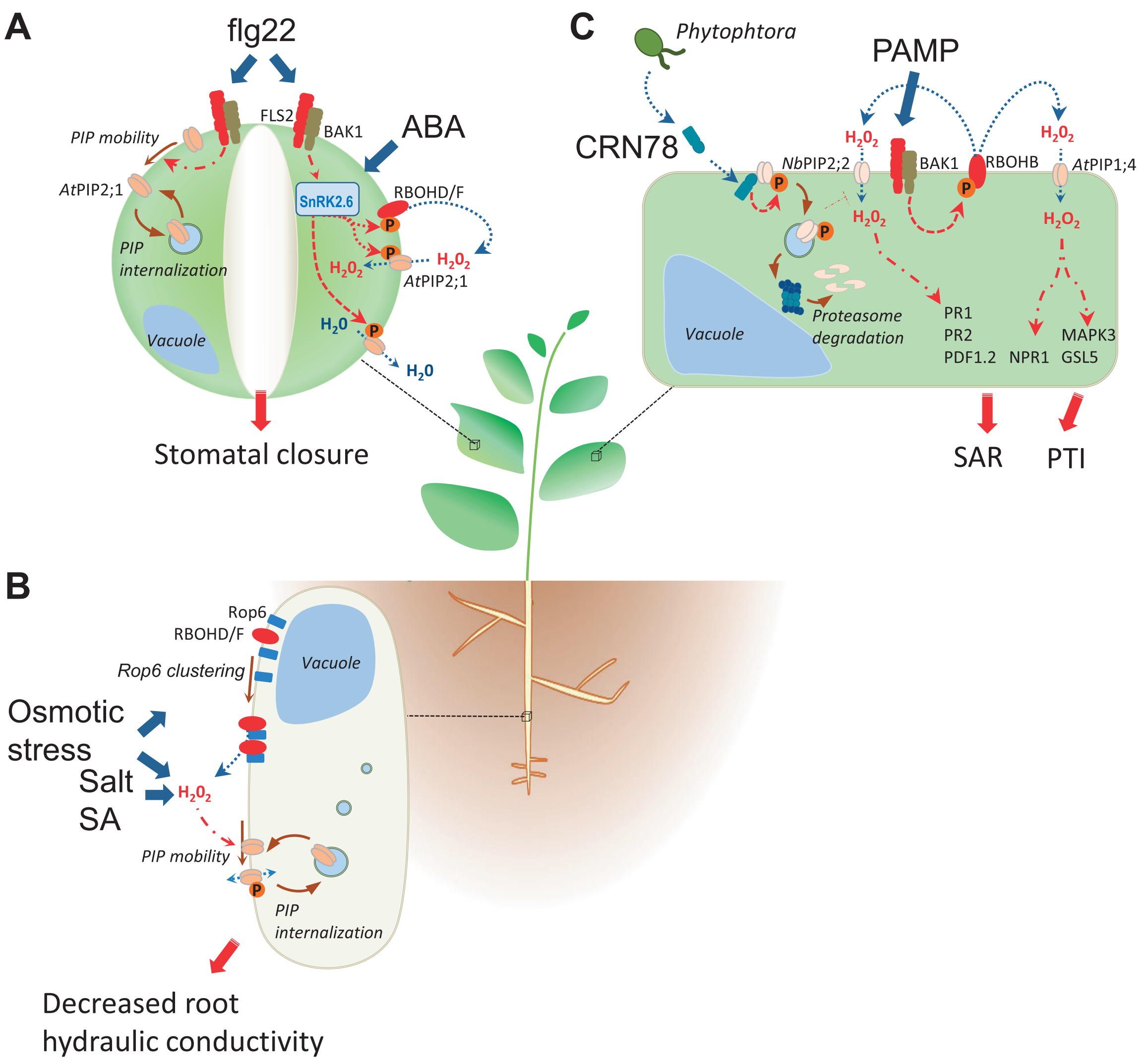

Figure 1 Cellular responses to biotic or abiotic stimuli involving H_2O_2 and aquaporins. A, In guard cells (right), the SnRK2.6 protein kinase is a key component of ABA- and flg22-induced stomatal closure. flg22 is a PAMP recognized by the plasma membrane FLS2 receptor, which acts in concert with BAK1. Upon activation, SnRK2.6 phosphorylates two NADPH oxidases, RBOHD and RBOHF, and the *At*PIP2;1 aquaporin. The latter facilitates the influx of H_2O_2 and the efflux of water, thereby promoting stomatal closure (Grondin et al., 2015; Rodrigues et al., 2017). fgl22 also enhances the lateral mobility of *At*PIP2;1 at the guard cell plasma membrane (left), thereby promoting its internalization (Cui et al., 2021). B, The root hydraulic conductivity (Lp_r) is strongly decreased in response to abiotic stresses such as osmotic and salt stress, or hormones such as SA. All these stimuli act through accumulation of H_2O_2 which lowers *At*PIP2;1 phosphorylation, alters its plasma membrane mobility, and promotes its internalization (Boursiac et al., 2008; Wudick et al., 2015). In contrast, PIP phosphorylation promotes its trafficking to the plasma membrane. The increased apoplastic H_2O_2 production observed in response to an osmotic stress is mediated through the clustering in nanodomains of the Rop6 together with the RBOHD and F NADPH oxidases (Martiniere et al., 2019; Smokvarska et al., 2020). C, In mesophyll cells, PAMPs are perceived by plasma membrane receptors, which trigger, in combination with BAK1 co-receptor and other nonrepresented cellular components, the activation of RBOHB NADPH oxidase and, as a consequence, apoplastic accumulation of H_2O_2. The *At*PIP1;4 aquaporin plays a key role in facilitating the diffusion of apoplastic H_2O_2 into the cytoplasm (Tian et al., 2016). The increase in cytoplasmic H_2O_2 concentration ultimately leads to activation of key proteins (Pathogenesis-Related protein 1 [PR1]; PR2; Plant Defensin 1.2; NPR1; Mitogen-Activated Protein Kinase 3; Glucan Synthase-like 5) involved in SAR or pathogen triggered immunity (PTI), as indicated. The figure also shows how the CRN78 effector of *Phytophthora* can penetrate the plant cell where it interacts with phosphorylated *Nb*PIP2;2, to induce its internalization and further degradation, thereby preventing its role in PAMP signaling (Ai et al., 2021). Thick blue arrows and thick red arrows indicate initial effects of stimuli and their output responses, respectively; thin dotted blue line arrows refer to diffusion of the indicated molecules; red dotted line arrows represent protein phosphorylation by indicated protein kinases; red dashed-dotted line arrows and red dashed-dotted line blunt arrows describe indirect activation and inactivation pathways, respectively; brown line arrows indicate movements of proteins or membrane compartments.

