Biochimica et Biophysica Acta 1793 (2009) 985–992

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Plant calcineurin B-like proteins and their interacting protein kinases

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ARTICLE INFO

Article history:
Received 24 September 2008
Accepted 15 October 2008
Available online 29 October 2008

Keywords:
CBLs
CIPKs
Calcium signaling
Calcium sensor
Protein kinase
Arabidopsis

ABSTRACT

Calcium serves as a critical messenger in many adaptation and developmental processes. Cellular calcium signals are detected and transmitted by sensor molecules such as calcium-binding proteins. In plants, the calcineurin B-like protein (CBL) family represents a unique group of calcium sensors and plays a key role in decoding calcium transients by specifically interacting with and regulating a family of protein kinases (CIPKs). Several CBL proteins appear to be targeted to the plasma membrane by means of dual lipid modification by myristoylation and S-acylation. In addition, CBL/CIPK complexes have been identified in other cellular localizations, suggesting that this network may confer spatial specificity in Ca^{2+} signaling. Molecular genetics analyses of loss-of function mutants have implicated several CBL proteins and CIPKs as important components of abiotic stress responses, hormone reactions and ion transport processes. The occurrence of CBL and CIPK proteins appears not to be restricted to the plant kingdom raising the question about the function of these Ca^{2+} decoding components in non-plant species.

1. Introduction

Almost every biological process in eukaryotic organisms is controlled by Ca^{2+} [1]. In plants Ca^{2+} regulates important developmental processes from polar pollen tube growth before fertilization to programmed cell death [2]. Among them, root hair elongation and nodulation responses to symbionts are accompanied by distinct spatio-temporal changes in cytosolic Ca^{2+} concentration [3,4]. Moreover, physiological responses to abiotic stresses, light, pathogens and hormones, such as abscisic acid (ABA) have been shown to modulate the intracellular Ca^{2+} concentration in different cell types, including guard cells [5,6]. However, despite the central importance of Ca^{2+} for most signaling and regulatory processes in plants, the molecular mechanisms leading to the generation of Ca^{2+} signals and the subsequent decoding of this information are largely unknown. The evolution of calcium signals, from simple single release events to timely and spatially regulated puffs, sparks and waves, coincided with the evolution of an assortment of calcium binding proteins for decoding these signals. An enormous and diverse range of target proteins that belong to different protein classes like transporters, cell structure proteins, metabolic enzymes, signalling proteins and transcription factors are regulated by calcium binding proteins [7]. Consequently, the interplay of calcium release and calcium sensing generates the uniqueness and versatility of calcium signaling.

Small calcium binding proteins, typically consisting of four calcium binding EF hand domains, are the most prominent type of calcium binding proteins. The prototype of these calcium binding proteins is provided by the calcium sensor calmodulin [8,9]. Similar calcium binding proteins already exist in prokaryotic organisms where they function mostly as calcium buffer proteins [10–12]. The evolution of calcium binding proteins resulted in the formation of different protein classes, like classical calmodulins (CaM), CaM-related/like proteins (CMLs), calcineurin-B (CNB) and CNB homologous proteins (CHPs), neuronal calcium sensors (NCS) and others [13–15]. While plants appear to lack the CNB and NCS protein classes, or important relay proteins like CaM regulated kinases (CaMKs), they evolved special types of calcium binding proteins, like Calcium-dependent protein kinases (CDPKs) [16] and calcineurin-B like (CBL) proteins [17]. CBL proteins are most related to CNB and NCS proteins from animal and fungal organisms. This review focuses on the structural and functional properties of the plant CBL proteins and their CBL-interacting protein kinases (CIPKs). We discuss emerging mechanistic principles that contribute to generating signaling specificity within this signaling system and discuss novel insights into the evolution of the CBL/CIPK network.

2. Organization of the CBL–CIPK network

CBL proteins have been initially identified from *Arabidopsis thaliana* [18]. Subsequent genomics analyses revealed that the *Arabidopsis* and rice genomes each encode a complement of 10 distinct CBL proteins that form an interaction network with 25 (*Arabidopsis*) or 30 (rice) CIPKs, respectively [19]. All CBL proteins share a rather conserved core region consisting of four EF hand calcium binding domains that are separated by spacing regions encompassing an absolutely conserved number of amino acids in all CBL Proteins (Fig. 1) [17]. However, CBL proteins can be further classified into two major

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doi:10.1016/j.bbamcr.2008.10.006

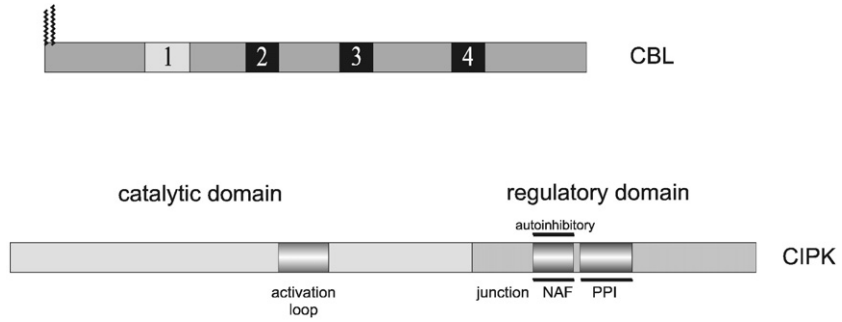

Fig. 1. General domain structure of CBLs and CIPKs. The overall structure of CBLs consists of four EF-hands (dark grey boxes with numbers). Spacing of EF-hands in all CBLs is invariable, while the N- and C-terminal extensions vary in length. In CBLs, the first EF hand has a unconventional structure, encompassing 14 amino acids instead of 12 in a canonical EF hand (light grey box). The overall structure of CIPKs consists of an N-terminal kinase domain, which harbors the activation loop. The regulatory C-terminal domain is partitioned into the two interaction domains, the NAF domain, which is responsible for CBL–CIPK interaction, and the adjacent PPI domain. Between the two domains the "junction domain" is further responsible for activation of the kinases.

subgroups according to their N-terminal domains. The first group consists of CBL1, CBL4, CBL5, CBL8 and CBL9. These proteins harbor a rather short (43- to 48 aa) N-terminal domain with a lipid modification site that appears to have been degenerated in CBL8. Indeed, CBL1, CBL4, CBL5 and CBL9 are myristoylated at their N-terminus like CNB and many NCS proteins [20,21]. The second group is formed by the proteins CBL2, CBL3, CBL6, CBL7 and CBL10. These proteins harbor an extended N-terminal domain that is similar to the K^+-channel interacting proteins (KChIP) from the NCS group and does not contain discernable lipid modification motifs. In this group CBL10 is special in that it harbors a very long N-terminal extension forming a potential single transmembrane domain important for localization of CBL10 [22] (Batistic et al., submitted).

