Plasma Membrane and Abiotic Stress

Bronwyn J. Barkla and Omar Pantoja

Abstract Environmental factors exert influence on nearly every aspect of plant function throughout its life cycle. In response to changing and often unfavorable conditions, stress perception in plants initiates signal transduction events that lead to expression of specific stress-related genes and generation of stress-protecting metabolites. Some of these responses are evidently adaptive and lead to changes that increase the chance of survival under adverse conditions, while others are symptoms of stress injury and are pathological in nature. As stressful abiotic environmental conditions can range from exposure to drought, salinity, cold, freezing, high temperature, anoxia, high light intensity, and nutrient imbalance, a complex and overlapping network of molecular machinery must regulate plant responses to these conditions.

The plasma membrane (PM) of the plant cell acts as an important barrier that separates and shields the cell from its environment. However, the PM is also the site of sensors that interpret environmental conditions and transduce signals to other sites on the membrane, inside the cell, and distal portions of the plant to provide for direct and rapid responses to changing environmental conditions. PM sensors can respond directly to alleviate a stress condition, signal secondary changes at the membrane, or activate signaling cascades that potentiate tertiary changes in stress-regulated gene expression.

1 Plasma Membrane Abiotic Stress Sensing

There is abundant information regarding plant abiotic stress-inducible signaling cascades and gene expression, but much less is known about plant abiotic stress sensors. Abiotic stress sensors in bacteria, cyanobacteria, and the yeast *Saccharomyces*

B.J. Barkla (✉) and O. Pantoja
Instituto de Biotecnología, UNAM, A.P. 510-3 Colonia Miraval, Cuernavaca, Morelos 62250, México

A.S. Murphy et al. (eds.), *The Plant Plasma Membrane*, Plant Cell Monographs 19,
DOI 10.1007/978-3-642-13431-9_21,

cereviseae are primarily made up of receptor kinase two- or multicomponent systems consisting of a sensory histidine kinase linked directly or indirectly via a phosphorelay molecule to a response regulator (Murata and Los 2006; Lohrmann and Harter 2002). In some particular cases, the response regulator may also function directly as a transcription factor, thus eliminating the requirement for intermediate signaling cascades (Lohrmann and Harter 2002).

In *Arabidopsis thaliana*, accumulating evidence suggests that two members of the histidine kinase family are involved in osmosensing: AHK1, a protein that detects changes in water status and desiccation (Wohlbach et al. 2008), and CRE1/AHK4, which responds to hyperosmotic stress-related changes in turgor pressure in the presence of cytokinin. Both putative sensors complement a deletion mutant of the yeast osmosensing histidine kinase SLN1 (Reiser et al. 2003; Urao et al. 1999), and *AHK1* can act as a positive regulator in osmotic stress signaling in Arabidopsis plants (Tran et al. 2007). While the assumption can be made that these sensors are PM-localized, it has not been confirmed experimentally, and neither protein has appeared in PM proteomic analyses. Moreover, AHK5, a related histidine kinase that responds to H_2O_2, has been shown to localize to both the cytoplasm and the PM in Arabidopsis (Desikan et al. 2008), and recently, the histidine kinase subfamily of ETR ethylene receptors were shown to be targeted to the endomembrane network and did not localize to the PM (Grefen et al. 2008). Two other related histidine kinase members have also been implicated in abiotic stress sensing; *AHK2* was shown to be regulated by abscisic acid (ABA), osmotic stress, and salt stress, while *AHK3* was shown to be induced by salt and cold stress (Wohlbach et al. 2008).

Other possible abiotic stress sensors at the plant PM are the receptor-like kinases, an unusually large family of proteins that consist of an extracellular receptor domain, a single transmembrane domain, and an intracellular serine/threonine kinase domain. While expression of the genes encoding these kinases has been shown to be regulated by different abiotic stress signals, there is no evidence of a direct role of these kinases in environmental sensing (Tamura et al. 2003). Rather, the evidence points to a more likely role in hormone signaling and host–pathogen recognition (Morris and Walker 2003).

Sensing of abiotic stress may not be limited to protein kinases. In yeast and mammals, PM proteins have been identified, which not only function as transporters but also act as sensors and signal relay proteins (Özcan et al. 1998; Diez-Sampedro et al. 2003). Interestingly, these proteins have unusually long C-terminal cytoplasmic domains, a trait shared by the Arabidopsis PM Na^+/H^+ exchanger SOS1/NHX7, which has been proposed to be an Na^+ sensor (Zhu 2002). However, as of yet, a sensor function has not been clearly demonstrated for any of these proteins. Evidence that SOS1 is a downstream target of a CBL–CIPK calcium-sensing network (see below) suggests that SOS1 is not likely to function as a primary salt stress sensor.

Some insights into the role of the SOS1 C-terminus have come from a yeast two-hybrid screen which detected that the cytoplasmic tail portion binds RCD1/CEO1 (Katiyar-Agarwal et al. 2006), a salt and oxidative stress-responsive nuclear protein that interacts with transcription factors such as STO and DREB2A (Belles-Boix

et al. 2000). Under stress conditions, RCD1/CEO1 localizes to both the nucleus and cytoplasm, thus suggesting it may relay a signal between the two compartments (Katiyar-Agarwal et al. 2006).