ABA-independent pathways

Drought can also exert ABA-independent effects on aquaporin expression and function. For instance, several aquaporin gene promoters harbor Dehydration Responsive Elements (DRE) that are targetted by DRE binding transcription factors (Rae et al., 2011). Other classes of transcription factors (e.g. AP2/EREBP and ASR1) also confer drought-dependent expression on aquaporin genes (Ricardi et al., 2014; Zhu et al., 2014). Furthermore, induction under severe drought of ubiquitin-conjugating enzyme UBC32 and RING-type E3 ligase, which both interact with *At*PIP2;1 and *At*PIP2;2, leads to ubiquitination and degradation of these aquaporins, thereby preventing their negative impact on drought tolerance (Chen et al., 2021).

Functional interaction between an aquaporin (*Rh*PIP2;1) and a membrane-tethered MYB protein named *Rh*PTM was recently described in rose (Zhang et al., 2019b). In brief, dehydration stress-induced C-terminal phosphorylation of *Rh*PIP2;1 at Ser273, which in turn led to membrane release and nuclear activation of *Rh*PTM. RhPTM itself acts as a transcription factor with general growth inhibitory effects. This landmark work establishes an unexpected link between hydraulics and a transcriptionally determined metabolic control of growth that promotes water deficiency tolerance. Yet, the protein kinases/phosphatases that act on Ser273 of *Rh*PIP2;1 and their drought-dependent activation/inhibition mechanisms remain to be identified. Similarly, some PIP aquaporins of Arabidopsis (e.g. *At*PIP2;4) show enhanced phosphorylation as early as 5 min after exposure to a hypertonic stress (0.3 M mannitol; Stecker et al., 2014).

Cell biological approaches are now developed to address associated regulatory mechanisms. An improved super-resolution microscopic technique called single particle tracking Photo-Activated Localization Microscopy (sptPALM) was used to monitor the lateral mobility of proteins at the plasma membrane (LTi6a and *At*PIP2;1) and tonoplast (*At*TIP1;1; Hosy et al., 2015). In contrast to the other membrane proteins, *At*PIP2;1 showed a low lateral mobility, was confined to membrane nanodomains and its mobility was enhanced upon a hyperosmotic stress. sptPALM further showed how reactive oxygen species (ROS) control specific diffusion and nano-organization of membrane cargo proteins (Martiniere et al., 2019). In particular, ROS initiated clustering of *At*PIP2;1 and its removal from the plasma membrane. This process is achieved in part by clathrin-mediated endocytosis. It is linked to further aquaporin internalization and transfer into the endocytic pathways (Zwiewka et al., 2015). In the longer term, the same aquaporins are subjected to ubiquitination and further degraded (Lee et al., 2009) in a phosphorylation-dependent manner (Chen et al., 2021). In addition, a role of autophagic pathways was recently uncovered in *Medicago truncatula* under water deficit (Li et al., 2020b). A dehydrin (*Mt*CAS31) was shown to act as a cargo receptor for the *Mt*PIP2;7 aquaporin. *Mt*CAS31 further promoted complex formation with the autophagy-related protein ATG8a to facilitate *Mt*PIP2;7 degradation. This, in turn, reduced water loss under water deficit and improved drought tolerance (Li et al., 2020b). The possible relation of this mechanism with the ABA-dependent TPSO pathway (Hachez et al., 2014b) mentioned above is not yet clear.

Finally, a bottom-up approach was used to further explore the signaling mechanisms acting upstream of aquaporins in the Arabidopsis root under hyperosmotic stress. A RESPIRATORY BURST OXIDASE HOMOLOG (RBOH) pathway defined by two NADPH oxidases and an additional pathway involving apoplastic ascorbate and iron were found to mediate osmotic stress-induced ROS production (Martiniere et al., 2019). Furthermore, osmotic stress was shown to trigger interaction of a Rho GTPase, Rho-of-Plant 6 with the two NADPH oxidases (Smokvarska et al., 2020). This interaction occurs in specific plasma membrane nanodomains that are necessary and sufficient to transduce production of ROS and several plant adaptive responses to osmotic constraints (Figure 1B).

Salinity

Under salt stress, PIP aquaporins are subjected to cellular regulations that are reminiscent of those seen under a purely osmotic stress. In Arabidopsis roots for instance, a 100 mM NaCl treatment decreased C-terminal phosphorylation at Ser283 of *At*PIP2;1 after 2–4 h (Prak et al., 2008). On a shorter timescale (30 min), a salt treatment also enhanced the lateral diffusion of *At*PIP2;1 at the plasma membrane (Li et al., 2011) and its constitutive cycling (Luu et al., 2012). These movements lead to internalization of *At*PIP2;1 from the plasma membrane, and its transfer to the prevacuolar and vacuolar compartments, a process that is mediated by ROS (Boursiac et al., 2008; Figure 1B) and is dependent on clathrin, phosphatidylinositol 3-kinase (PI3K), and PI4K (Ueda et al., 2016). These mechanisms may well be generalized: *At*PIP2;7, another major aquaporin isoform in the Arabidopsis root, showed a combined transcriptional down-regulation and protein internalization that resulted in a dramatic decrease in cell and tissue hydraulics (Pou et al., 2016). As in the case of osmotic stress, the primary signaling events triggering salt-dependent aquaporin regulation are not elucidated. Yet, salt-dependent expression of *At*PIP2;3 and *At*PIP2;5 is in part mediated by SnRK2s of subclass 1 (Kawa et al., 2020). Some of these protein kinases (SnRK2.4 and SnRK2.10) interact with and phosphorylate VARICOSE (VCS) and VCS RELATED (Soma et al., 2017; Kawa et al., 2020), two components of the mRNA decapping complex that catalyzes the first step of mRNA decay.