In contrast to CNB from animals and fungi, CBLs do not interact with a PP2B-type phosphatase that appears to be absent in plants. Instead, CBL proteins interact with a group of serine–threonine protein kinases that evolutionary belong to the superfamily of CaM-dependent kinases (CaMKs), and form a phylogenetically separate cluster within the group of SNF1 related kinases. Therefore, this group has also been designated as Snf1 related kinase group 3 (SnRK3; [23]). Earlier studies revealed that *Arabidopsis* contains 25 kinases [19]. Our recent re-evaluation of the *Arabidopsis* genome identified one additional SnRK3-type kinase encoding CIPK26 (At5g21326; Weinl S and Kudla J, unpublished). As in other kinases of the CaMK group, the kinase domain in CIPKs is separated by a junction domain from the less-conserved C-terminal regulatory domain (Fig. 1). Within the regulatory region of CIPKs a conserved NAF domain (designated according to the prominent amino acids N, A and F) mediates binding of CBL proteins and simultaneously functions as an auto-inhibitory domain [24]. Binding of CBLs to the NAF motif removes the autoinhibitory domain from the kinase domain, thereby conferring autophosphorylation and activation of the kinase [25]. Additional phosphorylation of the activation loop within the kinase domain by a yet unknown kinase further contributes to the activation of CIPKs [26].

Kinases related to CIPKs, like the AMP-activated protein kinase (AMPK), are dephosphorylated by type 2C protein phosphatases (PP2C) [27,28]. Interestingly, also CIPKs can associate with PP2Cs like ABI1 and ABI2 via a C-terminal protein-phosphatase interaction (PPI) domain [29]. Currently it is not known if PP2Cs may dephosphorylate CIPKs or if phosphorylation of PP2Cs by CIPKs occurs in vivo. Alternatively, generation of CIPK/PP2C complexes could serve the formation of signaling kinase/phosphatase modules allowing for rapid alternating phosphorylation/dephosphorylation of target proteins. In this regard, crystallization studies of CBL4 in complex with the regulatory domain of CIPK24 suggest that either CBLs or PP2Cs may mutually exclusive interact with the regulatory domain of CIPKs, and that formation of a trimeric complex is unlikely [30]. Therefore, it is tempting to speculate that PP2C interaction with the PPI domain of CIPKs leads to competitive replacing of the CBL protein, which binds to the NAF and partly to the PPI domain. Dissociation of the CBL protein would release the otherwise masked auto-inhibitory domain of the CIPK resulting in inactivation of the kinase. Alternative Ca^{2+}-dependent (see below) binding of CBL proteins to the CIPK would favor phosphorylation of a given substrate by out-competing the PP2C from the complex. However, as the target docking domains are still unknown for CIPKs and PP2Cs such models can currently not consider the influence of substrate binding.

Interestingly, the PPI domain was shown to be structurally related to the kinase associated domain1 (KA1) of the kinase KIN2/PAR-1/MARK subfamily [30–32]. Moreover, SnRK1, the SNF1 homologue in plants also contains such a structural domain [32]. Although the function of this domain is not known, this finding may point to a mechanism of protein regulation that is conserved from animal to plants [30,32].

3. Evolution and expansion of the CBL–CIPK network

Extensive CBL and CIPK gene families have been identified in the genomes of all higher plants that have been completely sequenced [19,33]. Rice, *Arabidopsis* and *Populus trichocarpa* each encode 10 CBL calcium sensors and a complement of 26 to 30 CIPKs. In lower plants, like the moss *Physcomitrella patens*, that encodes 4 CBLs and 7 CIPKs this signaling network appears to be less elaborate (Batistic et al., submitted). Remarkably, the green algae like *Ostreococcus tauri* and the related species *O. lucimarinus* encode only a single CBL protein and single CIPK (Batistic et al., submitted) (Fig. 2A). This observation suggests that the complexity of the CBL/CIPK system concurrently evolved with the increasing morphological and developmental sophistication of land plants that enabled the colonization of ecological diverse and environmental fluctuating habitats.

Surprisingly, our inspection of the recently sequenced genomes of several protozoan eukaryotes identified genes that potentially encode functional CBL and CIPK proteins. In the genomes of *Naegleria gruberi* and the related species *N. fowleri*, but also in the genome of the human parasite *Trichomonas vaginalis* we detected singular genes encoding calcium binding proteins that are unlikely to represent CNB proteins (Fig. 2A). In *T. vaginalis*, we found overall four calcium binding proteins, while in *N. gruberi* and *N. fowleri* we identified one CBL protein. The presence of several proteins in *T. vaginalis* most likely results from the extensive duplication events shaping this genome [34]. In phylogenetic analysis using the Clustal X algorithm this proteins cluster with the plant CBL proteins and display a more distant relation to animal and yeast CNB and NCS-related proteins. Importantly, the first EF of these calcium binding proteins clearly resembles the EF hand structure of the respective calcium-binding domain from