Cold, drought, and salt stress can induce production of the phytohormone/plant growth regulator ABA, which itself may be sensed at the PM. Candidate PM proteins for sensing and relaying the ABA signal are two proteins, *At*GTG1 and *At*GTG2 (Pandey et al. 2009). While these proteins show homology to a human Golgi-localized anion channel (Maeda et al. 2008), they share some properties of G protein-coupled receptors (GPCRs), as both proteins interacted with *At*GPA1, a G-protein α subunit, itself localized to the PM by a lipid anchor (Temple and Jones 2007). As would be expected for loss of ABA sensing, mutants lacking both proteins were shown to be hyposensitive to ABA and impaired in upstream ABA signaling responses. It was reported that *At*GTG1 and *At*GTG2 bind ABA with high affinity (Pandey et al. 2009); however, a reevaluation of ABA-binding assays cautions the interpretation of these studies under conditions of suboptimal protein purification (Risk et al. 2009). The proteins are widely expressed in most tissues, although neither *At*GTG1 nor *At*GTG2 are transcriptionally regulated by abiotic stress (Pandey et al. 2009). ABA perception at the PM in turn regulates a wide variety of plant physiological and developmental processes that have been well-studied, especially as they pertain to guard cell physiology. These include the production of reactive oxygen species (ROS), increases in cytosolic free Ca^{2+} levels ($[Ca^{2+}]_i$), protein kinase cascades, activation and inhibition of PM channels, including anion channels, and K^+ influx and K^+ efflux channels; some of which are PM delimited events (Fan et al. 2004).

Perception of most abiotic stresses results in the generation of calcium signals within the plant cell, which, in turn, elicit distinct concentration-dependent responses that depend on the duration, oscillation, and location of the calcium signal (McAinsh and Pittman 2009). In plants, the calcineurin B-like protein (CBL) family represents a unique group of small calcium-binding proteins that act as calcium sensors. They play a key role in decoding calcium transients by interacting with and regulating members of a family of serine/threonine protein kinases known as CBL-interacting protein kinases (CIPKs) and generating a localized signaling cascade that results in phosphorylation of specific target proteins (Batistic and Kudla 2009; Luan 2009). Unique calcium signatures are obtained by the programmed mixing and matching of CBL proteins with CIPKs at specific locations throughout the cell, which include the PM (Kim et al. 2000). PM localization of particular CBL proteins occurs via lipid modifications, as has been demonstrated for both CBL4 and CBL1 (Ishitani et al. 2000; Batistic et al. 2008). Loss-of-function studies of PM CBLs highlight their role in plant abiotic stress tolerance. Disruption of *CBL4*/*SOS3* in Arabidopsis results in plants that are hypersensitive to salt (Shi et al. 1999), disruption of *CBL1* results in plants that are hypersensitive to drought, high salt, and hyperosmotic stress (Albrecht et al. 2003; Cheong et al. 2003), and disruption of *CBL9* results in plants that are hypersensitive to ABA, salt, and osmotic stress (Pandey et al. 2004). Expression profiling also shows the importance of these sensors in abiotic stress response. In rice, expression analysis of ten *CBL* family members identified only two members, which showed no changes under any stress conditions,

with most showing regulation by multiple stresses, and many containing stress-related *cis*-elements in their promotor (Gu et al. 2008).

2 PM Targets of Abiotic Stress Signals

Many targets of stress signaling mechanisms are localized to the PM. These PM delimited events mediate regulation of membrane-resident channels, transporters, pumps, and other target proteins. Recruitment of CIPK family members to the PM is thought to be orchestrated by specific CBL proteins, as the CIPK protein has no membrane spanning domains and no recognizable lipid modification sites (Batistic et al. 2008). Rather, a C-terminal regulatory domain of CIPK contains a 24-amino acid conserved NAF/FISL motif that mediates its interaction with the CBL protein by binding to a hydrophobic crevice (Akaboshi et al. 2008). Moreover, this domain exerts autoinhibitory effects on the kinase activity of the CIPK protein when in the unbound formation (Guo et al. 2001). CBL binding and subsequent targeting of CIPK's to the PM allows for activation of the CIPK and subsequent phosphorylation of membrane-associated substrates. Many members of the CIPK family have been shown to be regulated at the level of transcription by one or more abiotic stress condition (Pandey 2008). In rice, of the 27 *CIPK* genes detected, 20 are regulated by salt, osmotic stress, low temperature, ABA, nutrient deficiency, or a combination of these (Xiang et al. 2007).

The physiological roles of several CBL–CIPK-target networks at the PM have been established in Arabidopsis, and these have been implicated in abiotic stress tolerance. The first elucidation of a CBL–CIPK-target interaction in plants was obtained from a genetic screen for salt overly sensitive mutants in Arabidopsis (Zhu et al. 1998) and resulted in the cloning of three genes (SOS1, SOS2, and SOS3), which are all required for salt tolerance. Analysis of these genes determined that the interaction of CBL4/SOS3 with CIPK24/SOS2 directed the kinase to the PM where it was shown to stimulate the activity of the Na^+/H^+ exchanger NHX7/SOS1 resulting in removal of excess cytoplasmic Na^+ (Qiu et al. 2002). Reconstitution of the pathway in yeast confirmed that CIPK24 phosphorylated the NHX7/SOS1 protein in vitro (Quintero et al. 2002), and a phosphoproteomic study of the PM identified a putative phosphorylation site on the long hydrophilic C-terminal of the NHX7/SOS1 protein (Nühse et al. 2004).

The PM K^+-uptake channel AKT1 has also been shown to be a target protein for phosphorylation by a CBL/CIPK signaling network involved in regulating the protein under K^+-deficiency. Through combined genetic, biochemical, and electrophysiological studies, it was shown that two CBL proteins, CBL1 and CBL9, interacted independently with CIPK23 at the PM to phosphorylate and activate inward K^+ currents through AKT1 (Xu et al. 2006). This multistep signaling cascade also includes the 2C-type protein phosphatase AIP1, which acts as a signaling terminator in conjunction with CIPK23 to dephosphorylate the AKT1 protein and thereby deactivate the K^+-channel (Lee et al. 2007). Both *cipk23* and *cbl1*/*cbl9*

double mutants are sensitive to low K^+, while, conversely, overexpression of *CIPK23, CBL1*, or *CBL9* resulted in plants that accumulated significantly more K^+ than wild type and were hypertolerant to K^+ deficiency (Xu et al. 2006). Moreover, *CIPK23* expression increased under low K^+ growth conditions, although this was not seen with either *CBL1* or *CBL9* (Xu et al. 2006; Cheong et al. 2007). Interestingly, *cipk23* mutants are drought-tolerant and show reduced transpirational water loss and ABA hypersensitivity (Cheong et al. 2007). The complexity of this network was further demonstrated by Lee et al. (2007), who showed that AKT1 interacts with two additional CIPKs, CIPK6 and CIPK16, in addition to CIPK23, and that interaction with these CIPKs results in activation of inward K^+ currents, albeit with reduced magnitude. No physiological function has been ascribed to the binding of CIPK6 or CIPK16 to the AKT1 to date.

A third, well characterized CBL–CIPK-target network localized to the PM is one resulting in the phosphorylation and subsequent negative regulation of an Arabidopsis P-type H^+-ATPase, AHA2, which results in perturbations of apoplastic pH (Fuglsang et al. 2007). CBL2/SCaBP1 and CIPK11/PKS5 have been implicated as upstream mediators of this signaling cascade. Studies established that CIPK11/PKS5 phosphorylated AHA2 in vitro, with the putative phosphorylation site localized to a serine residue in the C-terminal. Yeast reconstitution of the CBL2–CIPK11–AHA2 pathway confirmed the phosphorylation and negative regulation of AHA2 and showed that phosphorylation-dependent changes in AHA2 structure destabilized the binding of a 14-3-3 protein (Fuglsang et al. 2007). However, phosphoproteomics studies have not detected an AHA2 phosphopeptide (Nühse et al. 2004), to corroborate these in vitro studies. Also, while differential regulation of *CIPK11*/*PKS5* transcripts have been observed following treatment with NaCl, drought, ABA, and glucose, *cipk11*/*pks5* mutant plants did not show any phenotypic defect under these conditions (Fuglsang et al. 2007), making it difficult to assign a physiological role in abiotic stress tolerance to the signaling pathway. Nevertheless, some insight can be gained from the observation that *cipk11*/*pks5* mutants are more tolerant to high extracellular pH due to the ability of the deregulated AHA2 to acidify the extracellular space (Fuglsang et al. 2007).

PM transporters could function directly as relay mechanisms or effectors during the adaptive response of plant cells to stress conditions, mainly in cases where ions or metabolites must be transported across the membrane in order to maintain cell homeostasis and to remove toxic ions. One well-studied, but still controversial, mechanism implicated in adaptation to salinity stress is mediated by HKT transporters that have been variously identified as K^+ and/or Na^+ transport systems at the PM (Schachtman and Schroeder 1994; Rubio et al. 1995; Horie et al. 2001; Golldack et al. 2002; Rodríguez-Navarro and Rubio 2006). These transporters belong to a small family with only one member in Arabidopsis and nine members in rice (Garciadeblás et al. 2003). Initial characterization of HKT1;1 from Arabidopsis and HKT2;1 from rice demonstrated a large Na^+-selective transport activity relative to K^+ when expressed in yeast and Xenopus oocytes (Uozumi et al. 2000; Horie et al. 2001; Garciadeblás et al. 2003). However, Golldack et al. (2002) showed that HKT2;1 is competent in the transport of both K^+ and Na^+. More recent

studies have demonstrated that HKT1;1 from Arabidopsis and its closest homolog SKC1 (HKT1;5) in rice function by removing Na^+ from the xylem sap in the root, thus reducing both long distance Na^+ transport and Na^+ accumulation in the leaves (Ren et al. 2005; Sunarpi et al. 2005; Horie et al. 2007). *SKC1* is linked with a QTL in rice that is associated with the maintenance of K^+ content in the shoot under salt stress conditions (Ren et al. 2005). The *SKC1*/*HKT1;5* locus encodes a functional Na^+-selective transporter, whose activity was shown to be K^+-independent. Contrary to expectations, the isoform encoded by the *NSKC1* gene from the salt-resistant Nona Bokra rice variety was more active than KSKC1 from salt-sensitive Koshihikari variety (Ren et al. 2005). This difference in transport activity was attributed to four amino acid changes that exist in the sequence between the two rice varieties (Ren et al. 2005). GUS expression analyses demonstrated that *SKC1* was mainly present in the xylem parenchyma cells surrounding the xylem vessels, where it could serve in the removal of Na^+ from the xylem sap. Consistent with these results and supporting a direct role of HKT transporters in salt tolerance, Ren et al. (2005) reported lower levels of Na^+ in the xylem and shoot tissues upon salt stress in salt-tolerant Nona Bokra as compared to the salt-sensitive Koshihikari. Similar observations have been made with the homologous HKT1;1 transporter from Arabidopsis (Sunarpi et al. 2005). HKT1;1 is expressed mainly in the cells surrounding the xylem vessels, with higher expression observed in the root than in the shoot. Analyses of three different mutants of *HKT1*, *athkt1-3* in the Wassilewskija (Ws) background and *athkt1-1*, *athkt1* (disruption allele FN1148; Gong et al. 2004) in the Columbia background, showed clear increases in the Na^+ content of the xylem sap under control and Na^+ stress conditions, indicating a role of *At*HKT1;1 in lowering xylem Na^+ content (Sunarpi et al. 2005). Associated with these changes, Sunarpi et al. (2005) also reported a decrease in Na^+ levels in the phloem sap of the three different *hkt1;1* mutants, thus confirming an earlier observation reported by Berthomieu et al. (2003) for a partial loss-of-function *hkt1;1* allele, *sas 2-1*.

The decrease in Na^+ phloem content observed in the different *hkt1;1* mutants has been used to postulate that *Arabidopsis* HKT1;1 participates in the recirculation of Na^+ from the shoot to the root via the phloem, thus decreasing the toxic effects of Na^+ in shoot tissues. Further confirmation of the role of HKT1;1 in xylem Na^+ removal was provided by its localization to the PM of leaf xylem parenchyma cells by immunoelectron microscopy. This confirmed previous work that showed PM localization of *Mesembryanthemum crystallinum* HKT1;1 (Su et al. 2003). However, as expression of Arabidopsis *HKT1;1* was induced by 30 mM NaCl or KCl and equimolar concentrations of mannitol or sorbitol, HKT1 transporters may also play an additional role in osmotic adaptation (Sunarpi et al. 2005).

3 Stomatal Guard Cells and Stress Responses at the PM

Studies of stomatal guard cell physiology have played a central role in efforts to identify stress-responsive adaptive mechanisms at the PM, as guard cells play a central role in the control of transpiration and water loss under conditions of

drought, heat, salinity, and cold. In a mutant screen for ozone sensitivity, Vahisalu et al. (2008) identified the mutant *slac1-1* that showed constitutively higher stomatal conductance than the wild type. Contemporaneously, Negi et al. (2008) isolated the allelic *cdi3*/*slac1-2* mutant, which is impaired in CO_2-dependent leaf temperature changes. *SLAC1* encodes a membrane protein with ten predicted transmembrane helices and hydrophilic amino- and carboxy-terminals. SLAC1 is also predicted to possess a C_4-dicarboxylate/malic acid transport protein domain, similar to that identified in the TehA and Mae1 proteins from *Escherichia coli* and *Schizosaccharomyces pombe*, respectively (Guzzo and Dubow 2000; Grobler et al. 1995). *SLAC1* promoter GUS fusions showed the gene to be primarily expressed in guard cells, and GFP fusion studies demonstrated that it was localized to the PM (Vahisalu et al. 2008; Negi et al. 2008). Information on the participation of SLAC1 in stomatal control was gained from studies in *slac1-1* plants, demonstrating alterations in stomatal conductance and stomatal closing upon transfer to the dark or upon exposure to low relative air humidity conditions, and a lack of response to large increases in atmospheric CO_2 (Vahisalu et al. 2008). Similar results were also reported for the *slac1-2* mutant (Negi et al. 2008). Moreover, *slac1-1* guard cells were insensitive to ABA and downstream second messengers such as H_2O_2, NO, and oscillating cytoplasmic Ca^{2+}.

Whole cell patch-clamp experiments demonstrated that *slac1-1* guard cells lack the activity of the so-called S-type anion currents, which were clearly activated by μM levels of Ca^{2+} in wild type. From these results, it appears that SLAC1 plays a central role in stomatal closing under adverse environmental conditions (Vahisalu et al. 2008), although it is still not clear if this protein corresponds to the actual anion channel or to a regulatory subunit of the channel complex. In Arabidopsis, there are three other *SLAC1*-like genes (40–50% identity at the amino acid level) that encode proteins localized to the PM, but none of the genes are expressed in guard cells (Negi et al. 2008). However, two of the *SLAC1* homologs, At1g62280 and At5g24030, complemented the CO_2-insensitive phenotype of the *slac1-2* mutant when expressed under the control of the *SLAC1* guard cell-specific promoter. These findings indicate a conserved function despite tissue-specific expression. These studies highlight the importance of PM ion transport processes as direct adaptive mechanisms that mediate plant response to stress.

4 Membrane-Bound Transcription Factor and Response to Abiotic Stress

Another abiotic stress signaling target at the PM could be the recently described membrane-bound transcription factors (MBTF's). Like other transcription factors, MBTFs are proteins that regulate the expression of downstream genes in response pathways and are latent until activation by internal or environmental signals. Signal-induced activation of transcription factors generally leads to a

rapid transcriptional response. Until recently, it was assumed that transcription factors are present as cytosolic pools and that entry to the nucleus is mediated by protein–protein interactions, resulting in their phosphorylation (Leonard and O'Shea 1998) or release from an associated protein (e.g. NF-κB, IκB) (Ghosh et al. 1998). However, MBTFs are membrane-bound proteins that are inactive until undergoing a proteolytic step, which releases them from the resident membrane and allows their entry into the nucleus to modulate gene expression (Kim et al. 2006, 2007a, b; Chen et al. 2008; Yoon et al. 2008). Selective proteolysis of MBTFs to release the trans-acting DNA-binding domain from the membrane anchor adds another level of posttranslational regulation that can respond to abiotic stress at the PM.

As the role of MBTFs in Arabidopsis has been recently been reviewed (Chen et al. 2008), here we only describe the work that has demonstrated the association of stress-related MBTFs to the PM.

Structural analysis of NAC-type transcription factors was used to identify more than 13 members in Arabidopsis and 6 members in rice that possess strong α-helical domains suggestive of MBTF activity. These were designated *NTL*s for *NTM1*-like (Kim et al. 2007a), in reference to the well characterized intracellular membrane-associated *NTM1* MTBF (Kim et al. 2006). Expression of most *NTL*s was detected in leaves, stems, and in roots, with apparently lower expression levels seen in flower and shoot apex tissues (Kim et al. 2006). Eleven *NTL*s were found to be differentially regulated by abiotic stress; *NTL1* and *NTL11* were found to be regulated by heat, and *NTL4* and *NTL7* by cold. *NTL3, NTL6, and NTL8* were strongly upregulated by NaCl, *NTL9* expression increased with osmotic stress, *NTL5* was upregulated by drought and NaCl, and expression of *NTL2* and *NTL3* were influenced by cold, drought, and NaCl. Together, these responses indicate that individual *NTL*s play distinct roles in responses to abiotic stress conditions. However, *NTL* expression in response to hydrogen peroxide was low, suggesting that *NTL*s are not directly associated to responses to ROS (Kim et al. 2007a).

Evidence for the PM localization of NTL8 was demonstrated by GFP fusions to both full length NTL8 or to a truncated version of the protein (Kim et al. 2007a). The full length NTL8 GFP fusion was found to be associated with the PM, while the fusion lacking the NTL8 membrane anchor was detected in the nucleus, in agreement to the NTM1-like nature of NTL8 (Kim et al. 2007b). Overexpression of the truncated form of NTL8 caused a delay in flowering time, associated with a downregulation of specific flowering time genes including FLOWERING LOCUS T (*FT*), FRUITFUL (*FUL*), and CAULIFLOWER (*CAL*) (Kim et al. 2007b). Additionally, *FT* transcript levels were significantly reduced in plants exposed to NaCl, indicating that high salt delayed flowering by repressing *FT* expression via signaling mediated by NTL8. However, *ntl8-1* mutants exhibited only slight differences from wild type in lateral root growth and flowering times (Kim et al. 2007b). Expression of *NTL8* was also shown to be developmentally regulated, with maximal expression observed in germinating seeds (Kim et al. 2007b). A possible role of *NTL8* in germination under salt stress was indicated by an observed decrease in the germination rate of seeds

overexpressing the truncated form of *NTL8*, while germination rate increased in the *ntl8* mutant (Kim et al. 2008).

Another well-studied MBTF of the NTL family, NTL9, is also localized to the PM and appears to be involved in abiotic stress gene regulation (Yoon et al. 2008). Plants overexpressing full length *NTL9* did not show a clear phenotype, which contrasted to the dwarf phenotype with small and curled leaves often associated with abiotic stress that was observed in plants overexpressing *NTL9* lacking the membrane anchor (Yoon et al. 2008). Increased expression of the stress-related genes *SIAH*-Interacting Protein (*SIP*) and Cold-Regulated 15a (*COR15a*) in plants overexpressing the truncated form of *NTL9* further implicated *NTL9* in stress responses (Yoon et al. 2008) as did evidence of posttranscriptional processing of NTL9 and increased abundance of both full length and truncated NTL9 with osmotic stress (Yoon et al. 2008).

5 Other Posttranslational Regulation of PM Proteins

Evidence exists for posttranslational regulation of other plant PM proteins in response to abiotic stress, although the associated upstream signaling is poorly characterized. The turnover of populations of integral PM proteins by trafficking to and from the membrane can be regulated by a variety of environmental stimuli. In the case of the KAT1 inward rectifying K^+ channel, ABA induces the rapid and transient internalization of the channel to an endomembrane compartment from which it can be recycled to the PM upon removal of ABA (Sutter et al. 2007). The soluble *N*-ethylmaleimide-sensitive factor protein attachment protein receptor (SNARE) protein, SYP121, has been proposed to mediate the process (Grefen and Blatt 2009). It has been suggested that this trafficking is a posttranslational adaptive mechanism to allow rapid changes in osmotic solute flux required in response to abiotic stress.

Ubiquitination can also be induced by abiotic stress and alter the turnover and subcellular localization of PM target proteins such as the Arabidopsis aquaporin PIP2;1 (Lee et al. 2009). Overexpression of Arabidopsis RMA1, an ER-localized homolog of a RING membrane anchor E3 ubiquitin ligase, was shown to reduce PIP2;1 protein levels at the PM and inhibited trafficking of PIP2;1 from the ER in protoplasts. In addition, protoplasts from RNA interference (RNAi) plants with reduced expression of *RMA1* showed increased levels of PIP2;1 (Lee et al. 2009). However, increased water permeability was not seen in these protoplasts, and compensatory expression of other *PIP* genes was not investigated. RMA1-mediated ubiquitination was further implicated in abiotic stress responses by the finding that *Capsicum annuum* plants exposed to cold, high salinity, and drought stress (but not ABA) showed rapid induction of transcripts for *Rma1H1*, and overexpression of the *CaRMA1H1* gene in Arabidopsis resulted in plants that exhibited an increased tolerance to drought (Lee et al. 2009).

6 Abiotic Stress and Effects on PM Integrity

Exposure to abiotic stress can result in damage to PM lipids resulting in lesions and membrane leakiness, particularly when osmotic or cold stress factors are involved. Damaged PMs are thought to be repaired by fusion of exocytotic vesicles triggered by a rapid elevation in cytoplasmic Ca^{2+} resulting from entry through the damaged membrane and mediated by the calcium-sensing synaptotagmin protein functioning in parallel with SNARE proteins (Andrews 2005). In a proteomic study of PM proteins from Arabidopsis leaves induced by cold stress, a synaptotagmin protein was identified by its increased abundance (Kawamura and Uemura 2003). Subsequently, it was shown that loss-of-function mutations in *Synaptotagmin1* (*SYT1*) exhibited reduced cellular viability as a consequence of compromised PM integrity and consequent increases in electrolyte leakage and hypersensitivity to NaCl, high osmotic stress, and freezing (Schapire et al. 2008). Further support for the role of this SYT1 in abiotic stress tolerance came from independent studies demonstrating that protoplasts or leaves from *SYT1*-RNA interference (RNAi) plants were freezing-sensitive even in the presence of calcium (Yamazaki et al. 2008).

7 Conclusion

It is evident that plants employ diverse mechanisms, many of which are localized to the PM; to contend with the adverse environmental conditions, they are continuously exposed. The PM not only functions as a protection barrier or interface involved in the maintenance of ionic and metabolite gradients, but it is directly involved in sensing the diversity of external signals, in the transduction of these signals, and in activating mechanisms that help maintain the cellular homeostasis that is disrupted by the imposition of multiple stress conditions.

Acknowledgments Work in the lab is funded by CONACyT (49735 to BJB and 79191 to OP) and DGAPA (IN212410 to BJB and IN218308 to OP).

References

Akaboshi M, Hashimoto H, Ishida H, Saijo S, Koizumi N, Sato M, Shimizu T (2008) The crystal structure of plant-specific calcium-binding protein AtCBL2 in complex with the regulatory domain of AtCIPK14. J Mol Biol 377:246–257

Albrecht V, Weinl S, Blazevic A, D'Angelo C, Batistic O, Kolukisaoglu Ü, Bock R, Schulz B, Harter K, Kudla J (2003) The calcium sensor CBL1 integrates plant responses to abiotic stresses. Plant J 36:457–470

Andrews NW (2005) Membrane resealing: synaptotagmin VII keeps running the show. Sci Signal 3:pe15

Batistic O, Kudla J (2009) Plant calcineurin B-like proteins and their interacting protein kinases. Biochim Biophys Acta 1793:985–992

Batistic O, Sorek N, Schultke S, Yalovsky S, Kudla J (2008) Dual fatty acyl modification determines the localization and plasma membrane targeting of CBL/CIPK Ca^{2+} signaling complexes in *Arabidopsis*. Plant Cell 20:1346–1362

Belles-Boix E, Babiychuk E, Van Montagu M, Inzé D, Kushnir S (2000) CEO1, a new protein from *Arabidopsis thaliana*, protects yeast against oxidative damage. FEBS Lett 482:19–24

Berthomieu P, Conejero G, Nublat A, Brackenbury WJ, Lambert C, Savio C, Uozumi N, Oiki S, Yamada K, Cellier F, Gosti F, Simonneau T, Essah PA, Tester M, Very AA, Sentenac H, Casse F (2003) Functional analysis of AtHKT1 in Arabidopsis shows that Na^+ recirculation by the phloem is crucial for salt tolerance. EMBO J 22:2004–2014

Chen YN, Slabaugh E, Brandizzi F (2008) Membrane-tethered transcription factors in *Arabidopsis thaliana*: novel regulators in stress response and development. Curr Opin Plant Biol. 11:695–701

Cheong YH, Kim KN, Pandey GK, Gupta R, Grant JJ, Luan S (2003) CBL1, a calcium sensor that differentially regulates salt, drought, and cold responses in Arabidopsis. Plant Cell 15:1833–1845

Cheong YH, Pandey GK, Grant JJ, Batistic O, Li L, Kim B-G, Lee SC, Kudla J, Luan S (2007) Two calcineurin B-like calcium sensors, interacting with protein kinase CIPK23, regulate leaf transpiration and root potassium uptake in Arabidopsis. Plant J 52:223–239

Desikan R, Horák J, Chaban C, Mira-Rodado V, Witthöft J, Elgass K, Grefen C, Cheung MK, Meixner AJ, Hooley R, Neill SJ, Hancock JT, Harter K (2008) The histidine kinase AHK5 integrates endogenous and environmental signals in *Arabidopsis* guard cells. PLoS ONE 3:e2491

Diez-Sampedro A, Hirayama BA, Osswald C, Gorboulev V, Baumgarten K, Volk C, Wright EM, Koepsell H (2003) A glucose sensor hiding in a family of transporters. Proc Natl Acad Sci USA 100:11753–11758

Fan L-M, Zhao Z, Assmann SM (2004) Guard cells: a dynamic signaling model. Curr Opin Plant Biol 7:537–546

Fuglsang AT, Guo Y, Cuin TA, Qiu Q, Song C, Kristiansen KA, Bych K, Schulz A, Shabala S, Schumaker KS, Palmgren MG, Zhu JK (2007) *Arabidopsis* protein kinase PKS5 inhibits the plasma membrane H^+-ATPase by preventing interaction with 14-3-3 protein. Plant Cell 19:1617–1634

Garciadeblás B, Senn ME, Bañuelos MA, Rodríguez-Navarro A (2003) Sodium transport and HKT transporters: the rice model. Plant J 34:788–801

Ghosh S, May MJ, Kopp EB (1998) NF-kB and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol 16:225–260

Golldack D, Su H, Quigley F, Kamasani UR, Muñoz-Garay C, Balderas E, Popova OV, Bennett J, Bohnert HJ, Pantoja O (2002) Characterization of a HKT-type transporter in rice as a general alkali cation transporter. Plant J 31:529–542

Gong JM, Waner DA, Horie T, Li SL, Horie R, Abid KB, Schroeder JI (2004) Microarray-based rapid cloning of an ion accumulation deletion mutant in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 101:15404–15409

Grefen C, Blatt MR (2009) SNAREs – molecular governors in signalling and development. Curr Opin Plant Biol 11:600–609

Grefen C, Städele K, Ruzicka K, Obrdlik P, Harter K, Horak J (2008) Subcellular localization and *in vivo* interactions of the *Arabidopsis thaliana* ethylene receptor family members. Mol Plant 1:308–320

Grobler J, Bauer F, Subden RE, Van Vuuren HJ (1995) The mae1 gene of Schizosaccharomyces pombe encodes a permease for malate and other C_4 dicarboxylic acids. Yeast 11:1485–1491

Gu Z, Ma B, Jiang Y, Chen Z, Su X, Zhang H (2008) Expression analysis of the calcineurin B-like gene family in rice (*Oryza sativa* L.) under environmental stresses. Gene 415:1–12

Guo Y, Halfter U, Ishitani M, Zhu JK (2001) Molecular characterization of functional domains in the protein kinase SOS2 that is required for plant salt tolerance. Plant Cell 13:1383–1400

Guzzo J, Dubow MS (2000) A novel selenite- and tellurite-inducible gene in *Escherichia coli*. Appl Environ Microbiol 66:4972–4978

Horie T, Yoshida K, Nakayama H, Yamada K, Oiki S, Shinmyo A (2001) Two types of HKT transporters with different properties of Na^+ and K^+ transport in *Oryza sativa*. Plant J 27:129138

Horie T, Costa A, Kim TH, Han MJ, Horie R, Leung H-Y, Miyao A, Hirochika H, Gynheung A, Schroeder JI (2007) Rice OsHKT2;1 transporter mediates large Na^+ influx component into K^+-starved roots for growth. EMBO J 26:1–12

Ishitani M, Liu J, Halfter U, Kim CS, Shi W, Zhu JK (2000) SOS3 function in plant salt tolerance requires N-myristoylation and calcium binding. Plant Cell 12:1667–1678

Katiyar-Agarwal S, Zhu J, Kim K, Agarwal M, Fu X, Huang A, Zhu JK (2006) The plasma membrane Na^+/H^+ antiporter SOS1 interacts with RCD1 and functions in oxidative stress tolerance in *Arabidopsis*. Proc Natl Acad Sci USA 103:18816–18821

Kawamura Y, Uemura M (2003) Mass spectrometric approach for identifying putative plasma membrane proteins of *Arabidopsis* leaves associated with cold acclimation. Plant J 36: 141–154

Kim KN, Cheong YH, Gupta R, Luan S (2000) Interaction specificity of Arabidopsis calcineurin B-like calcium sensors and their target kinases. Plant Physiol 124:1844–1853

Kim YS, Kim SG, Park JE, Park HY, Lim MH, Chua N-H, Park C-M (2006) A membrane-bound NAC transcription factor regulates cell division in Arabidopsis. Plant Cell 18:3132–3144

Kim SY, Kim SG, Kim YS, Seo PJ, Bae M, Yoon HK, Park CM (2007a) Exploring membrane-associated NAC transcription factors in Arabidopsis: implications for membrane biology in genome regulation. Nucleic Acids Res 35:203–213

Kim SG, Kim SY, Park C-M (2007b) A membrane-associated NAC transcription factor regulates salt-responsive flowering via FLOWERING LOCUS T in Arabidopsis. Planta 226:647–654

Kim SG, Lee AK, Yoon HK, Park CM (2008) A membrane-bound NAC transcription factor NTL8 regulates gibberellic acid-mediated salt signaling in Arabidopsis seed germination. Plant J 55:77–88

Lee SC, Lan WZ, Kim BG, Li L, Cheong YH, Pandey GK, Lu G, Buchanan BB, Luan S (2007) A protein phosphorylation/dephosphorylation network regulates a plant potassium channel. Proc Natl Acad Sci USA 104:15959–15964

Lee HK, Cho SK, Son O, Xu Z, Hwang I, Kim WT (2009) Drought stress-induced RMA1h1, a ring membrane-anchor E3 ubiquitin ligase homolog, regulates aquaporin levels via ubiquitination in transgenic *Arabidopsis* plants. Plant Cell 21:622–641

Leonard WJ, O'Shea JJ (1998) JAKS AND STATS: biological Implications. Annu Rev Immunol 16:293–322

Lohrmann J, Harter K (2002) Plant two-component signaling systems and the role of response regulators. Plant Physiol 128:363–369

Luan S (2009) The CBL–CIPK network in plant calcium signaling. Trends Plant Sci 14:37–42

Maeda Y, Ide T, Koike M, Uchiyama Y, Kinoshita T (2008) GPHR is a novel anion channel critical for acidification and functions of the Golgi apparatus. Nat Cell Biol 10:1135–1145

McAinsh MR, Pittman JK (2009) Shaping the calcium signature. New Phytol 181:275–294

Morris ER, Walker JC (2003) Receptor-like protein kinases: the keys to response. Curr Opin Plant Biol 6:339–342

Murata N, Los DA (2006) Histidine kinase HiK33 is an important participant in cold-signal transduction in cyanobacteria. Physiol Plant 126:17–27

Negi J, Matsuda O, Nagasawa T, Oba Y, Takahashi H, Kawai-Yamada M, Uchimiya H, Hashimoto M, Iba K (2008) CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. Nature 452:483–486

Nühse TS, Stensballe A, Jensen ON, Peck SC (2004) Phosphoproteomics of the Arabidopsis plasma membrane and a new phosphorylation site database. Plant Cell 16:2394–2405

Özcan S, Dover J, Johnston M (1998) Glucose sensing and signaling by two glucose receptors in the yeast *Saccharomyces cerevisiae*. EMBO J 17:2566–2573

Pandey GK (2008) Emergence of a novel calcium signaling pathway in plants: CBL-CIPK signaling network. Physiol Mol Biol Plants 14:51–68

Pandey GK, Cheong YH, Kim KN, Grant JJ, Li L, Hung W, D'Angelo C, Weinl S, Kudla J, Luan S (2004) The calcium sensor calcineurin B-like 9 modulates abscisic acid sensitivity and biosynthesis in Arabidopsis. Plant Cell 16:1912–1924

Pandey S, Nelson DC, Assmann SM (2009) Two novel GPCR-type G proteins are abscisic acid receptors in *Arabidopsis*. Cell 136:136–148

Qiu QS, Guo Y, Dietrich MA, Schumaker KS, Zhu JK (2002) Regulation of SOS1, a plasma membrane Na^+/H^+ exchanger in Arabidopsis thaliana, by SOS2 and SOS3. Proc Natl Acad Sci USA 99:8436–8441

Quintero FJ, Ohta M, Shi H, Zhu JK, Pardo JM (2002) Reconstitution in yeast of the *Arabidopsis* SOS signaling pathway for Na^+ homeostasis. Proc Natl Acad Sci USA 99:9061–9066

Reiser V, Raitt DC, Saito H (2003) Yeast osmosensor Sln1 and plant cytokinin receptor Cre1 respond to changes in turgor pressure. J Cell Biol 161:1035–1040

Ren ZH, Gao JP, Li LG, Cai XL, Huang W, Chao DY, Zhu MZ, Wang ZY, Luan S, Lin HX (2005) A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nat Genet 37:1141–1146

Risk JM, Day CL, Macknight RC (2009) Reevaluation of abscisic acid-binding assays shows that G-protein-coupled receptor2 does not bind abscisic acid. Plant Physiol 150:6–11

Rodríguez-Navarro A, Rubio F (2006) High-affinity potassium and sodium transport systems in plants. J Exp Bot 57:1149–1160

Rubio F, Gassmann W, Schroeder JI (1995) Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. Science 270:1660–1663

Schachtman DP, Schroeder JI (1994) Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants. Nature 370:655–658

Schapire AL, Voigt B, Jasik J, Rosado A, Lopez-Cobollo R, Menzel D, Salinas J, Mancuso S, Valpuesta V, Baluska F, Botella MA (2008) *Arabidopsis s*ynaptotagmin 1 is required for the maintenance of plasma membrane integrity and cell viability. Plant Cell 20:3374–3388

Shi J, Kim KN, Ritz O, Albrecht V, Gupta R, Harter K, Luan S, Kudla J (1999) Novel protein kinases associated with calcineurin B-like calcium sensors in Arabidopsis. Plant Cell 11:2393–2405

Su H, Balderas E, Vera-Estrella R, Golldack D, Quigley F, Zhao C, Pantoja O, Bohnert HJ (2003) Expression of the cation transporter McHKT1 in a halophyte. Plant Mol Biol 52:967–980

Sunarpi HT, Motoda J, Kubo M, Yang H, Yoda K, Horie R, Chan WY, Leung HY, Hattori K, Konomi M, Osumi M, Yamagami M, Schroeder JI, Uozumi N (2005) Enhanced salt tolerance mediated by AtHKT1 transporter-induced Na^+ unloading from xylem vessels to xylem parenchyma cells. Plant J 44:928–938

Sutter JU, Sieben C, Hartel A, Eisenach C, Thiel G, Blatt MR (2007) Abscisic acid triggers the endocytosis of the Arabidopsis KAT1 K^+ channel and its recycling to the plasma membrane. Curr Biol 17:1396–1402

Tamura T, Hara K, Yamaguchi Y, Koizumi N, Sano H (2003) Osmotic stress tolerance of transgenic tobacco expressing a gene encoding a membrane-located receptor-like protein from tobacco plants. Plant Physiol 131:454–462

Temple BR, Jones AM (2007) The plant heterotrimeric G-protein complex. Annu Rev Plant Biol 58:249–266

Tran LS, Urao T, Qin F, Maruyama K, Kakimoto T, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in *Arabidopsis*. Proc Natl Acad Sci USA 104: 20623–20628

Uozumi N, Kim EJ, Rubio F, Yamaguchi T, Muto S, Tsuboi A, Bakker EP, Nakamura T, Schroeder JI (2000) The Arabidopsis HKT1 gene homolog mediates inward Na^+ currents in Xenopus laevis oocytes and Na^+ uptake in *Saccharomyces cerevisiae*. Plant Physiol 122: 1249–1259

Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T, Shinozaki K (1999) A transmembrane hybrid-type histidine kinase in *Arabidopsis* functions as an osmosensor. Plant Cell 11:1743–1754

Vahisalu T, Kollist H, Wang Y-F, Nishimura N, Chan W-Y, Valerio G, Lamminmäki A, Brosché M, Moldau H, Desikan R, Schroeder JI, Kangasjärvi J (2008) SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling. Nature 452:487–491

Wohlbach DJ, Quirino BF, Sussman MR (2008) Analysis of the Arabidopsis histidine kinase ATH1 reveals a connection between vegetative osmotic stress sensing and seed maturation. Plant Cell 20:1101–1117

Xiang Y, Huang Y, Xiong L (2007) Characterization of stress-responsive *CIPK* genes in rice for stress tolerance improvement. Plant Physiol 144:1416–1428

Xu J, Li HD, Chen LQ, Wang Y, Liu LL, He L, Wu WH (2006) A protein kinase, interacting with two calcineurin B-like proteins, regulates K^+ transporter AKT1 in *Arabidopsis*. Cell 125:1347–1360

Yamazaki T, Kawamura Y, Minami A, Uemura M (2008) Calcium-dependent freezing tolerance in Arabidopsis involves membrane resealing via synaptotagmin SYT1. Plant Cell 20:3389–3404

Yoon HK, Kim SG, Kim SY, Park CM (2008) Regulation of leaf senescence by NTL9-mediated osmotic stress signaling in Arabidopsis. Mol Cells 25:438–445

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–273

Zhu JK, Liu J, Xiong L (1998) enetic analysis of salt tolerance in Arabidopsis. Evidence for a critical role of potassium nutrition. Plant Cell 10:1181–1191