A role of PIP aquaporins in ion transport, tentatively associated to the so-called Nonselective Cation Channels described by patch clamp in many cell types, was recently proposed in Arabidopsis and barley (Byrt et al., 2017; Tran et al., 2020). As thoughtfully discussed by (Tyerman et al., 2021), this activity may well be central during plant response to salinity stress. The ionic conductance of *At*PIP2;1 is repressed by calcium and strongly enhanced upon C-terminal phosphorylation (Qiu et al., 2020). In barley, *Hv*PIP2;8 shows similar regulation, and, in addition, its gene is induced in shoots of salt-stressed plants, specifically in a tolerant cultivar (Tran et al., 2020). The connections of these regulatory processes to upstream salt stress signaling events and, possibly, to water transport regulation remains to be investigated.

Other abiotic constraints and stimuli

Oxygen availability

Flooding stress, whether due to water logging or submergence, deeply impacts plant water relations by favoring a massive inflow of water through roots while transpiration is directly or indirectly impaired (Tan et al., 2018). Two pathways leading to aquaporin regulation have been uncovered in this context (Maurel and Nacry, 2020). On the short term, a pronounced cytosolic acidosis can be observed under oxygen (O_2) deprivation due to the metabolic production of organic acids while a drop in cellular respiration and ATP production hampers efficient proton (H^+) extrusion by H^+-ATPases. This in turn induces protonation of a His residue located on the second cytosolic loop of PIPs, triggering channel gating. It is remarkable that this His residue is perfectly conserved in PIPs of all species, supporting the generality of the H^+-dependent gating mechanism (Tournaire-Roux et al., 2003). We note that certain plant species do not show any effect of hypoxia on root hydraulic conductivity (Lp_r). In the case of two lupin species (*Lupinus angustifolius* and *Lupinus luteus*), hypoxia induced a marked inhibition of cortical cell hydraulic conductivity but had no substantial effect at the whole root level, suggesting the predominance of an apoplastic pathway during water uptake (Bramley et al., 2010).

The second pathway was recently uncovered through quantitative genetic analysis of Arabidopsis root hydraulics. It is under the control of *Hydraulic Conductivity of Root 1* (*HCR1*; Shahzad et al., 2016). HCR1 mediates root responses to hypoxia in relation to potassium (K^+) availability, thereby acting as a negative regulator of Lp_r and root aquaporins. HCR1 encodes a RAF-type MAP3K protein kinase that specifically accumulates in the presence of K^+ and absence of O_2. HCR1 enhances the abundance of RAP2.12, a key Ethylene Response Factor VII transcription factor that is stabilized under O_2 starvation and triggers the core anaerobic transcriptional response. We have proposed that HCR1 phosphorylates RAP2.12 or one of its direct regulators in planta and thereby potentiates the most early plant responses to O_2 starvation (Shahzad et al., 2016). However, the mode of aquaporin down-regulation through this pathway has not been elucidated.

Nitrogen

Nitrate (NO_3^-) is the major inorganic nitrogen (N) source absorbed by nonwetland plants and its uptake and metabolism are tightly associated with water utilization. Whereas ammonium has a rather negative effect on water absorption, NO_3^- was shown to enhance Lp_r in multiple plant species. Although posttranslational regulation may also be involved, the increase in Lp_r induced by NO_3^- was shown to correspond to upregulation of aquaporin expression (Gloser et al., 2007; Gorska et al., 2008). It has also been disputed whether NO_3^- acts directly or through its assimilation products. In Arabidopsis, the conserved Lp_r response of a NO_3^- reductase mutant (*nia*) supports the first hypothesis (Li et al., 2016). Furthermore, characterization of various Arabidopsis genotypes, altered in NO_3^- transport, assimilation, or signaling and growing under various N supply conditions revealed a strong positive relationship between Lp_r and NO_3^- accumulation, but in shoots rather than in roots (Li et al., 2016). This suggests a control of Lp_r by putative long-distance signals generated in the shoots. Such systemic regulation of aquaporins was also observed in split-root experiments with rice plants subjected to heterogeneous NO_3^- treatments (Ishikawa-Sakurai et al., 2014). We also note that Lp_r regulation by transpiration or wounding (Laur and Hacke, 2013; Ishikawa-Sakurai et al., 2014; Liu et al., 2014; Vandeleur et al., 2014) may also be mediated by shoot-borne signals acting on root hydraulics.

Although NRT1.1 is considered as the major NO_3^- sensor, the high-affinity NO_3^- transporter NRT2.1 is also thought to have a signaling role. In contrast with *nrt1.1* plants, *nrt2.1* plants showed a pronounced reduction in Lp_r, by up to 30% (Li et al., 2016). Surprisingly, this regulation was independent of NO_3^- and hence, of *NRT2.1* expression level. Thus, the signaling mechanisms involved in this newly discovered pathway remain fairly mysterious.

Cold

Both moderate cold and freezing challenge the plant water status. For instance, low temperature, which reduces Lp_r due to a higher viscosity of water and aquaporin downregulation, causes water deficit and growth arrest in leaves (Lee et al., 2012). In maize, aquaporins seem to play a key role in the recovery of Lp_r that is specifically observed in chilling tolerant genotypes (Aroca et al., 2005). Similarly, cold acclimation of Arabidopsis seedlings is accompanied by upregulation of two specific PIP genes (*AtPIP1;4* and *AtPIP2;5*; Rahman et al., 2020), which also show mRNA accumulation in leaves under water deficit (Alexandersson et al., 2005). In rice, proper expression and function of Calcium-dependent Protein Kinase (CDPK) *Os*CPK17 appears to be key for plant adaptation to low-temperature stress (Almadanim et al., 2017). A comparative phosphoproteomic approach further identified *Os*PIP2;1 and *Os*PIP2;6 as putative targets of *Os*CPK17. A direct activation by *Os*CPK17 is further supported by in vitro assays showing that the two aquaporins can be phosphorylated by the protein kinase in a calcium-dependent manner. Since *Os*CPK17 also targets sugar metabolism, it was proposed that cold stress-activated *Os*CPK17 phosphorylates *Os*PIP2;1 and *Os*PIP2;6 to coordinate membrane water transport with cell osmotic regulation (Almadanim et al., 2017).

Circadian control

The circadian clock allows the fine tuning and synchronization of key physiological, metabolic, and signaling pathways, in phase with daily variations of environmental parameters. While circadian regulation of tissue hydraulics has been largely documented (Henzler et al., 1999), it is only recently that the corresponding modes of aquaporin regulation have been investigated. In roots of Arabidopsis and maize, the control of aquaporin expression seems to be largely transcriptional and depends on well-identified clock components such as ELF3 (Takase et al., 2011; Caldeira et al., 2014). In maize, the oscillation amplitude of aquaporin expression and plant hydraulic conductance was strongly enhanced under water deficit, while oscillating expression of the core circadian clock genes was not altered (Caldeira et al., 2014). Interestingly, this specific potentiation of the hydraulic outputs, which possibly depends on ABA, appears to be beneficial for cumulated plant growth under water deficit whereas steady tissue hydraulic properties are more appropriate under water replete conditions. These data show how adaptive responses of plant hydraulics to specific environmental variations rely on exquisite coupling to endogenous signaling mechanisms.

In contrast with roots, the circadian control of leaf hydraulics seems to be mediated through posttranslational modifications of proteins (Choudhary et al., 2015; Prado et al., 2019). In particular, the rosette hydraulic conductivity (K_{ros}) of Arabidopsis shows circadian oscillations with a peak at midday, in phase with quantitative variations of PIP2 aquaporin phosphorylation (Prado et al., 2019). Remarkably, this modification is necessary but not sufficient for circadian control of K_{ros}, which requires the additional contribution of 14-3-3 proteins (Prado et al., 2013, 2019). Two of these (GRF4 and GRF10) can physically interact with *At*PIP2;1 and modulate its function in *Xenopus* oocytes depending on the phosphorylation status of the aquaporin C-terminal tail, with more pronounced effects on phosphomimetic than on phosphodeficient forms (Prado et al., 2019). Much remains to be learned about the protein kinase/protein phosphatase machinery that ensures oscillations in aquaporin phosphorylation and, possibly, a synchronized activation of 14-3-3 proteins.

A multiplicity of endogenous signals

Auxin

Transcriptomic analyses in Arabidopsis have revealed a dramatic downregulation of aquaporin expression during lateral root formation (Péret et al., 2012). This effect is mediated in part by auxin which, more generally, downregulates most aquaporin expression in roots within 5–10 h, and, consequently, Lp_r. Auxin also induces, after 24 h, a drop in turgor of cortical cells. Interestingly, mutant analyses have shown that all these molecular and physiological responses are mediated through Auxin Response Factor 7 (ARF7), a transcription factor that serves as a master regulator of lateral root formation and emergence. Yet, the down-stream targets of ARF7 in these responses remain to be identified. Our current understanding is that a finely tuned spatial and temporal control of PIP expression favors water entry into the lateral root primordium. This inflow is made at the expense of overlaying cells, thereby reducing their mechanical resistance and facilitating lateral root emergence.

Methyl jasmonate

Contrasting effects of Methyl jasmonate (MeJa) on root water transport have been reported by different groups. Sanchez-Romera et al. (2014) observed that exogenously supplied MeJA increases Lp_r in bean, tomato, and Arabidopsis. These effects are associated with an increased phosphorylation of PIP2s and partly depend on ABA, which accumulates in response to MeJa. In beans, the fluoridone inhibitor counteracted the effects of MeJA on both ABA accumulation and Lp_r. In contrast, exogenous MeJa induced a decrease in cell hydraulic conductivity in the cortex of Arabidopsis roots (Lee and Zwiazek, 2019). Surprisingly, *fad3-2 and fad7-2* plants, which are defective in MeJa endogenous biosynthesis, were insensitive to this external treatment. These contrasting results suggest that effects of MeJa on root hydraulics are cell-type specific, somewhat indirect, and involve important hormonal cross-talks that remain to be uncovered.

Ethylene

The effects of ethylene on aquaporin function in various plant species and organs have revealed the contribution of multiple signaling pathways. In rose, ethylene acts as a repressor of flower opening. It negatively controls petal cell expansion through downregulation of *Rh*PIP2;1 (Ma et al., 2008) and *Rh*PIP1;1 (Chen et al., 2013), which functionally interacts with the former. Consistent with these negative hormonal effects, silencing of ethylene receptors (*Rh*ETRs) which, in the absence of the hormone function as repressors of ethylene effects, resulted in an ethylene-independent decrease in *Rh*PIP1;1 expression (Chen et al., 2013). In Arabidopsis leaves, ethylene exerts positive effects on water transport through enhanced C-terminal phosphorylation of *At*PIP2;1 (Qing et al., 2016). These posttranslational effects require the ETR-1 receptor and its interacting partner Constitutive Triple-Response 1 (CTR1). They are independent of the major transcriptional pathway involving Ethylene-Insensitive 1, and Ethylene-Insensitive 3-like, which would in turn mediate the transcriptional downregulation of PIPs, as mentioned above. Tentative regulatory networks acting on aquaporin phosphorylation, under the control of ethylene and through balanced effects on protein kinases and phosphatases, were inferred in part from a differential phosphoproteomics approach in Col-0 and *ctr1* plants (Yang et al., 2013). Since ethylene enhances auxin accumulation, hormonal interactions will also have to be taken into account to comprehend the multiple pathways leading to ethylene-dependent aquaporin regulation (Yang et al., 2013; Qing et al., 2016).

Sugars

Sugars act as metabolites but also signaling molecules to regulate various whole plant and cellular processes such as photosynthesis and stomatal movements. With respect to tissue water transport, sucrose was shown to enhance Arabidopsis Lp_r specifically in the dark, whereas it inhibited Lp_r in the light, as did other osmotica (Di Pietro et al., 2013). Sucrose also induced changes in *At*PIP2;6 phosphorylation (Niittylä et al., 2007), thereby providing a tentative link between light, sugars, and aquaporins. This link was corroborated by the high negative correlation existing between the expression of aquaporins and either exogenous glucose or the expression of Hexokinase 1 in over-expressing or knockout transgenic Arabidopsis (Kelly et al., 2017). Protein kinases that are involved in sucrose-induced phosphorylation responses, among which RLKs (e.g. Sucrose Induced Receptor Kinase 1 [SIRK1]) and receptor-like cytoplasmic kinases (RLCKs e.g. Brassinosteroid Signaling Kinase 8 [BSK8]) have been identified through typical changes in their phosphorylation status within the few minutes following sucrose resupply. Some aquaporins were subsequently identified as downstream targets of these protein kinases (Wu et al., 2013, 2014, 2019). For instance, molecular interactions of SIRK1 with *At*PIP2;6 were established through pull down experiments and phosphorylation assays using an *At*PIP2;6-specific peptide. The mesophyll protoplast water permeability of Arabidopsis lines showing different expression levels of active SIRK1 revealed that the RLK directly regulates aquaporins via phosphorylation in a sucrose-dependent manner (Wu et al., 2013). In addition, SIRK1 forms a complex with its co-receptor Qian Shou Kinase 1 (QSK1). SIRK1 shows autophosphorylation, subsequently trans-phosphorylates QSK1, which in turn enhances and stabilizes the interaction of aquaporins with the receptor kinase complex (Wu et al., 2019). Our understanding of BSK8 action on aquaporins is not as precise, but the RLCK possibly acts on another isoform, *At*PIP2;7, that shows reduced phosphorylation in a *bsk8* mutant. Although still incomplete, the whole set of data suggests intricate osmotic, metabolic, and signaling effects of sugars acting on aquaporins through multiple receptor pathways and time frames (Wu et al., 2014). At the whole plant level, the somewhat opposite effects of sugar on hydraulics depending on organs, timing, or environmental conditions surely reflect contrasting contexts where maintenance of growth or of water content dominates one another.

Peptidic hormones and other unknown ligands

The endodermis functions as a major barrier within the root, determining fundamental features of water and nutrient absorption from the soil (Doblas et al., 2017). These properties follow from the typical differentiation of endodermal cells, with an initial formation of Casparian strips made out of lignin and further deposition of suberin lamellas that wrap the whole cell. Casparian strips mostly block apoplastic solute movements and favor xylem loading by preventing solute backflow from the stele to the cortex. Suberin lamellas, rather, limit transcellular transport of nutrients and possibly water at the endodermis. These barriers, which are at the cross-roads of mineral nutrient and water uptake, are therefore under tight control by endogenous and environmental factors (Barberon et al., 2016). For instance, the loss of Casparian strip integrity is detected by diffusible vasculature-derived peptides CASPARIAN STRIP INTEGRITY FACTORS 1 and 2 (CIF1 and 2) which act through the SCHENGEN3 receptor-like kinase (Pfister et al., 2014; Doblas et al., 2017; Nakayama et al., 2017) to rebalance water and mineral nutrient uptake. More specifically, CIF1 and CIF2 induce a reduction in Lp_r driven by the deactivation of aquaporins (Wang et al., 2019). They also limit ion leakage through deposition of suberin in endodermal cell walls. Inhibition of aquaporins seems to be a primary effect of CIFs, which in turn may promote suberin deposition. Interestingly, CIF1 and CIF2 act independently of ABA signaling, which can also be a potent regulator of aquaporins in endodermal cells.

Casparian strip deposition is guided by the local expression of a family of CAsparian Strip domain Proteins (CASPs). Related proteins named CASP-like are expressed throughout the plant. Four of these (CASPL1B1, CASPL1B2, CASPL1D1, and CASPL1D2) were identified as interacting with *At*PIP2;1 (Bellati et al., 2016). Although the physiological relevance of these molecular interactions is not known, CASPL1B1 was shown to specifically activate phosphorylated *At*PIP2;1 (Champeyroux et al., 2019), pointing to a putative composite regulatory pathway.

Biotic interactions

Plant immunity

In recent years, several landmark works have reported a crucial role of aquaporins in plant defense (Tian et al., 2016; Wang et al., 2018; Zhang et al., 2019a; Li et al., 2020a; Ai et al., 2021). *At*PIP1;4 was identified as one of the PIPs, the expression of which is induced by infection with *Pseudomonas syringae* pv. *Tomato* (Tian et al., 2016). The use of intracellular and extracellular ROS dyes in yeast and transgenic Arabidopsis further revealed the capacity of *At*PIP1;4 to mediate the diffusion of apoplastic H_2O_2 into the cytoplasm. This process represents a major step in the signaling response of plants to pathogen-associated molecular patterns (PAMPs), which activate RBOH NADPH oxidases present on the plasma membrane. More generally, analysis of knockout mutants and overexpressing plants revealed that *At*PIP1;4 is necessary for PAMP-triggered immunity (Tian et al., 2016) and mediates systemic-acquired resistance (SAR) through a parallel pathway involving activation of NONINDUCER OF PR GENES1 (NPR1). These findings establish a major signaling role of PIPs in plant defense (Figure 1C). Interestingly, a mechanism allowing bacterial effectors to counteract this aquaporin signaling function was recently uncovered (Ai et al., 2021). In brief, a Crinkler 78 (CRN78) effector from *Phytophthora* displaying a protein kinase activity was shown to phosphorylate the C-terminal tail of several H_2O_2-transporting PIPs of soybean or *Nicotiana benthamiana*, thereby triggering their degradation through a proteasome-dependent pathway. This led to inhibition of H_2O_2-accumulation and reduced immunity of these plants when infected by *Phytophthora* oomycetes (Ai et al., 2021).

A signaling role for aquaporins during plant defense was also uncovered for *At*PIP2;1 in guard cells, with this aquaporin being necessary for flg22-induced stomatal closure. In this case, a pathway was proposed, whereby the bacterial PAMP flg22 binds to its receptor flg22-sensitive 2 (FLS2) leading to the successive activation of the BRI1-Associated Kinase 1 (BAK1) co-receptor and SnRK2.6 protein kinase. This in turn phosphorylates *At*PIP2;1 at Ser121 to promote both water and H_2O_2 transport as shown in the context of ABA-induced stomatal closure (Rodrigues et al., 2017; Figure 1A). Flagellin (flg22) also enhanced lateral diffusion of *At*PIP2;1 at the guard cell plasma membrane through effects on microtubules, resulting in its internalization to the cell (Cui et al., 2021). It is not yet clear whether these effects contribute to the primary role of *At*PIP2;1 in stomatal closure or reflect a feedback inhibition of its function due to flg22-induced ROS accumulation. Interestingly, *At*PIP2;1 dynamics at the plasma membrane of adjacent (subsidiary) cells showed a distinct dependency on the cytoskeleton and were somewhat insensitive to flg22 (Cui et al., 2021). Thus, cell-specific regulation of aquaporins probably contributes to an integrated control of water exchange between cell types during stomatal movements.

PAMPs such as chitin are produced during plant attack by fungi and insects. Aquaporin-mediated water transport in leaf tissues such as the mesophyll and bundle sheath was shown to be downregulated by chitin (Attia et al., 2020). This inhibition could contribute to plant defense by hampering vascular pathogen propagation in xylem vessels and by promoting stomatal closure through long-distance hydraulic signaling. Although the mode of aquaporin regulation by chitin remains as yet unknown, the contribution of two chitin receptor-like kinases, chitin elicitor receptor kinase 1 and lysine motif receptor kinase 5, could be established (Attia et al., 2020).

Recent work also points to another molecular role of aquaporin during bacterial infection. For instance, rice *Os*PIP1;3 and Arabidopsis *At*PIP1;4 molecularly interact with the bacterial protein harpin (Hpa1) which is secreted by Gram-negative pathogenic bacteria. Thus, PIPs serve as receptors for harpins at the plant plasma, which in turn mediate the delivery of effector proteins (such as PthXo1) into the plant cell cytoplasm (Li et al., 2015b, 2019). Along the same lines, *At*PIP2;7 was recently shown to form a molecular complex with the protease domain of the tobacco etch potyvirus NIa protein (Martinez et al., 2016). Due to its numerous additional connections to stress responses, the aquaporin seems to be a preferential target during viral infection.

The overall data show that aquaporins are at the heart of the arms race between plants and their aggressors. On the one hand, aquaporins help plants resist pathogens by contributing to defense signaling pathways and by preventing their ingress through promotion of stomatal closure (Zhang et al., 2019a; Li et al., 2020a). On the other hand, some aquaporins can be hijacked by bacterial or viral pathogenic machineries, thereby promoting pathogen propagation.

Myccorhizae

Aquaporins also contribute to positive plant–microbe interactions whereby they play important roles in nutrient transport and abiotic stress responses. In particular, arbuscular mycorrhizal symbiosis can notably improve the plant water status. A fine dialog exists between the fungus and the host plants, leading to precise transcriptional and posttranslational regulation of plant aquaporins, particularly under drought (Quiroga et al., 2019). Similarly, the sub-cellular expression of specific tonoplast aquaporins seems to be subtly targeted in N-fixing symbiotic nodules (Gavrin et al., 2014). Although strong hormonal interplays are suspected, the signaling pathways leading to these regulations are as yet unknown.

Commonalities of signaling pathways

The survey above provides a broad but scattered knowledge of signaling processes targeting cell and tissue hydraulics in plants (Table 1). Yet, a few main principles do emerge from this information.

Whereas transcriptional control is definitely key for long-term adjustment of plant hydraulics in response to environmental or developmental cues, posttranslational mechanisms seem to dominate short term (minutes and hours) regulations, as these are more tailored for fast and reversible responses to the multiple factors (including water availability) acting on the plant water status. Here, we discuss a few common players of the latter regulatory mechanisms.

First, ROS plays a central signaling role in such regulation of plant water transport (Figure 1). They are produced in response to numerous stimuli including osmotic, salt and cold stress, nutrient deprivation, and hormones (salicylic acid [SA] and ABA). Although several ROS generating systems may operate at the plasma membrane (Martiniere et al., 2019), RBOH NADPH oxidases seem to occupy a central position in this device (Li et al., 2011; Martiniere et al., 2019). By enhancing constitutive cycling of aquaporins at the plasma membrane and shifting the equilibrium toward endocytosis, ROS promote aquaporin internalization, thereby reducing their density at the cell surface and, as a consequence, cell hydraulic conductivity (Boursiac et al., 2008; Wudick et al., 2015). As suggested by recent work in guard cells (Cui et al., 2021), this mechanism could provide a feedback regulation in contexts where aquaporins contribute to the initial steps of ROS signaling.

The sub-cellular trafficking and regulated degradation of aquaporins also appear to be a main component of plant responses to environmental stimuli. Syntaxins and other traffic proteins, such as adaptor proteins, play well-established roles in these processes (Besserer et al., 2012; Hachez et al., 2014a; Pertl-Obermeyer et al., 2016). While some internalized aquaporins accumulate in prevacuolar compartments (Luu et al., 2012), a stress–response pathway under the control of TPSO (Hachez et al., 2014b) can relay the above-mentioned trafficking to promote aquaporin degradation. The autophagic pathways described in *M. truncatula* under water deficit (Li et al., 2020b) may also operate under other stressing conditions. In addition, two recent works have pointed to a control by phosphorylation of ubiquitin-dependent degradation of aquaporins in plants under abiotic or biotic stress (Ai et al., 2021; Chen et al.,

Table 1 Molecular signaling mechanisms involved in aquaporin regulation under abiotic and biotic stresses and by endogenous signals

Type of Signal	Stimulus	Signaling Intermediate	Molecular Mechanism on Aquaporins	Aquaporin Regulation	References
Abiotic	Drought (ABA-dependent)	SnRK2.6	*At*PIP2;1 loop B phosphorylation	Up-regulated activity	Grondin et al. (2015); Rodrigues et al. (2017)
		LP2	Interaction with *Os*PIP1;1, *Os*PIP1;3 and *Os*PIP2;3	Downregulated activity	Wu et al. (2015)
	Drought (ABA-independent)	*Rh*PTM	Interaction with *Rh*PIP2;1	ND	Zhang et al. (2019a, 2019b)
		TPSO	ND	*At*PIP2;7 protein degradation	Hachez et al. (2014b)
		*Mt*Cas31/*Mt*ATG8	*Mt*Cas31 as a cargo receptor for *Mt*PIP2;7	Protein degradation by autophagy	Li et al. (2020b)
	Osmotic	ND	Increased C-ter phosphorylation of *At*PIP2;4	ND	Stecker et al. (2014)
		RBOHs and ROP6	Membrane lateral mobility	Clustering in nano-domains	Hosy et al. (2015); Martiniere et al. (2019); Smokvarska et al. (2020)
		clathrin	ND	Protein internalization	Zwiewka et al. (2015)
		Rma1H1	*At*PIP2;1 ubiquitination	Protein degradation	Lee et al. (2009)
		UBC32	*At*PIP2;1 ubiquitination	Protein degradation	Chen et al. (2021)
	Salinity	clathrin, PI3K, PI4K, SnRK2.4, SnRK2.10	C-ter phosphorylation of PIPs; enhanced mRNA decay	Protein internalization and down-regulated activity	Prak et al. (2008) Boursiac et al. (2008); Ueda et al., (2016); Pou et al., (2016); Kawa et al. (2020)
	O_2 Availability	cytosolic protons	His protonation of PIPs	Downregulated activity	Tournaire-Roux et al. (2003)
		HCR1	ND	Downregulated activity	Shahzad et al. (2016)
	N	NRT2;1	ND	Enhanced gene expression, protein accumulation and upregulated activity	Li et al. (2016); Ishikawa-Sakurai et al. (2014)
	Cold	ND	*AtPIP1;4* and *AtPIP2;5* gene expression	Upregulation of gene expression	Rahman et al. (2020)
		*Os*CPK17	*Os*PIP2;1 and *Os*PIP2;6 C-ter phosphorylation	Upregulated activity	Almadanim et al. (2017)
	Circadian Clock	14-3-3	*At*PIP2;1 C-ter phosphorylation	Upregulated activity	Prado et al. (2019)
		ELF3	*AtPIP1;2* and *AtPIP2;1* gene expression	Oscillating activity	Caldeira et al. (2014); Takase et al. (2011)
Biotic	flg22	BAK1 and SnRK2.6	Increased *At*PIP2;1 loop B phosphorylation	Upregulated activity	Rodrigues et al. (2017)
	Phytophtora sojae	CRN78	*Gm*PIP2;13 and *Nb*PIP2;2 phosphorylation	Protein degradation	Ai et al. (2021)
	Chitin	CERK1 and LYK5	ND	Upregulated activity	Attia et al. (2020)
	Xanthomonas oryzae	Harpin	Interaction with *Os*PIP1;3 and *At*PIP1;4	ND	Li et al. (2015b); Li et al. (2019)
Endogenous	Auxin	ARF7	*PIP* gene expression	Downregulated activity	Péret et al. (2012)
	MeJa	ND	PIP2 C-ter phosphorylation	Upregulated activity	Lee and Zwiazek (2019)
	Ethylene	*Rh*ETRs	*RhPIP1;1* and *RhPIP2;1* gene expression	Downregulated gene expression	Ma et al. (2008); Chen et al. (2013)
		ETR1 and CTR1	*At*PIP2;1 C-ter phosphorylation	Upregulated activity	Yang et al. (2013); Qing et al. (2016)
	Sucrose	SIRK1, QSK1	*At*PIP2;6 C-ter phosphorylation	Upregulated activity	Niittylä et al. (2007); Wu et al. (2013, 2014, 2019)
		BSK8	*At*PIP2;7 C-ter phosphorylation	ND	Wu et al. (2014)
	CIF1 and CIF2	SGN3	ND	Downregulated activity	Wang et al. (2019)
	Other	CASPL1B1	Physical interaction with *At*PIP2;1	Upregulated activity	Champeyroux et al. (2019)

2021). This mechanism, which allows control of aquaporin abundance, further expands the multiple, and possibly opposite, roles of phosphorylation in controlling aquaporin function.

In relation to the central role of phosphorylation, aquaporins are known to be targeted by multiple classes of protein kinases. This feature holds promises to further decipher aquaporin signaling pathways in bottom-up approaches

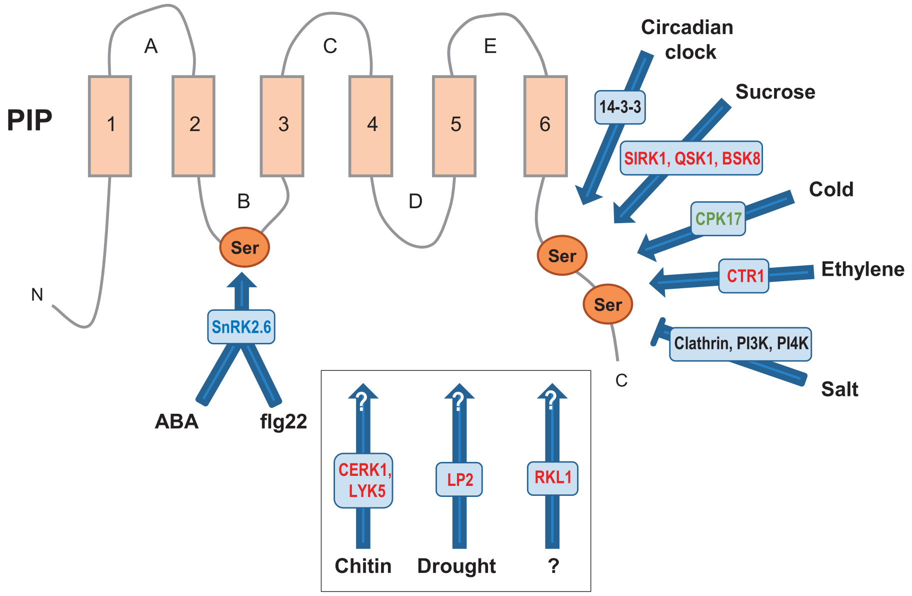

Figure 2 Signaling pathways converging on PIP phosphorylation. The figure represents a prototypal PIP aquaporin with its transmembrane domains (1–6) and extracellular (A, C, and E) or cytosolic (B and D) loops. The indicated stimuli lead to enhanced (arrows) or reduced (blunt arrow) phosphorylation of Ser residues in loop B or C-terminal tail. The inset shows three pathways acting on unknown PIP phosphorylation sites (white question marks). Signaling intermediates are indicated with the following color code: SnRK2: blue; CDPK, green; RLK, red; others, black. Note that the indicated protein kinases do not necessarily act directly on the indicated sites. For more details and references see text and Table 1.

(Figure 2). CDPKs have long been proposed as major players of aquaporin regulation (Johnson and Chrispeels, 1992; Johansson et al., 1996). Yet, there are very few cases where their role was formally established (Almadanim et al., 2017; Ji et al., 2017). We also note that CDPKs can be involved in upstream steps and therefore indirectly act on aquaporin regulation (Li et al., 2015a). Due to their key role during stomatal movements (Yip Delormel and Boudsocq, 2019), it will be worth exploring the action of CDPKs on guard cell aquaporins in closer detail.

SnRK2s represent another potentially important class of protein kinases acting on plant aquaporins. Besides SnRK2.6 (OST1), which mediates ABA- and flg22-dependent activation of *At*PIP2;1 in guard cells (Grondin et al., 2015; Rodrigues et al., 2017), other SnRK2s may delineate additional ABA- or osmotic stress-dependent pathways acting on aquaporins. In support for this, functional expression of plant signaling pathways in yeast using a transcriptional readout indicated that four SnRK2s initially thought to be specific for osmotic responses can also contribute to ABA signaling (Ruschhaupt et al., 2019). Thus, the model of ABA-dependent regulation of *At*PIP2;1 may be extended to additional pairs of SnRK2s and PIPs.

RLKs constitute another major a class of serine/threonine kinases that perceive environmental and extracellular developmental signals and transduce them via their intracellular kinase domain. While their role in aquaporin regulation in the endodermis (SGN3) and during sugar response (SIRK1 and BSK8) can serve as models, we anticipate a role for RLKs in many other cellular contexts including water stress (see "Outstanding questions"). For instance, two RLKs from rice (LP2, *Os*RLCK311) were shown to interact with PIPs in vivo (Wu et al., 2015; Sade et al., 2020), but the functional role of these interactions was not definitively elucidated. Moreover, Arabidopsis RKL1, the activating signals of which remain to be identified, was shown to molecularly interact with *At*PIP2;1 (Bellati et al., 2016). Co-expression of *At*PIP2;1 with RKL1 in *Xenopus* oocytes resulted in enhanced *At*PIP2;1 water transport activity by an as yet unknown mechanism (Bellati et al., 2016). RLKs may also regulate aquaporins by noncanonical mechanisms. For instance, co-expression of FERONIA and *At*PIP2;1 in *Xenopus* oocytes leads to inactivation of the latter (Bellati et al., 2016). This inactivation seems to require the protein kinase activity of FERONIA and is favored by two C-terminal phosphorylation sites of *At*PIP2;1 that, in contrast, have been described as activating sites. Thus, these phosphorylated residues may favor the FERONIA–*At*PIP2;1 interaction, which in turn would lead to aquaporin inhibition.

Finally, CBL-interacting protein kinases represent major regulators of plant nutrition and response to abiotic stresses (Dong et al., 2020). It is rather surprising that, despite efforts from several laboratories including ours, no aquaporin has ever been identified as one of their numerous substrates. In addition, protein phosphatases, which surely play a key role in phosphorylation-dependent regulation of aquaporins, will deserve specific attention in future studies.

Lipid signaling represents another emerging component targeting aquaporins (see "Outstanding questions"). For instance, genetic alteration of phosphoinositides (phosphatidyl inositol [4,5] bisphosphate: PtdInsP2) in tobacco (*Nicotiana tabacum*) increased the osmotic water permeability and aquaporin activity of protoplasts isolated from suspension cells (Ma et al., 2015). The lipid effects may operate through direct interaction with aquaporins or through indirect cellular effects of PtdInsP2. For instance, interaction of PtdInsP2 with TPSO is required for interaction with *At*PIP2;7 and its subsequent degradation (Jurkiewicz et al., 2020). A role for lipid signaling in PIP regulation is also suggested by the physical interaction between *At*PIP2;1 and two phospholipases D (PLDδ and PLDγ1) (Bellati et al., 2016). In plants, PLDs and their enzymatic product phosphatidic acid (PA) play key roles in cellular responses to hormonal and abiotic stimuli. Along these lines, *At*PIP2;1 and *At*PIP2;2 were identified as PA-binding proteins (McLoughlin et al., 2013) but the functional effects of these interactions remain to be elucidated.

In complement to these targeted studies, we believe that systems biology approaches will help uncover previously unidentified signaling pathways targeting aquaporins. For instance, protein interaction networks involving aquaporins have pointed to links with clusters associated with brassinosteroid signaling (Bellati et al., 2016). A genome-wide search for membrane–protein complexes has revealed other clusters that are enriched in aquaporins and have potential roles in stimulus-dependent regulation of water transport (Jones et al., 2014). Finally, a close inspection of aquaporin gene position in gene regulation networks will for sure reveal other modes of developmental or environmental regulation of aquaporins (Alexandersson et al., 2010).

Perspectives

Whereas this overview principally deals with chemical signals, whether abiotic or hormonal, there is still much to be learned about how physical cues lead to aquaporin regulation (see “Outstanding questions”). Particularly, challenging seems the direct link between tissue hydraulics and transpiration that may well be mediated through hydraulic signals, or in other words, pressure-dependent regulation of aquaporins (Laur and Hacke, 2013; Pou et al., 2013). Such dependency may also be on the basis of wound-induced alterations in root or leaf hydraulic conductivity (Liu et al., 2014; Vandeleur et al., 2014). Plant defense is another emerging context in which aquaporin regulation will have to be investigated. While the plant water status is crucial for resilience of plants under attacks, it is also a key target for pathogens to facilitate their proliferation within the plant (Xin et al., 2016). We now have some ideas on how aquaporins serve as both signaling components and targets during innate immunity (Zhang et al., 2019a). In contrast, the action of effectors on aquaporins and possible resistance mechanisms associated to these are just emerging (Ai et al., 2021).

Beyond regulation of water transport in a strict sense, we realize that mechanisms targeting other aquaporin transport functions may have some relevance for plant water relations or share similarities with mechanisms targeting the latter. For instance, aquaporin-mediated CO_2 transport is regulated through interaction with βCA4 carbonic anhydrase (Wang et al., 2016), indicating that the various molecular functions of aquaporin interactants and their ability to associate aquaporin regulation to upstream signaling cascades still represent a gold mine of information. Also, the ion transport of *At*PIP2;1, which is strongly dependent on divalent cations and pH (Byrt et al., 2017), confirms that both Ca^{2+} and H^+ are definitely crucial second messengers acting on aquaporin activity.

Because plants are continuously exposed to combinations of environmental constraints, we realize that our analytic representation of single signaling pathways remains largely inappropriate. Although we emphasized how conserved pathways and their cross talks ultimately act on aquaporins, our knowledge of these processes remains very poor. More generally, basic information on how plant water transport is adjusted in response to combined stresses is still lacking. This large field of investigation, combining fundamental plant physiology and systems biology, is now necessary to properly comprehend the amazing capacity of plants to acclimate to a large array of stressful environments.

OUTSTANDING QUESTIONS

- Is aquaporin-mediated H_2O_2 transport a common process in plant cell signaling?
- Which cellular signals trigger RLK-dependent aquaporin regulation?
- What is the role of lipid signaling in water transport regulation?
- What are the mechanisms for coupling physical stimuli to aquaporin regulation?
- How do aquaporins contribute to plant defense? What are their active roles in plant immunity, and how can they be hijacked by pathogens?
- What are the effects of combined stresses on plant water transport, and which signaling pathways are involved?

Acknowledgments

The authors thank their colleagues of the Aqua team for fruitful discussions.

Funding

This work was supported in part by the Agence Nationale de la Recherche (ANR- 18-CE92-0055).

Conflict of interest statement. None declared.

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