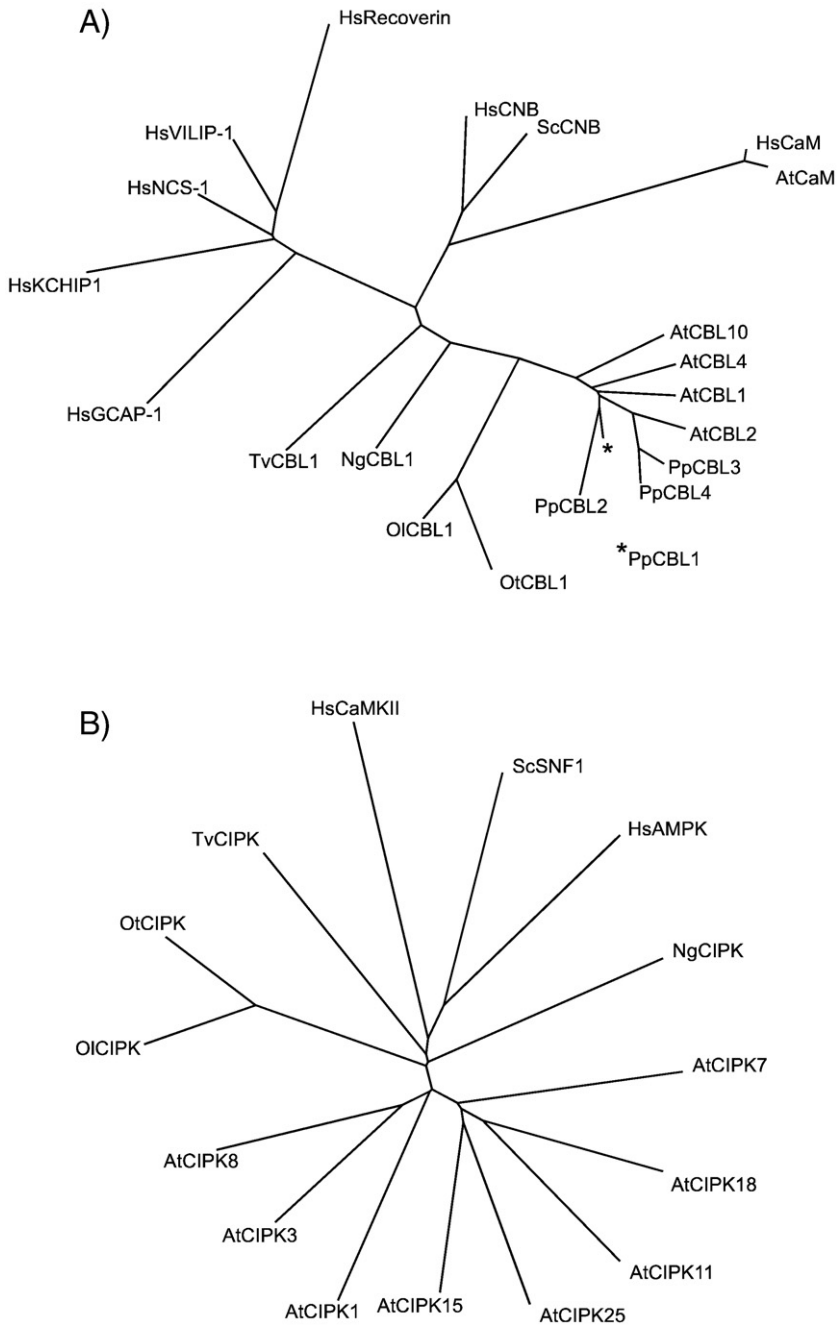

Fig. 2. A) Phylogenetic relationships of *Arabidopsis* CBLs with related calcium-binding proteins. Alignment of full-length protein sequences and phylogenetic analyses were performed as described in [19]. The accession numbers are: *Arabidopsis* CaM1 (AtCaM1, accession no. NP_198594), human CaM 1 (HsCaM1, accession no. AAA51918), yeast CNB (ScCNB, accession no. P25296), human CNB (HsCNB, accession no. AAB08721), human NCS 1 (HsNCS1, accession no. NP_NP055101), human Recoverin (HsRecoverin, accession no. BAA19460), human VILIP-1 (HsVILIP-1, accession no. BAA86891), human KChIP1 (HsKChIP1, accession no. AAH50375), human GCAP-1 (HsGCAP-1, accession no. AAA60541), *T. vaginalis* CBL1 (TvCBL1, accession no. XP_001320932), *N. gruberi* CBL1 (NgCBL1, accession no. AAF78251), *O. lucimarinus* CBL1 (OlCBL1, accession no. XP_001421712), *O. tauri* CBL1 (OtCBL1, accession no. CAL58089), *P. patens* CBLs (PpCBL1, accession no. EDQ77528; PpCBL2, accession no. EDQ71345; PpCBL3, accession no. EDQ66285; PpCBL4 , accession no. EDQ82786) and *Arabidopsis* CBLs (AtCBL1, accession no. AAC26008; AtCBL2, accession no. AAC26009; AtCBL4, accession no. AAG28402; AtCBL10, accession no. AAO72364). B) Phylogenetic relationships of *Arabidopsis* CIPKs with related kinases of the CaMK super-family. Alignment of full-length protein sequences and phylogenetic analyses were performed as described in [19]. The accession numbers are: human CaMKII (HsCaMKII, accession no. Q9UQM7), yeast SNF1 (ScSNF1, accession no. A26030), human AMPK (HsAMPK, accession no. AAA64745), *T. vaginalis* CIPK (TvCIPK, accession no. XP_001309947), *N. gruberi* CIPK (NgCIPK, JGI accession jgi|Naegr1|80321), *O. lucimarinus* CIPK (OlCIPK, accession no. XP_001420913), *O. tauri* CIPK (OtCIPK, accession no. CAL56411), *Arabidopsis* CIPKs (AtCIPK1, accession no. AAG28776; AtCIPK3, accession no. AAF86507; AtCIPK07, accession no. AAK16682; AtCIPK08, accession no. AAK16683; AtCIPK11. accession no. AAK16686; AtCIPK15, accession no. AAK16692; AtCIPK18, accession no. AAK59695; AtCIPK25, accession no. AAL41008).

CBL proteins, that consists of 14 amino acids instead of 12 amino acids typical for canonical EF hands (Fig. 3B).

The existence of a functional CBL/CIPK system in *Naegleria* and *Trichomonas* would presuppose the presence of kinases that are related to CIPKs. Indeed, our inspection of the genomes of both *Naegleria* species as well as of the genome of *T. vaginalis* specifically identified one kinase in each genome that represented candidate CIPKs. Phylogenetic analysis revealed that these kinases indeed cluster within the group of CIPKs and this association was clearly distinguishable from their relation to SNF1 kinases and AMPK (Fig. 2B). Moreover, comparison of their regulatory domains revealed the presence of a conserved NAF domain that is a distinguishing feature of CIPK-type protein kinases. Although it remains to be investigated if these protist CBL and CIPK proteins interact with each other the simultaneous

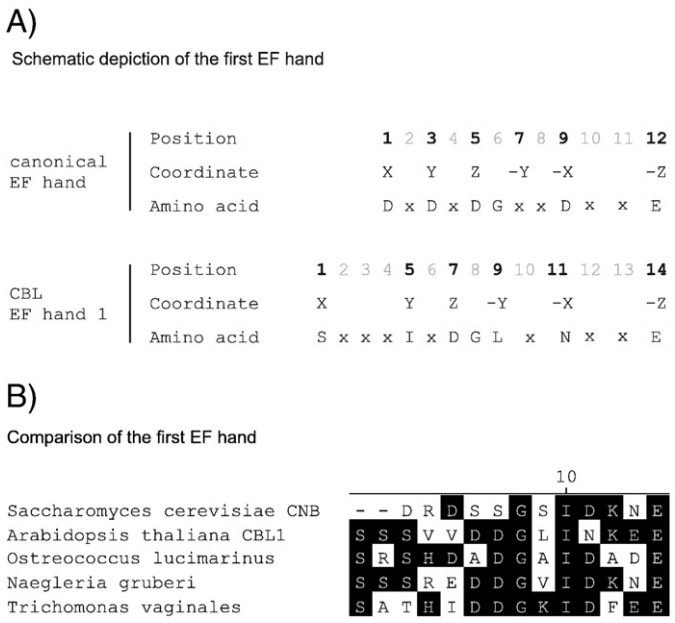

Fig. 3. A) Schematic depiction of the unconventional first EF hand in CBLs. The unconventional EF hand is extended to 14 amino acids in contrast to 12 amino acids in the conventional EF hand. B) Comparison of the first EF hands of yeast CNB, *Arabidopsis* CBL1, and the CBL proteins from *Ostreococcus lucimarinus*, *Naegleria gruberi* and *Trichomonas vaginalis*.

presence of both protein classes suggests the existence of an ancient CBL/CIPK based Ca^{2+} decoding system in these protozoan species. These findings extend the function of the CBL/CIPK system beyond the plant kingdom and may in the future enable a further investigation about the descensus and evolution of these calcium sensors and their interacting protein kinases. Moreover, the simultaneous presence of both protein classes in all these genomes makes a horizontal gene transfer as the cause of their presence in all these genomes rather unlikely. This raises further questions about the evolutionary positioning of these basal eukaryotes. As more genome sequences become available it will be most interesting to search additional genomes of primitive eukaryotic species for the presence of this calcium decoding system.

4. Structure features and calcium binding properties of CBL proteins

As typical for CNB and NCS proteins, CBL proteins contain four EF hands. However, only a few members of this family contain several canonical EF hands. Two canonical EF hands are present in CBL1 and CBL9, while CBL6, CBL7 and CBL10 harbor only one canonical EF hand. The remaining proteins CBL2, CBL3, CBL4, CBL5 and CBL8 from *Arabidopsis* do not possess any canonical EF hand. Moreover, several CBL proteins contain EF hands with amino acids substitutions at the important $-Z$ coordinate that generally impair calcium ion binding [17,19]. In addition, a deletion within the first EF hand of CBL6 most likely abolishes Ca^{2+} binding to this domain [19,35]. These differences in number and arrangement of canonical to non-canonical EF hands in each specific CBL protein suggest that CBL proteins may exhibit distinct affinities to calcium that would allow for differential activation of CBL–CIPK complexes depending on the respective Ca^{2+} concentration imposed by a specific stimulus. However, experimental investigations of the Ca^{2+} binding affinities and kinetics of plant CBL proteins and especially comparative side-by-side analyses of different proteins still have to be performed.

Nevertheless, several structural analyses of CBL proteins already revealed some insights into the structural properties and calcium binding mechanism of these calcium sensors. Determination of the crystal structure of CBL2 and of CBL4/SOS3 revealed that both CBL proteins form globular protein structures like previously observed for NCS proteins [35,36]. In NCS-1 the four EF hands are arranged in two pairs positioned in a tandem linear array on one side of the molecule. Moreover, Frequenin/NCS-1 possess a wide hydrophobic crevice at their surface situated between the two EF-hand containing lobes, as well as solvent exposed N and C termini [37]. Interestingly, this structural feature is shared by the CBL proteins [35,36] and most hydrophobic residues forming the hydrophobic crevice in NCS proteins are conserved or exchanged by other hydrophobic residues in CBLs (10 of 12 residues). In the non-CIPK bound form, the C-terminal tail of the CBL proteins normally binds to and blocks the hydrophobic crevice thereby preventing non-specific protein interactions.

Upon interaction with a CIPK this block is released and exchanged by the hydrophobic α-helix of the NAF domain [32]. This basically hydrophobic interaction is further stabilized by several hydrogen bonds. Remarkably, a similar mechanism mediates interaction between PI4Kβ and NCS-1/Frequenin proteins [37], and yeast two hybrid analyses suggest that plant CBL1 also can interact with the *Arabidopsis* PI4Kβ01 protein [38]. Like Frequenin and NCS-1, the CBL1 protein does not exhibit a calcium-myristoyl switch mechanism, that has been described for Recoverin and Hippocalcin [21,39,40].

The crystal structure analyses also revealed that CBL proteins contain an un-common calcium binding loop in the first EF hand [35]. Based on the structure of a canonical EF hand, the first EF hand in all CBL proteins should be not able to bind calcium. However, the first EF hand binds calcium. This is accomplished by the restructuring of the binding loop, which does not consist of 12 amino acids but instead of 14 amino acids. In this un-common extended EF hand, calcium is coordinated by the side chain carboxylates of aspartate (64) and glutamate (71) at the positions Z and $-Z$, respectively. Coordinates X (serine 58), Y (isoleucine 62) and $-Y$ (leucine 66) bind the calcium via the main chain carbonyl and $-$X via a water molecule ($X_1{*}_2{*}_3{*}_4Y_5{*}_6Z_7{*}_8-Y_9{*}_{10}-X_{11}{*}_{12}{*}_{13}-Z_{14}$) (Fig. 3A) [35]. Calcium binding could be further promoted by binding of the respective CIPK. In its non-CIPK-interacting form CBL2 was shown to bind only two calcium ions via the un-common first and last EF hand, respectively. EF hands two and three, which are not canonical because of lysine residues at the coordinate Y, do not bind calcium ions [35]. In contrast, in complex with CIPK14, all four EF hands of CBL2 are occupied by calcium ions [32]. Although binding of the regulatory domain of CIPK14 imposes a large structural change in the EF hands 2 and 3, it is unclear if this change is responsible for calcium binding to EF hands 2 and 3 [32]. This observation may suggest that complex formation or activation of CIPKs by CBL proteins may either occur at rather low calcium concentrations or that this interaction may be maintained during declining calcium levels to further promote signaling.

In contrast to CBL2, CBL4 was found to bind four calcium ions in the CIPK-unbound state [36]. In complex with CIPK24, CBL4 binds only two calcium ions to the EF hands 1 and 4 [30]. This is in contrast to CBL2–CIPK14, and resembles the situation of unbound CBL2, where EF hands 1 and 4 are occupied by calcium ions. However, when interpreting these results it needs to be considered that these studies were performed with a CBL4 that was purified from *Escherichia coli*. As CBL4 represents a myristoylated protein [20], a modification that does not occur in *E. coli*, and because lipid groups can introduce cooperativity into a calcium binding protein, it remains possible that the lipid-modified CBL4 in complex with a kinase exhibits different features of calcium binding.

5. Localization of CBL proteins and CIPKs

Spatial specificity is an important aspect of cellular information processing. Localization studies of *Arabidopsis* CBL proteins revealed that most of these proteins are localized either to the plasma membrane or vacuolar membrane [21,22,41,42] (Batistic et al.,

submitted). Considering that these membranes represent the borders to the two main calcium storage compartments of the plant cell (extracellular apoplast, and intracellular vacuole), CBL–CIPK complexes could function as fast responders to local calcium release events at these different membranes.

The distinct cellular distribution is also reflected by the phylogenetic separation of the CBL proteins into two groups (see above). While CBL2 and CBL10 (group two) are localized to the vacuolar membrane, CBL1 and CBL9 (group one) are localized to the plasma membrane [21,22,41]. In the latter case, lipid modifications were implicated as important structural modifications essential for plasma membrane binding [17]. It has been experimentally confirmed that CBL1, CBL4, CBL5 and CBL9 are N-myristoylated proteins [21]. Moreover, CBL1 was further shown to be S-acylated by either palmitate or stearate at the cysteine residue at position three. This modification is also implicated to affect CBL4, 5 and CBL9, since all these proteins harbor a similar N-terminal domain with a conserved cysteine residue [19]. CBL1 is besides CaBP from *Trypanosoma cruzi* [43] the only myristoylated–palmitoylated calcium binding protein of the NCS/CNB type. Both lipid modifications are important for *in planta* function of CBL1, because expression of mutant cDNAs that cannot undergo lipid modification did not restore salt tolerance of a *cbl1* mutant [21]. Sub-cellular localization GFP fusion proteins of G2A mutant and C3S mutant versions of CBL1 implicate that myristoylation of CBL1 occurs in the cytosol or at the rough ER. The myristoyl group allows targeting of CBL1 to the ER, where the protein most likely becomes modified by an ER bound palmitoyltransferase. After the second lipid group is added to the protein, CBL1 is further transported to the plasma membrane. Interestingly, this transport to the plasma membrane is appears not to be affected by Brefeldin A, an inhibitor of vesicle trafficking in eukaryotic cells. Moreover plasma membrane targeting of CBL1 does not involve COPII vesicles [21], as revealed by experiments using a dominant-negative protein form of Sar1, a G-protein regulating the assembly of the COPII vesicles at the ER [44,45]. This is in contrast to the NCS protein KChIP1. This protein, which is important for trafficking of the Kv4.2 channels, is transported from the ER to the Golgi independently of COPII vesicles. However, further transport to the plasma membrane of the KChIPI–Kv4.2 complex then depends on the Golgi function [46].

CIPKs do not possess recognizable localization signals. Most CIPKs when expressed as GFP fusion exhibit a cytoplasmic and nucleoplasmic localization (Batistic et al., in preparation). However CBL–CIPK interaction analyses using Bimolecular fluorescence complementation (BiFC) show that CIPKs are targeted to different membranous compartments of the cell by their respective interacting CBL proteins. For example, CIPK1 is targeted to the plasma membrane by CBL1 or CBL9 [41]. BiFC analyses of CIPK1 with CBL1 mutant versions that cannot undergo myristoylation or palmitoylation revealed that interaction of CBL1 and CIPK1 occurs independently of the lipid modification of the calcium sensor, but that the localization of the CBL1/CIPK1 complex still depends on the lipid modification status of CBL1 [21].

Yeast two hybrid investigations have suggested that each CBL protein can interact with a defined subset of the 25 CIPKs from *Arabidopsis* and conversely that each CIPK can interact with several CBL proteins [24]. Considering the spatial distinct localization of the CBL proteins this could lead to alternative complex formation of a given CIPK with certain CBL proteins in one compartment or at different membranes of the cell. Indeed, BiFC analyses established that, for example the kinase CIPK1 is targeted to the plasma membrane upon interaction with CBL1, while this kinase forms complexes with the calcium sensor CBL2 at the vacuolar membrane [21]. Most importantly, the development of multicolor BiFC analyses for plant cells allowed to confirm that the formation of these complexes can occur simultaneously within one and the same cell [47]. Simultaneous complex formation within one cell was recently unambiguously established for CBL1–CIPK1 and CBL9–CIPK1 at the plasma membrane, and for CBL1–CIPK24 and CBL10–CIPK24 at the plasma membrane and the vacuolar membrane, respectively [47]. These analyses clearly indicated that the CBL–CIPK system can indeed exist as a complex network within the cell that can simultaneously decode calcium signals in different compartments and thereby contributes to spatial specificity in calcium signaling. In this network, distinct CBL proteins form the docking station for CIPKs at the different membranous compartments, and CIPKs are mobile signal-relay proteins interacting with different CBL proteins. This differential interaction of CIPKs with CBL proteins at the same or different compartments within a single cell may be critical for the differential regulation of various targets by one and the same kinase.

6. Function of CBL–CIPK complexes

A current bottleneck in our understanding of the function of the CBL/CIPK system is still the lack of identified target proteins. Although it is reasonable to assume that several hundred cellular proteins will be phosphorylated by the plethora of distinct CBL/CIPK complexes, only very few targets have been unambiguously identified. Nevertheless, it is an emerging view that CBL–CIPK complexes are critical for regulation of membrane channels and transporters [48]. The founding example for the CBL/CIPK/substrate paradigm is the SOS ("salt overly sensitive") pathway. These mutants were identified in a forward screen of *Arabidopsis* plants which were sensitive to high salt conditions [49]. Therefore, the identified mutants were named "salt overly sensitive" 3 (SOS3), SOS2 and SOS1, respectively. Cloning of the genes underlying these phenotypes revealed that SOS3 encodes a CBL-type calcium sensor (CBL4) and that SOS2 represents a member of the CIPK family (CIPK24). The calcium sensor CBL4 (SOS3) targets its interacting kinase CIPK24 (SOS2) to the plasma membrane where this calcium sensor/protein kinase complex activates the H^+/Na^+ exchanger SOS1 leading to extrusion of toxic sodium ions from the cell [50–52]. The activation of SOS1 ensures low sodium concentrations in the cytosol and survival of the plant organism.

An additional CBL/CIPK regulatory system was recently identified in a break through study by the group of Wei-Hua Wu [53]. In a genetic screen, a mutant sensitive to low potassium conditions was isolated and designated as Lks1. Cloning of the gene identified Lks1 to encode CIPK23, a component of the CBL–CIPK system. Subsequent analysis revealed that this protein is targeted to the plasma membrane and activated by two highly related calcium sensors, CBL1 and CBL9 [42,53]. These complexes then regulate the activity of the shaker-like potassium channel AKT1. Interestingly, in animals the regulation of shaker channels is mediated by KChIPs, which directly regulate the activity of the channels [54]. It appears that plants lacking functional KChIPs have evolved a K^+ channel regulatory system relying on the related CBL proteins, in complex with CIPKs. In addition, recent reports have identified novel potential target proteins of CBL/CIPK complexes, like the vacuolar proton/calcium antiporter 1 (CAX1) [55] and a plasma membrane H^+-ATPase AHA1 [56].

7. Mechanisms generating target specificity in the CBL–CIPK signaling network

Every calcium signaling network has to cope with the same problem: the generation of specificity in signal to response coupling in a situation of multiple simultaneously incoming signals that all generate calcium transients. Besides warranting signaling specificity a calcium decoding system still has to enable an integrated organismal response that ensures plant development and reproduction.

7.1. Differential calcium response, expression and localization of CBLs and CIPKs

The multiplicity of CBL proteins and CIPKs allows for several mechanisms generating specificity in plant calcium signaling. In this

regard, the divergence of single family members as well as the combination of distinct CBLs with specific CIPKs represent two levels on which specificity can originate. CBL proteins could respond to different calcium signals due to their different affinities to calcium. Although this is suggested by the occurrence of EF hands with different sequences in each CBL protein this facet of calcium signaling still requires experimental investigation. Another aspect contributing to generating specificity in calcium decoding is the differential expression of CBLs and CIPKs in different plant tissues and their different transcriptional regulation in response to signals like for example to hormones, salt or cold [17]. The alternative complex formation of CIPK24 with either CBL4 or CBL10 provides an intriguing example of specificity generation that combines the aspects of tissue specific expression with the spatial separation of calcium decoding processes. CIPK24 is expressed in every tissue of the plant cell, and an important regulator of salt signaling in roots and leaves [57]. In contrast, the calcium sensors that interact and regulate CIPK24 activity are differentially expressed. CBL4 is mainly expressed in the root, while CBL10 is mainly expressed in leaves [22]. CBL4/CIPK24 assemble at the plasma membrane where this complex regulates the SOS1 Na^+/H^+ exchanger during high salt conditions to extrude toxic sodium out of the cell to the apoplast of the root [51]. In leaves, CIPK24 interacts with CBL10 at the vacuolar membrane leading to intracellular sodium sequestration into the vacuole [22]. Thereby, interaction with different CBL proteins creates a dual functioning kinase that differentially regulates ion homeostasis in different tissues of the plant and in compartments of the cell (Fig. 4) [22].

7.2. Differential interaction and activation of CBL–CIPK complexes

Another specificity mechanism that relies on the combination of calcium sensor and kinases is the differential interaction between CBLs and CIPKs. Yeast two-hybrid analyses revealed that at least in yeast CBLs interact with not each CIPKs, and vice versa [24]. These observations have been confirmed by *in planta* interaction analyses (Batistic et al., submitted). These findings suggest differential interaction affinity and consequently preferential complex formation of defined CBLs with specific CIPKs as one of the mechanisms generating spatial and temporal specificity in plant calcium signaling.

This differential interaction affinity of CBLs to CIPKs appears to result from the interplay of different factors. Biochemical studies using

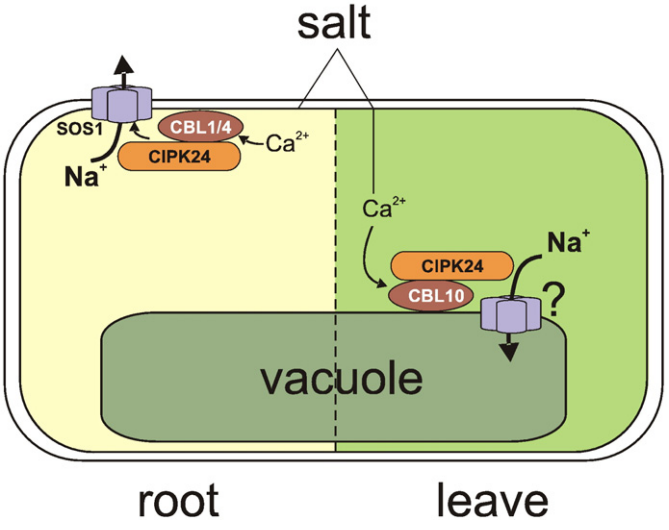

Fig. 4. Model of CBL/CIPK function in salt stress responses. Alternative complex formation of CIPK24 with either CBL1/CBL4 or CBL10 creates a dual functioning kinase. While CBL4–CIPK24 regulate Na^+-extrusion via the Na^+/H^+ exchanger SOS1 at the plasma membrane of roots, CBL10–CIPK24 complexes are localized at the vacuole in shoots where they potentially regulate Na^+ sequestration into this organelle.

full-length CIPK24, and crystal studies of CBL4 and the regulatory domain of CIPK24 revealed that this complex is formed in a calcium independent manner [30]. However, activation of CIPK24 only occurs in presence of calcium [20,50]. A similar complex formation has been described for CBL2 and the regulatory domain of CIPK14 [32]. Again, here the interaction occurs calcium independent, even if EF hand domains of CBL2 are impaired in calcium binding by mutations. In contrast to this type of interaction, in vitro interaction analysis of CBL1 and CIPK1 indicated that this complex is only formed in presence of calcium [58]. This conclusion is further supported by the fact that CBL1 is not able to interact with CIPK1 when all EF hands are disabled by mutations (Batistic and Kudla, unpublished). Interestingly, using only the regulatory domain of CIPK1 in in vitro pull down assays, calcium independent interaction was observed, similar to the observed interaction between CBL2 and CIPK14 [32,58]. Therefore, it is important to clarify the art of interaction between CBL2 and full-length CIPK14.

These findings indicate that the kinase domain seems to have a important role in stabilizing and defining interaction specificity. In fact, in earlier studies using two hybrid analysis, it was shown that CBL4 also interacts with the regulatory domains of CIPK5 and CIPK6 [59]. Using full-length protein kinases, only CIPK6 was still able to bind to CBL4. To further clarify the role of the kinase domain in the interaction mechanism, a chimeric protein was constructed using the regulatory domain of CIPK6 and the kinase domain of CIPK5. This chimeric CIPK5/6 was also not able to interact with CBL4, implicating a negative influence of the CIPK5 kinase domain to the interaction [59]. This observations may point to intramolecular interactions of the different kinase domains that may contribute to specificity in CBL interaction.

7.3. Differential target activation by specific CBL–CIPK complexes

An intriguing aspect of specificity generation is that only specific CBL–CIPK complexes regulate the activity of targets. This was nicely exemplified in complementation studies of the salt sensitive *cbl1* mutant line. In these experiments only a CBL1 wild type cDNA could restore salt tolerance. In contrast, a fusion protein of the 12 N-terminal amino acids of CBL1 with CBL2 (CBL1nCBL2) did not restore salt tolerance [21]. This observation is significant, because in yeast two-hybrid and in *in planta* BiFC analyses, both CBL1 and CBL2 interact with CIPK24/SOS2, the CBL-interacting protein kinase mediating salt tolerance in *Arabidopsis* [19,60]. Replacement of the N terminus of CBL2 with the N terminus of CBL1 redirects the localization of the resulting fusion protein to the plasma membrane, where CBL1 usually exerts its function. The failure of this fusion protein to functionally compensate for the loss of CBL1 function, therefore, suggests that, in addition to subcellular localization and specificity in CIPK interaction, factors that are inherent to the particular version of the CBL protein determine the functional specificity of the resulting CBL/CIPK complex toward its substrate(s). Such a functional specificity determinant may lie in the differences in the EF hand composition of CBL1 and CBL2, which could lead to distinct Ca^{2+} binding abilities [17].

Another example for CBL-dependent target specificity of CBL/CIPK complexes is provided by studies of CIPK1 that revealed that this kinase is differentially regulated by two highly related CBL proteins [41]. While interaction of CBL1 with CIPK1 is important for regulating downstream processes during salt stress, complex formation of CBL9 with CIPK1 mediates the response to the stress-hormone ABA [41,61]. These results suggest that the calcium sensor moiety of the CBL/CIPK complex is crucial for determining the activity towards defined phosphorylation targets. This conclusion is further corroborated by the finding that only CBL1 and CBL9 activate CIPK23 to phosphorylate AKT1 [53]). Remarkably, CIPK23 also interacts with CBL5 and CBL8, but these sensors are not able to achieve the activity towards the target AKT1 [53]. This implicates that interaction of CIPKs with the

CBLs modulates the target specificity of CIPKs. It will be very interesting and important to elucidate the mechanisms responsible for this remarkable specificity.

8. Conclusions

In plants, environmental cues like high salt, cold, low nutrition, and phytohormones involve calcium as an important signaling intermediate [4]. Genetic studies of CBL and CIPK mutants clearly revealed that these proteins are important regulators of plant stress response reactions [17]. Recent studies established that CBL/CIPK complexes regulate downstream targets like ion channels and transporters, and thereby ensure plant survival under unfavorable conditions. The specific regulation of target proteins is achieved by a complex interplay of mechanisms generating spatial and temporal specificity in the CBL/CIPK signaling network. The potential of generating specificity in this system is exemplified by the two highly related proteins CBL1 and CBL9 that share 90% identical amino acids, but reveal distinct functions when the phenotypes of single knock-out mutants were analyzed. The most striking difference between the two mutants is the response to the phytohormone ABA. While CBL1 mediates signals in response to salt and drought independently of the stress-hormone ABA, CBL9 represents a critical component regulating ABA synthesis and responses [60,61]. Evidently, subtle differences between these two calcium sensor proteins are responsible for this striking functional difference. Interestingly, while both proteins function differently in ABA responses, both proteins function synergistically in activating the kinase CIPK23 to regulate of the shaker-like channel AKT1. These striking differences in the function of structurally closely related CBL protein may point to additional levels of functional regulation by protein modifications like phosphorylation and sumoylation that need to be addressed in the future.

Acknowledgements

Work in the authors laboratory is supported by grants from the Deutsche Forschungsgemeinschaft the DAAD, the German Israeli Science foundation and the Human Frontier of Science Program to J.K.

References

[1] M.J. Berridge, M.D. Bootman, P. Lipp, Calcium—a life and death signal, Nature 395 (1998) 645–648.
[2] S. Gilroy, A. Trewavas, Signal processing and transduction in plant cells: the end of the beginning? Nat. Rev. Mol. Cell Biol. 2 (2001) 307–314.
[3] N.H. Evans, M.R. McAinsh, A.M. Hetherington, Calcium oscillations in higher plants, Curr. Opin. Plant Biol. 4 (2001) 415–420.
[4] D. Sanders, J. Pelloux, C. Brownlee, J.F. Harper, Calcium at the crossroads of signaling, Plant Cell 14 (Suppl.) (2002) S401–S417.
[5] J.F. Harper, Dissecting calcium oscillators in plant cells, Trends Plant Sci. 6 (2001) 395–397.
[6] H. Knight, M.R. Knight, Abiotic stress signalling pathways: specificity and cross-talk, Trends Plant Sci. 6 (2001) 262–267.
[7] V.S. Reddy, A.S. Reddy, Proteomics of calcium-signaling components in plants, Phytochemistry 65 (2004) 1745–1776.
[8] D. Chin, A.R. Means, Calmodulin: a prototypical calcium sensor, Trends Cell Biol. 10 (2000) 322–328.
[9] M. Ikura, J.B. Ames, Genetic polymorphism and protein conformational plasticity in the calmodulin superfamily: two ways to promote multifunctionality, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 1159–1164.
[10] V. Norris, S. Grant, P. Freestone, J. Canvin, F.N. Sheikh, I. Toth, M. Trinei, K. Modha, R.I. Norman, Calcium signalling in bacteria, J. Bacteriol. 178 (1996) 3677–3682.
[11] J. Michiels, C. Xi, J. Verhaert, J. Vanderleyden, The functions of $Ca^{(2+)}$ in bacteria: a role for EF-hand proteins? Trends Microbiol. 10 (2002) 87–93.
[12] D.C. Dominguez, Calcium signalling in bacteria, Mol. Microbiol. 54 (2004) 291–297.
[13] H. Kawasaki, S. Nakayama, R.H. Kretsinger, Classification and evolution of EF-hand proteins, Biometals 11 (1998) 277–295.
[14] S. Luan, J. Kudla, M. Rodriguez-Concepcion, S. Yalovsky, W. Gruissem, Calmodulins and calcineurin B-like proteins: calcium sensors for specific signal response coupling in plants, Plant Cell 14 (Suppl.) (2002) S389–S400.
[15] R.D. Burgoyne, D.W. O'Callaghan, B. Hasdemir, L.P. Haynes, A.V. Tepikin, Neuronal Ca^{2+}-sensor proteins: multitalented regulators of neuronal function, Trends Neurosci. 27 (2004) 203–209.
[16] J.F. Harper, G. Breton, A. Harmon, Decoding $Ca^{(2+)}$ signals through plant protein kinases, Annu. Rev. Plant Biol. 55 (2004) 263–288.
[17] O. Batistic, J. Kudla, Integration and channeling of calcium signaling through the CBL calcium sensor/CIPK protein kinase network, Planta 219 (2004) 915–924.
[18] J. Kudla, Q. Xu, K. Harter, W. Gruissem, S. Luan, Genes for calcineurin B-like proteins in *Arabidopsis* are differentially regulated by stress signals, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4718–4723.
[19] U. Kolukisaoglu, S. Weinl, D. Blazevic, O. Batistic, J. Kudla, Calcium sensors and their interacting protein kinases: genomics of the *Arabidopsis* and rice CBL–CIPK signaling networks, Plant Physiol. 134 (2004) 43–58.
[20] M. Ishitani, J. Liu, U. Halfter, C.S. Kim, W. Shi, J.K. Zhu, SOS3 function in plant salt tolerance requires N-myristoylation and calcium binding, Plant Cell 12 (2000) 1667–1678.
[21] O. Batistic, N. Sorek, S. Schultke, S. Yalovsky, J. Kudla, Dual fatty acyl modification determines the localization and plasma membrane targeting of CBL/CIPK Ca^{2+} signaling complexes in *Arabidopsis*, Plant Cell 20 (2008) 1346–1362.
[22] B.G. Kim, R. Waadt, Y.H. Cheong, G.K. Pandey, J.R. Dominguez-Solis, S. Schultke, S.C. Lee, J. Kudla, S. Luan, The calcium sensor CBL10 mediates salt tolerance by regulating ion homeostasis in *Arabidopsis*, Plant J. 52 (2007) 473–484.
[23] E.M. Hrabak, C.W. Chan, M. Gribskov, J.F. Harper, J.H. Choi, N. Halford, J. Kudla, S. Luan, H.G. Nimmo, M.R. Sussman, M. Thomas, K. Walker-Simmons, J.K. Zhu, A.C. Harmon, The *Arabidopsis* CDPK-SnRK superfamily of protein kinases, Plant Physiol. 132 (2003) 666–680.
[24] V. Albrecht, O. Ritz, S. Linder, K. Harter, J. Kudla, The NAF domain defines a novel protein-protein interaction module conserved in Ca^{2+}-regulated kinases, EMBO J. 20 (2001) 1051–1063.
[25] Y. Guo, U. Halfter, M. Ishitani, J.K. Zhu, Molecular characterization of functional domains in the protein kinase SOS2 that is required for plant salt tolerance, Plant Cell 13 (2001) 1383–1400.
[26] D. Gong, Y. Guo, A.T. Jagendorf, J.K. Zhu, Biochemical characterization of the *Arabidopsis* protein kinase SOS2 that functions in salt tolerance, Plant Physiol. 130 (2002) 256–264.
[27] R.S. Westphal, K.A. Anderson, A.R. Means, B.E. Wadzinski, A signaling complex of Ca^{2+}-calmodulin-dependent protein kinase IV and protein phosphatase 2A, Science 280 (1998) 1258–1261.
[28] F. Moore, J. Weekes, D.G. Hardie, Evidence that AMP triggers phosphorylation as well as direct allosteric activation of rat liver AMP-activated protein kinase. A sensitive mechanism to protect the cell against ATP depletion, Eur. J. Biochem. 199 (1991) 691–697.
[29] M. Ohta, Y. Guo, U. Halfter, J.K. Zhu, A novel domain in the protein kinase SOS2 mediates interaction with the protein phosphatase 2C ABI2, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 11771–11776.
[30] M.J. Sanchez-Barrena, H. Fujii, I. Angulo, M. Martinez-Ripoll, J.K. Zhu, A. Albert, The structure of the C-terminal domain of the protein kinase AtSOS2 bound to the calcium sensor AtSOS3, Mol. Cell 26 (2007) 427–435.
[31] N. Tochio, S. Koshiba, N. Kobayashi, M. Inoue, T. Yabuki, M. Aoki, E. Seki, T. Matsuda, Y. Tomo, Y. Motoda, A. Kobayashi, A. Tanaka, Y. Hayashizaki, T. Terada, M. Shirouzu, T. Kigawa, S. Yokoyama, Solution structure of the kinase-associated domain 1 of mouse microtubule-associated protein/microtubule affinity-regulating kinase 3, Protein Sci. 15 (2006) 2534–2543.
[32] M. Akaboshi, H. Hashimoto, H. Ishida, S. Saijo, N. Koizumi, M. Sato, T. Shimizu, The crystal structure of plant-specific calcium-binding protein AtCBL2 in complex with the regulatory domain of AtCIPK14, J. Mol. Biol. 377 (2008) 246–257.
[33] Y. Yu, X. Xinli, Y. Weilun, Z. Hechen, Comparative genomics analysis of CIPK gene family in *Arabidopsis* and *Populus*, Plant Growth Regul. 52 (2007) 101–110.
[34] J.M. Carlton, R.P. Hirt, J.C. Silva, A.L. Delcher, M. Schatz, Q. Zhao, J.R. Wortman, S.L. Bidwell, U.C. Alsmark, S. Besteiro, T. Sicheritz-Ponten, C.J. Noel, J.B. Dacks, P.G. Foster, C. Simillion, Y. Van de Peer, D. Miranda-Saavedra, G.J. Barton, G.D. Westrop, S. Muller, D. Dessi, P.L. Fiori, Q. Ren, I. Paulsen, H. Zhang, F.D. Bastida-Corcuera, A. Simoes-Barbosa, M.T. Brown, R.D. Hayes, M. Mukherjee, C.Y. Okumura, R. Schneider, A.J. Smith, S. Vanacova, M. Villalvazo, B.J. Haas, M. Pertea, T.V. Feldblyum, T.R. Utterback, C.L. Shu, K. Osoegawa, P.J. de Jong, I. Hrdy, L. Horvathova, Z. Zubacova, P. Dolezal, S.B. Malik, J.M. LogsdonJr, K. Henze, A. Gupta, C.C. Wang, R.L. Dunne, J.A. Upcroft, P. Upcroft, O. White, S.L. Salzberg, P. Tang, C.H. Chiu, Y.S. Lee, T.M. Embley, G.H. Coombs, J.C. Mottram, J. Tachezy, C.M. Fraser-Liggett, P.J. Johnson, Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*, Science 315 (2007) 207–212.
[35] M. Nagae, A. Nozawa, N. Koizumi, H. Sano, H. Hashimoto, M. Sato, T. Shimizu, The crystal structure of the novel calcium-binding protein AtCBL2 from *Arabidopsis thaliana*, J. Biol. Chem. 278 (2003) 42240–42246.
[36] M.J. Sanchez-Barrena, M. Martinez-Ripoll, J.K. Zhu, A. Albert, The structure of the *Arabidopsis thaliana* SOS3: molecular mechanism of sensing calcium for salt stress response, J. Mol. Biol. 345 (2005) 1253–1264.
[37] T. Strahl, B. Grafelmann, J. Dannenberg, J. Thorner, O. Pongs, Conservation of regulatory function in calcium-binding proteins: human frequenin (neuronal calcium sensor-1) associates productively with yeast phosphatidylinositol 4-kinase isoform, Pik1, J. Biol. Chem. 278 (2003) 49589–49599.
[38] M.L. Preuss, A.J. Schmitz, J.M. Thole, H.K. Bonner, M.S. Otegui, E. Nielsen, A role for the RabA4b effector protein PI-4Kbeta1 in polarized expansion of root hair cells in *Arabidopsis thaliana*, J. Cell Biol. 172 (2006) 991–998.
[39] J.B. Ames, R. Ishima, T. Tanaka, J.I. Gordon, L. Stryer, M. Ikura, Molecular mechanics of calcium-myristoyl switches, Nature 389 (1997) 198–202.

[40] D.W. O'Callaghan, A.V. Tepikin, R.D. Burgoyne, Dynamics and calcium sensitivity of the Ca^{2+}/myristoyl switch protein hippocalcin in living cells, J. Cell Biol. 163 (2003) 715–721.
[41] C. D'Angelo, S. Weinl, O. Batistic, G.K. Pandey, Y.H. Cheong, S. Schultke, V. Albrecht, B. Ehlert, B. Schulz, K. Harter, S. Luan, R. Bock, J. Kudla, Alternative complex formation of the Ca-regulated protein kinase CIPK1 controls abscisic acid-dependent and independent stress responses in *Arabidopsis*, Plant J. 48 (2006) 857–872.
[42] Y.H. Cheong, G.K. Pandey, J.J. Grant, O. Batistic, L. