Plant Biotechnol Rep (2013) 7:1–8
DOI 10.1007/s11816-012-0228-1

REVIEW ARTICLE

Stress responses mediated by the CBL calcium sensors in plants

Kyung-Nam Kim

Received: 20 February 2012 / Accepted: 17 April 2012 / Published online: 3 May 2012

Abstract Calcium ions (Ca^{2+}) are involved as second messenger in plant responses to a broad array of environmental stimuli, including biotic and abiotic stresses. Therefore, understanding Ca^{2+}-signaling mechanisms may lead to the development of transgenic crops with enhanced tolerance to adverse environmental conditions. In order to initiate the signaling cascades and give rise to relevant cellular and physiological responses, changes in the parameters of Ca^{2+} transients should be first detected by appropriate Ca^{2+} sensors in plant cells. In this regard, elucidations of plant Ca^{2+} sensors and their target molecules are critical steps for unraveling the Ca^{2+} signal transduction pathways. Recent studies have revealed that plants possess many Ca^{2+}-binding proteins with different properties, which can serve as distinct Ca^{2+} sensors. This present review mainly focuses on a family of calcineurin B-like Ca^{2+} sensors which has been most recently identified from higher plants including Arabidopsis, rice, maize and pea.

Keywords CBL · CIPK · Calcium signaling · Stress response

Complexity of the Ca^{2+} signals and sensors

In plants, Ca^{2+} is involved in a variety of cellular and developmental processes such as guard-cell turgor control, pollen-tube growth, and root-hair elongation (Blatt 2000; Holdaway-Clarke et al. 1997; Wymer et al. 1997). Moreover, it also plays a critical role in mediating a wide range of extracellular stimuli such as light, plant hormones, pathogen attack, and abiotic stresses (Ehrhardt et al. 1996; Rudd and Franklin-Tong 2001; Sanders et al. 1999). How can the simple cation Ca^{2+} serve as a second messenger in so many diverse signal transduction pathways and yet manage to elicit appropriate plant responses? The answer to this question lies in the complexity of Ca^{2+} signal itself and numerous Ca^{2+} sensors with different properties.

Ca^{2+} signals can be complex because they are constituted not only of concentration but also the temporal and spatial parameters, which include frequency, magnitude, duration, and subcellular localization of the transient Ca^{2+} increases in the cytosol (Dolmetsch et al. 1997, 1998; Evans et al. 2001; Rudd and Franklin-Tong 2001).

Three major families of EF-hand Ca^{2+}-binding proteins

In order for the diverse Ca^{2+} signals to elicit stimulus-specific responses, plant cells should be equipped with various Ca^{2+} sensors which detect and transduce changes in the Ca^{2+} parameters. As a matter of fact, plants possess numerous Ca^{2+} sensors that are mostly proteins with the EF-hand Ca^{2+}-binding motif (Kawasaki et al. 1998). These Ca^{2+}-binding proteins can be classified into three major families; calmodulin (CaM) and CaM-like proteins, Ca^{2+}-dependent protein kinases (CDPKs), and calcineurin B-like (CBL) proteins.

First, the family members of CaM and CaM-like proteins, which do not possess any enzymatic activity themselves, act as "sensor relays" (Luan et al. 2002; Zielinski 1998). Upon Ca^{2+} binding, they undergo conformational changes to associate with and regulate various target proteins such as NAD kinase, glutamate decarboxylase,

K.-N. Kim (✉)
Department of Molecular Biology, PERI,
Sejong University, Seoul 143-747, Korea
e-mail: knkim@sejong.ac.kr

Ca^{2+}-ATPase, and protein kinases (Luan et al. 2002; Reddy et al. 2002; Snedden and Fromm 2001; Yang and Poovaiah 2003; Zielinski 1998). The Arabidopsis genome contains 50 CaM-like genes in addition to the six genomic loci encoding typical CaMs (Luan et al. 2002; McCormack and Braam 2003).

Second, the CDPK family members are classified as "sensor responders", because they harbor a kinase domain at the N-terminal end along with the C-terminal CaM-like Ca^{2+} sensor (Sanders et al. 2002). It is well known that Ca^{2+} can activate the kinase activity of CDPKs by binding to the CaM-like region (Chandran et al. 2006; Cheng et al. 2002; Harper et al. 2004; Hrabak et al. 2003). The Arabidopsis genome was predicted to carry 34 CDPK genes, which appear to possess different properties in terms of expression pattern, subcellular localization, Ca^{2+}-binding affinity, and substrate specificity (Asano et al. 2005; Harper et al. 2004; Hernandez Sebastia et al. 2004; Hrabak et al. 2003; Lee et al. 1998; Rodriguez Milla et al. 2006; White and Broadley 2003).

The third major family of EF-hand Ca^{2+} sensors is CBL proteins, which are most similar to the regulatory B subunit of the protein phosphatase calcineurin (CNB) in animals (Kudla et al. 1999; Liu and Zhu 1998). Following the initial discovery from Arabidopsis, many CBLs were identified from various plant species including rice, maize, and pea (Kolukisaoglu et al. 2004; Mahajan et al. 2006; Wang et al. 2007). Therefore, it is believed that the CBL proteins are ubiquitously present in the plant kingdom (Batistic and Kudla 2009). Interestingly, the CBL family is comprised of 10 genes in both Arabidopsis and rice plants (Kolukisaoglu et al. 2004; Luan et al. 2002).

CBLs associate with a group of serine/threonine protein kinases, CIPKs

The CBL members appeared to be sensor relays like CaM, and therefore they should function by interacting with and modulating their target proteins in a Ca^{2+}-dependent manner. In fact, several research groups demonstrated that CBLs have interaction partners which are mainly a group of serine/threonine protein kinases designated as CBL-interacting protein kinases (CIPKs) (Batistic and Kudla 2009; Cheong et al. 2007; Halfter et al. 2000; Huang et al. 2011; Kim et al. 2000; Kolukisaoglu et al. 2004; Quan et al. 2007; Shi et al. 1999; Waadt et al. 2008). The CIPK proteins are unique to plants in that they contain a distinct regulatory domain in the C-terminus, although their N-terminal kinase domain is most related to those of the yeast SNF1 protein kinase (Sucrose NonFermenting 1) and the mammalian AMP-dependent protein kinase (Shi et al. 1999).

Sequence analysis predicted that the CIPK family consists of 25 and 30 genes in the Arabidopsis and rice genomes, respectively (Kolukisaoglu et al. 2004). It appears that each CIPK can interact physically and specifically with more than one CBL members at different affinities through the conserved NAF (or FISL) motif present in the C-terminal non-kinase domain (Albrecht et al. 2001; Batistic and Kudla 2004; Halfter et al. 2000; Jeong et al. 2005; Kim et al. 2000; Kolukisaoglu et al. 2004; Luan et al. 2002).

CBLs activates target CIPKs in a Ca^{2+}-dependent manner

CBL1 formed a complex with CIPK1 only in the presence of Ca^{2+} (Shi et al. 1999). In contrast, other CBL members, such as CBL2 and CBL4 (also known as SOS3), did not display such Ca^{2+}-dependency: CBL2 and CBL4 interacted regardless of Ca^{2+} with CIPK14 and CIPK24 (also known as SOS2), respectively (Akaboshi et al. 2008; Halfter et al. 2000). These findings together suggest that the Ca^{2+} requirement for the complex formation can vary among the participating CBL and CIPK members. Nevertheless, it is very important to note that in both cases Ca^{2+} is still necessary for the CBL–CIPK complex to be active and phosphorylate substrates (Halfter et al. 2000) (Fig. 1).

Deletion analysis with CIPK24 provided a molecular mechanism by which the CBL–CIPK interaction results in activation of the CIPK enzyme activity (Guo et al. 2001). The results of this study indicated that the NAF-carrying C-terminal domain serves as an auto-inhibitory module by interacting intramoleculary with the kinase domain, and thereby blocks the active site from binding with a substrate. It seems that upon interaction with a Ca^{2+}-bound CBL partner, CIPKs undergo a conformational change which disrupts the auto-inhibitory effect and allows the kinase domain to gain the phosphorylation activity.

CBL–CIPK complexes mediate a variety of extracellular stimuli

Extensive studies with Arabidopsis mutants have demonstrated that the CBL–CIPK complexes are involved in mediating Ca^{2+} signals elicited by diverse stimuli such as cold, ABA, salinity, osmotic stress, low K^+ concentration, and high pH (Cheong et al. 2003, 2007; D'Angelo et al. 2006; Fuglsang et al. 2007; Kim et al. 2007; Li et al. 2006; Pandey et al. 2004; Quan et al. 2007; Xu et al. 2006). It appears that each pair of the CBL–CIPK complexes plays a specific role in relaying different signals (Fig. 2).

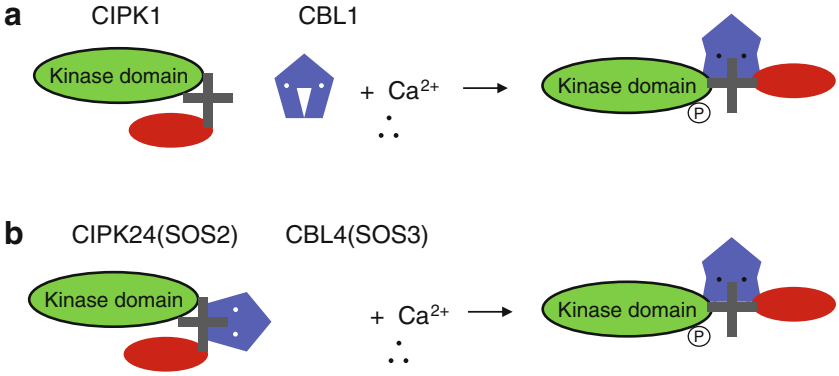

Fig. 1 Two hypothetical models for the activation of CIPKs by Ca^{2+}-bound CBLs. **a** Ca^{2+}-dependent interaction. Upon Ca^{2+} binding, CBL1 undergoes conformational change and can associate with the NAF motif (*cross*) of CIPK1. The association disrupts the intramolecular interaction of CIPK1, thereby activating the kinase activity. **b** Ca^{2+}-independent interaction. CBL4 can interact with the NAF motif of CIPK24 in the absence of Ca^{2+}. However, the interaction itself is not enough to block the autoinhibitory interaction of CIPK24. CBL4 requires Ca^{2+} to activate the enzyme activity

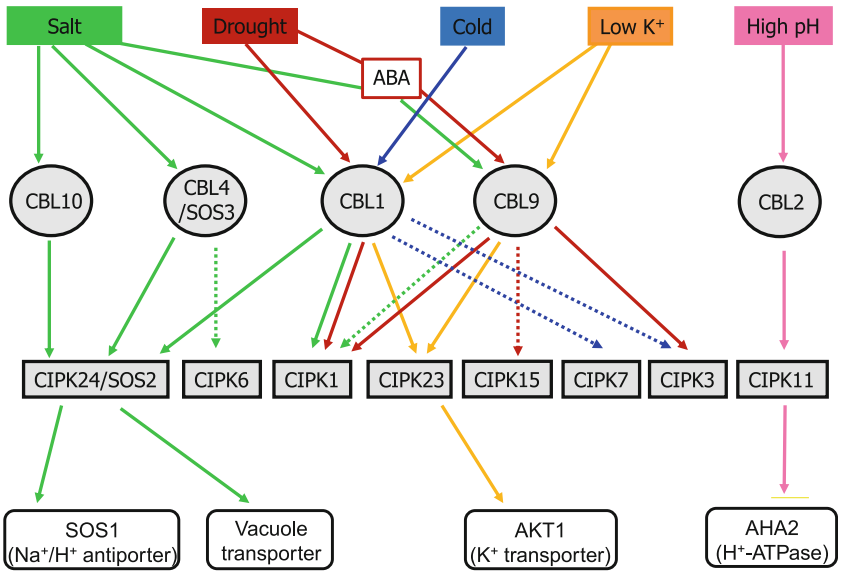

Fig. 2 The CBL–CIPK networks mediating various stress responses. The signaling network was generated using the results of genetic and biochemical analyses. *Different line colors* indicate different stress signaling pathways. *Broken lines* represent signaling pathways which need further solid evidence to make sure (color figure online)

Abscisic acid

Abscisic acid (ABA) is a phytohormone involved in plant response to abiotic stresses such as drought and high salt (Yamaguchi-Shinozaki and Shinozaki 2006; Zhu 2002). A specific Ca^{2+} signature is known to be implicated in an early step of the ABA signaling pathways (Allen et al. 2000, 2001; Leung and Giraudat 1998), suggesting involvement of Ca^{2+} sensors in this signaling pathway. In fact, several lines of recent evidence suggested that the CBL–CIPK pathways should play a role in the ABA signaling.

The Arabidopsis mutant plants with disrupted CBL9 (*cbl9*) were hypersensitive to ABA in the early developmental stages such as seed germination and post-germination seedling growth (Pandey et al. 2004). The *cbl9* mutant seedlings also accumulated much higher levels of ABA than the wild-type plants under the osmotic stress conditions caused by mannitol and salt. Furthermore, the expression levels of the genes involved in ABA signaling, such as *ABA-INSENSITIVE* 4 and 5, were increased to a greater extent in the *cbl9* mutant plants under the osmotic stress conditions or exogenous ABA (Pandey et al. 2004). These results strongly indicate that CBL9 plays an important role in both the biosynthesis and sensitivity of ABA in Arabidopsis. It was demonstrated that CBL9 can form a specific complex with CIPK3 to act together in regulating the ABA responses (Pandey et al. 2008). The CIPK3 knock-out Arabidopsis mutant (*cipk3*) plants were more sensitive to exogenous ABA during seed germination, and they also expressed significantly lower levels of ABA-induced genes such as *RD22* and *RD29B* (Kim et al. 2003).

CBL1 is the most similar isoform of CBL9 in Arabidopsis. The Arabidopsis plants lacking the CBL1 activity (*cbl1*) did not show changes in the ABA responsiveness, although they exhibited less tolerance to drought and salt stress (Albrecht et al. 2003; Cheong et al. 2003). These findings indicated that CBL1 is not implicated in the ABA signaling processes unlike CBL9. Meanwhile, it is interesting to note that CIPK1, which can interact with CBL1 and CBL9, mediates ABA responses as well as osmotic stress responses such as drought and salt. Loss of CIPK1 rendered the plants hypersensitive to the osmotic stresses and it also impaired the ABA responsiveness (D'Angelo et al. 2006). Based on these findings, it is believed that CIPK1 can mediate ABA-independent and ABA-dependent stress responses by forming an alternative complex with either CBL1 or CBL9.

Silencing of the Arabidopsis CIPK15 gene (designated PKS3 in the paper) by an RNA interference technique rendered the plants hypersensitive to ABA in seed germination, seedling growth, stomatal closing, and gene expression (Guo et al. 2002). These findings implicated CIPK15 as a global negative regulator of ABA responses. Because yeast two-hybrid assays demonstrated that CIPK15 associates with a group of CBL family members including CBL1, 2, 3, 5, 8, and 9 (Kim, unpublished data), it is likely that CBL9 may also form a complex with CIPK15 to mediate the ABA responses. However, further genetic analysis is required to demonstrate this speculation.

Salt stress

A series of genetic and biochemical analyses with the salt overly sensitive (SOS) mutants provided a molecular mechanism by which a CBL–CIPK complex mediates the salt stress-induced Ca^{2+} signal and give rise to salt tolerance (Zhu et al. 1998). CBL4 and CIPK24, encoded, respectively, by the genomic loci *SOS3* and *SOS2*, form a complex and activate the CIPK24 kinase activity in a Ca^{2+}-dependent manner (Halfter et al. 2000; Liu et al. 2000; Liu and Zhu 1998). Then, the CBL4/SOS3-CIPK24/SOS2 complex phosphorylates and activates the downstream component SOS1, a Na^+/H^+ antiporter (Shi et al. 2000). The activated SOS1 protein functions to get rid of excess Na^+ in plant cells, thereby conferring salt tolerance (Qiu et al. 2002; Quintero et al. 2002).

Another CBL family member, CBL10, was later included in the salt-tolerance pathway. Like CBL4/SOS3, CBL10 also associates with and activates the kinase activity of CIPK24/SOS2 (Kim et al. 2007; Quan et al. 2007). Although both CBL4/SOS3 and CBL10 proteins are involved in mediating salt tolerance, they do differ in executing their functions due to their distinct expression patterns and subcellular localizations.

CBL4/SOS3 is expressed mainly in the roots and localized at the plasma membrane. Therefore, CIPK24/SOS2 is recruited to the plasma membrane of the root cells where it phosphorylates the Na^+/H^+ antiporter SOS1, thereby enhancing the Na^+ efflux rate (Fig. 3a). In contrast, CBL10 is expressed predominantly in the shoots and leaves, and it is localized at the vacuolar membrane (tonoplast). The knock-out Arabidopsis mutant lacking the CBL10 activity (*cbl10*) showed the salt-sensitive phenotype specifically in the leaves or shoots under the high salt conditions (Kim et al. 2007). Interestingly, the *cbl10* mutant plants accumulated much less Na^+ than the wild-type under either normal or high salt conditions. This unique feature of the *cbl10* mutant plants, along with the tonoplast localization of the CBL10 protein, suggests that CBL10 is required for the sequestration of Na^+ into the vacuole. It is likely that CIPK24/SOS2 recruited by CBL10

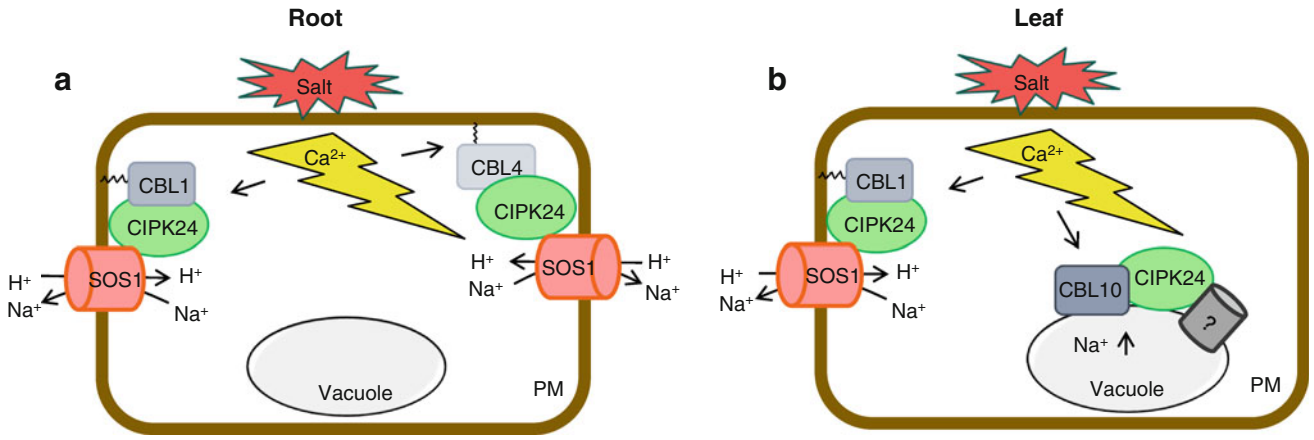

Fig. 3 Hypothetical model of CBL–CIPK function in salt stress response. **a** In the roots, CIPK24 activates the the Na^+/H^+ antiporter SOS1 at the plasma membrane by forming a complex with either CBL1 or CBL4. **b** In the leaves, CIPK24 regulates Na^+ sequestration into the vacuole by interacting with the tonoplast-bound CBL10. *Question mark* a component of unknown identity

to the tonoplast may phosphorylate and activate an as-yet-unknown Na^+ channel or transporter, which is tonoplast-bound and plays a role in transporting cytosolic Na^+ into the vacuole (Fig. 3b).

Meanwhile, it should be noted that another CBL family member, CBL1, is also involved in plant response to salt stress. The *cbl1* mutant plants showed less tolerance to the salt stress (Albrecht et al. 2003; Cheong et al. 2003). Like CBL4/SOS3, CBL1 is present in the plasma membrane and interacts with CIPK24/SOS2. Therefore, it is very likely that CBL1 also confers salt tolerance via CIPK24/SOS2-mediated SOS1 regulation (D'Angelo et al. 2006). Unlike CBL4/SOS3, however, CBL1 expression is not restricted the roots. CBL1 is actually expressed in both root and shoot tissues. Such an expression pattern implies that Na^+ extrusion mediated by the CIPK24/SOS2-SOS1 machinery may also occur in the shoots. In summary, CIPK24/SOS2 can regulate two different processes depending on its interacting CBL partners. Through the association with CBL1 or CBL4/SOS3, CIPK24/SOS2 facilitates Na^+ export across the plasma membrane by activating the Na^+/H^+ antiporter SOS1. Furthermore, CIPK24/SOS2 can also play a role in sequestrating Na^+ into the vacuole by forming a complex with CBL10.

Recently, an Arabidopsis mutant lacking CIPK6 activity was reported to be more sensitive to salt stress compared to the wild-type, suggesting involvement of CIPK6 in salt tolerance (Tripathi et al. 2009). Because CIPK6 was known to associate with CBL4/SOS3 in the yeast two-hybrid system (Kim et al. 2000), there is a possibility that CBL4/SOS3 may also target CIPK6 in vivo in addition to CIPK24/SOS2. Further genetic and biochemical analyses will provide solid evidence, which will determine whether or not the CBL4/SOS3-CIPK6 complex is actually working in plant cells to mediate salt tolerance. Anyway, it is obvious that multiple members of the CBL and CIPK families are implicated in salt tolerance.

Drought and osmotic stress

Loss-of-function Arabidopsis mutants lacking CBL1, CBL9, or CIPK1 were found to be more sensitive to drought and osmotic stress than the wild-type plants (Pandey et al. 2004; D'Angelo et al. 2006). Moreover, several lines of evidence demonstrated that CIPK1 interacts with either CBL1 or its closest isoform CBL9 at the plasma membrane (Shi et al. 1999; Kim et al. 2000; Albrecht et al. 2001; D'Angelo et al. 2006). These data together suggested that both CBL1 and CBL9 can target CIPK1 to form two distinct complexes, CBL1–CIPK1 and CBL9–CIPK1, thereby mediating the stress responses. As described above, they are, respectively, involved in ABA-independent and ABA-dependent signaling pathways.

Cold

The *cbl1* mutant plants exhibited more tolerance to cold stress and displayed enhanced cold induction of stress genes such as *RD29A* and *Kin1* (Albrecht et al. 2003; Cheong et al. 2003). These results indicate that CBL1 plays a negative role in regulating plant cold response. Recently, a study showed that CBL1 can interact with CIPK7 whose expression is induced by cold stress (Huang et al. 2011). This suggests that CBL1 may target CIPK7 to mediate cold response in plants.

Meanwhile, expression patterns of RD29A and Kin1/Kin2 genes were altered in the *cipk3* mutant plants in response to cold stress (Kim et al. 2003), implying the involvement of CIPK3 in cold response. Because expressions of the stress genes are independent of endogenous ABA production (Shinozaki and Yamaguchi-Shinozaki 2000; Thomashow 1999), these findings suggest that CIPK3 can act as a cross-talk node between the ABA-dependent and ABA-independent pathways in stress responses.

Potassium deficiency

Two independent research groups have provided solid evidence demonstrating that CBL1 and CBL9 calcium sensors and their target CIPK23 are involved in plant response to low K^+ conditions (Li et al. 2006; Xu et al. 2006). According to their work, the Arabidopsis mutant plants with the disrupted CIPK23 gene, *cipk23*, were more sensitive to low K^+ concentrations than the wild-type plants. The similar phenotype was also observed with the *cbl1/cbl9* double mutant plants, but not with the *cbl1* or *cbl9* single mutants, which implies that the two CBL members have redundant functions in the low K^+ signaling pathways.

Biochemical assays have demonstrated that CIPK23 can be activated by the association with either CBL1 or CBL9, thereby phosphorylating the C-terminal region of K^+ transporter AKT1 in the plasma membrane. Based on these findings, a model was proposed for the K^+ -deficiency response in plants: CBL1 and CBL9 detect the Ca^{2+} signatures elicited by low K^+ conditions. Upon binding with Ca^{2+}, they recruit CIPK23 to the plasma membrane and promote the kinase activity. The activated CIPK23 then phosphorylates the C-terminal region of the AKT1 transporter, thereby enhancing its K^+-transporting activity.

High pH

The Arabidopsis mutant plants lacking the functional protein kinase CIPK11 (designated PKS5 in the paper) displayed enhanced tolerance to high external pH compared with the wild-type plants because of the increased rate of

H^+ secretion to the extracellular space (Fuglsang et al. 2007). Further studies revealed that CIPK11 phosphorylates the Ser-931 residue in the C-terminal regulatory domain of the plasma membrane proton pump (PM H^+-ATPase 2; AHA2), which results in the inhibition of AHA2 activity by preventing its association with an activating 14-3-3 (Fuglsang et al. 2007). Although CBL2 was originally proposed to be responsible for activating CIPK11 in the presence of cytosolic Ca^{2+} signals elicited by external high pH conditions, the involvement of CBL2 was later ruled out due to its exclusive localization at the tonoplast (Batistic et al. 2008). Therefore, it needs to be discovered which member of the CBL family is responsible for Ca^{2+}-dependent modulation of the AHA2 activity in planta.

Biotic stress

Plants generate cytosolic Ca^{2+} signals in response to pathogen attacks (Rudd and Franklin-Tong 2001). Currently, however, little is known about whether the CBL family members are involved in mediating the Ca^{2+} signals elicited by the biotic stress. Only circumstantial or indirect evidence suggests that CBLs may be implicated in plant response to the biotic stress; salicylic acid, mediating biotic stress response in plants, stimulated the expression of pea CBL, called PsCBL (Mahajan et al. 2006).

Perspectives

Identification of the novel Ca^{2+} sensors CBLs and their interaction partners CIPKs has certainly enhanced our knowledge on the Ca^{2+} signal transduction pathways in higher plants. Furthermore, the findings of the in vivo substrates phosphorylated by the CBL4/SOS3–CIPK24/SOS2 and CBL1 (CBL9)–CIPK23 complexes have provided molecular mechanisms by which plants respond to salt and low K^+ stresses, respectively (Qiu et al. 2002; Quintero et al. 2002; Li et al. 2006; Xu et al. 2006). Currently, only three CIPK substrates are known, all of which happen to be membrane-bound proteins, SOS1, AKT1, and AHA2 (Fuglsang et al. 2007; Li et al. 2006; Qiu et al. 2002; Quintero et al. 2002; Xu et al. 2006). Therefore, it becomes critical to identify as yet unknown in vivo substrates or target molecules for the CIPK family members in order to gain further insight into the CBL–CIPK Ca^{2+} signaling network.

Analyses of the *CBL* and *CIPK* knock-out mutants have indicated that the CBL–CIPK Ca^{2+} signaling pathways are involved in regulating expression of stress genes such as *RD29A/B*, *Kin1/2,* and *DREB1A/B* (Albrecht et al. 2003; Cheong et al. 2003; D'Angelo et al. 2006; Kim et al. 2003; Pandey et al. 2004). However, little is known about how the CBL–CIPK complexes exert their influence on the transcriptional controls. In the cases of the CBL1 and CBL9 proteins, which recruit interacting CIPK partners to the plasma membrane (Albrecht et al. 2003; Cheong et al. 2003; D'Angelo et al. 2006; Pandey et al. 2004), we can speculate the existence of messenger molecules delivering information from the plasma membrane-bound CBL–CIPK complexes into the nucleus, where gene regulation occurs. In this regard, ECT1 can be considered as a strong candidate protein executing such function, because it can associate with CIPK1 in the cytoplasm and can be translocated into the nucleus (Ok et al. 2005). Further analyses will show whether ECT1 is actually involved in relaying a signal from the CBL1–CIPK1 complex.

Meanwhile, it is important to note a recent report that CBL3 can interact with and modulate the Arabidopsis 5′-methylthioadenosine nucleosidase (AtMTAN) in a Ca^{2+}-dependent manner (Oh et al. 2008). Since AtMTAN associates only with CBL3 but not with other CBL family members such as CBL1 and CBL4, it is reasonable to speculate that other CBL members may also have as yet unidentified interaction partners which do not belong the CIPK family. In this case, the CBL members can target a greater number of proteins with different biochemical properties. Such target diversity confers an additional level of complexity on the CBL-mediated Ca^{2+}-signaling pathways, thereby allowing the CBL Ca^{2+} sensors to regulate a wider range of cellular and physiological processes in higher plants. Anyway, the finding above clearly indicates that the CBL family can relay the Ca^{2+} signals in more diverse ways than currently known. Extensive further studies will be necessary to fully understand the Ca^{2+}-signaling networks mediated by the CBL family members.

Acknowledgments This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the ministry of Education, Science and Technology (2010-0008839). This work was also supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2011-0029568).

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, Ritz O, Linder S, Harter K, Kudla J (2001) The NAF domain defines a novel protein–protein interaction module conserved in Ca^{2+}-regulated kinases. EMBO J 20:1051–1063

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

Allen GJ, Chu SP, Schumacher K, Shimazaki CT, Vafeados D, Kemper A, Hawke SD, Tallman G, Tsien RY, Harper JF, Chory

J, Schroeder JI (2000) Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in Arabidopsis det3 mutant. Science 289:2338–2342

Allen GJ, Chu SP, Harrington CL, Schumacher K, Hoffmann T, Tang YY, Grill E, Schroeder JI (2001) A defined range of guard cell calcium oscillation parameters encodes stomatal movements. Nature 411:1053–1057

Asano T, Tanaka N, Yang G, Hayashi N, Komatsu S (2005) Genome-wide identification of the rice calcium-dependent protein kinase and its closely related kinase gene families: comprehensive analysis of the CDPKs gene family in rice. Plant Cell Physiol 46:356–366

Batistic O, Kudla J (2004) Integration and channeling of calcium signaling through the CBL calcium sensor/CIPK protein kinase network. Planta 219:915–924

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

Blatt MR (2000) Cellular signaling and volume control in stomatal movements in plants. Annu Rev Cell Dev Biol 16:221–241

Chandran V, Stollar EJ, Lindorff-Larsen K, Harper JF, Chazin WJ, Dobson CM, Luisi BF, Christodoulou J (2006) Structure of the regulatory apparatus of a calcium-dependent protein kinase (CDPK): a novel mode of calmodulin-target recognition. J Mol Biol 357:400–410

Cheng SH, Willmann MR, Chen HC, Sheen J (2002) Calcium signaling through protein kinases. The Arabidopsis calcium-dependent protein kinase gene family. Plant Physiol 129:469–485

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 BG, 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

D'Angelo C, Weinl S, Batistic O, Pandey GK, Cheong YH, Schultke S, Albrecht V, Ehlert B, Schulz B, Harter K, Luan S, Bock R, Kudla J (2006) Alternative complex formation of the Ca-regulated protein kinase CIPK1 controls abscisic acid-dependent and independent stress responses in Arabidopsis. Plant J 48:857–872

Dolmetsch RE, Lewis RS, Goodnow CC, Healy JI (1997) Differential activation of transcription factors induced by Ca^{2+} response amplitude and duration. Nature 386:855–858

Dolmetsch RE, Xu K, Lewis RS (1998) Calcium oscillations increase the efficiency and specificity of gene expression. Nature 392:933–936

Ehrhardt DW, Wais R, Long SR (1996) Calcium spiking in plant root hairs responding to *Rhizobium* nodulation signals. Cell 85:673–681

Evans NH, McAinsh MR, Hetherington AM (2001) Calcium oscillations in higher plants. Curr Opin Plant Biol 4:415–420

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

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

Guo Y, Xiong L, Song CP, Gong D, Halfter U, Zhu JK (2002) A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signaling in Arabidopsis. Dev Cell 3:233–244

Halfter U, Ishitani M, Zhu JK (2000) The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. Proc Natl Acad Sci USA 97:3735–3740

Harper JF, Breton G, Harmon A (2004) Decoding Ca^{2+} signals through plant protein kinases. Annu Rev Plant Biol 55:263–288

Hernandez Sebastia C, Hardin SC, Clouse SD, Kieber JJ, Huber SC (2004) Identification of a new motif for CDPK phosphorylation in vitro that suggests ACC synthase may be a CDPK substrate. Arch Biochem Biophys 428:81–91

Holdaway-Clarke TL, Feijo JA, Hackett GR, Kunkel JG, Hepler PK (1997) Pollen tube growth and the intracellular cytosolic calcium gradient oscillate in phase while extracellular calcium influx is delayed. Plant Cell 9:1999–2010

Hrabak EM, Chan CW, Gribskov M, Harper JF, Choi JH, Halford N, Kudla J, Luan S, Nimmo HG, Sussman MR, Thomas M, Walker-Simmons K, Zhu JK, Harmon AC (2003) The Arabidopsis CDPK-SnRK superfamily of protein kinases. Plant Physiol 132:666–680

Huang CL, Ding S, Zhang H, Du H, An LZ (2011) CIPK7 is involved in cold response by interacting with CBL1 in *Arabidopsis thaliana*. Plant Sci 181:57–64

Jeong HJ, Jwa NS, Kim KN (2005) Identification and characterization of protein kinase that interact with the CBL3 calcium sensor in Arabidopsis. Plant Sci 169:1125–1135

Kawasaki H, Nakayama S, Kretsinger RH (1998) Classification and evolution of EF-hand proteins. Biometals 11:277–295

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 KN, Cheong YH, Grant JJ, Pandey GK, Luan S (2003) CIPK3, a calcium sensor-associated protein kinase that regulates abscisic acid and cold signal transduction in Arabidopsis. Plant Cell 15:411–423

Kim BG, Waadt R, Cheong YH, Pandey GK, Dominguez-Solis JR, Schultke S, Lee SC, Kudla J, Luan S (2007) The calcium sensor CBL10 mediates salt tolerance by regulating ion homeostasis in Arabidopsis. Plant J 52:473–484

Kolukisaoglu U, Weinl S, Blazevic D, Batistic O, Kudla J (2004) Calcium sensors and their interacting protein kinases: genomics of the Arabidopsis and rice CBL–CIPK signaling networks. Plant Physiol 134:43–58

Kudla J, Xu Q, Harter K, Gruissem W, Luan S (1999) Genes for calcineurin B-like proteins in Arabidopsis are differentially regulated by stress signals. Proc Natl Acad Sci USA 96:4718–4723

Lee JY, Yoo BC, Harmon AC (1998) Kinetic and calcium-binding properties of three calcium-dependent protein kinase isoenzymes from soybean. Biochemistry 37:6801–6809

Leung J, Giraudat J (1998) Abscisic acid signal transduction. Annu Rev Plant Physiol Plant Mol Biol 49:199–222

Li L, Kim BG, Cheong YH, Pandey GK, Luan S (2006) A Ca^{2+} signaling pathway regulates a K^+ channel for low-K response in Arabidopsis. Proc Natl Acad Sci USA 103:12625–12630

Liu J, Zhu JK (1998) A calcium sensor homolog required for plant salt tolerance. Science 280:1943–1945

Liu J, Ishitani M, Halfter U, Kim CS, Zhu JK (2000) The Arabidopsis thaliana SOS2 gene encodes a protein kinase that is required for salt tolerance. Proc Natl Acad Sci USA 97:3730–3734

Luan S, Kudla J, Rodriguez-Concepcion M, Yalovsky S, Gruissem W (2002) Calmodulins and calcineurin B-like proteins: calcium sensors for specific signal response coupling in plants. Plant Cell 14(Suppl):S389–S400

Mahajan S, Sopory SK, Tuteja N (2006) Cloning and characterization of CBL–CIPK signalling components from a legume (*Pisum sativum*). FEBS J 273:907–925

McCormack E, Braam J (2003) Calmodulins and related potential calcium sensors of Arabidopsis. New Phytol 159:585–598

Oh SI, Park J, Yoon S, Kim Y, Park S, Ryu M, Nam MJ, Ok SH, Kim JK, Shin JS, Kim KN (2008) The Arabidopsis calcium sensor calcineurin B-like 3 inhibits the 5′-methylthioadenosine nucleosidase in a calcium-dependent manner. Plant Physiol 148:1883–1896

Ok SH, Jeong HJ, Bae JM, Shin JS, Luan S, Kim KN (2005) Novel CIPK1-associated proteins in Arabidopsis contain an evolutionarily conserved C-terminal region that mediates nuclear localization. Plant Physiol 139:138–150

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 GK, Grant JJ, Cheong YH, Kim BG, le Li G, Luan S (2008) Calcineurin-B-like protein CBL9 interacts with target kinase CIPK3 in the regulation of ABA response in seed germination. Mol Plant 1:238–248

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

Quan R, Lin H, Mendoza I, Zhang Y, Cao W, Yang Y, Shang M, Chen S, Pardo JM, Guo Y (2007) SCABP8/CBL10, a putative calcium sensor, interacts with the protein kinase SOS2 to protect Arabidopsis shoots from salt stress. Plant Cell 19:1415–1431

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

Reddy VS, Ali GS, Reddy AS (2002) Genes encoding calmodulin-binding proteins in the Arabidopsis genome. J Biol Chem 277:9840–9852

Rodriguez Milla MA, Uno Y, Chang IF, Townsend J, Maher EA, Quilici D, Cushman JC (2006) A novel yeast two-hybrid approach to identify CDPK substrates: characterization of the interaction between AtCPK11 and AtDi19, a nuclear zinc finger protein. FEBS Lett 580:904–911

Rudd JJ, Franklin-Tong VE (2001) Unravelling response-specificity in Ca^{2+} signalling pathways in plant cells. New Phytol 151:7–33

Sanders D, Brownlee C, Harper JF (1999) Communicating with calcium. Plant Cell 11:691–706

Sanders D, Pelloux J, Brownlee C, Harper JF (2002) Calcium at the crossroads of signaling. Plant Cell 14(Suppl):S401–S417

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

Shi H, Ishitani M, Kim C, Zhu JK (2000) The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na^+/H^+ antiporter. Proc Natl Acad Sci USA 97:6896–6901

Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Curr Opin Plant Biol 3:217–223

Snedden WA, Fromm H (2001) Calmodulin as a versatile calcium signal transducers in plants. New Phytol 151:35–66

Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol 50:571–599

Tripathi V, Syed N, Laxmi A, Chattopadhyay D (2009) Role of CIPK6 in root growth and auxin transport. Plant Signal Behav 4:663–665

Waadt R, Schmidt LK, Lohse M, Hashimoto K, Bock R, Kudla J (2008) Multicolor bimolecular fluorescence complementation reveals simultaneous formation of alternative CBL/CIPK complexes in planta. Plant J 56:505–516

Wang MY, Gu D, Liu TS, Wang ZQ, Guo XY, Hou W, Bai YF, Chen XP, Wang GY (2007) Overexpression of a putative maize calcineurin B-like protein in Arabidopsis confers salt tolerance. Plant Mol Biol 65:733–746

White PJ, Broadley MR (2003) Calcium in plants. Ann Bot (Lond) 92:487–511

Wymer CL, Bibikova TN, Gilroy S (1997) Cytoplasmic free calcium distributions during the development of root hairs of *Arabidopsis thaliana*. Plant J 12:427–439

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

Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu Rev Plant Biol 57:781–803

Yang T, Poovaiah BW (2003) Calcium/calmodulin-mediated signal network in plants. Trends Plant Sci 8:505–512

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) Genetic analysis of salttolerance in Arabidopsis: evidence for a critical role of potassium nutrition. Plant Cell 10:1181–1191

Zielinski RE (1998) Calmodulin and calmodulin-binding proteins in plants. Annu Rev Plant Physiol Plant Mol Biol 49:697–725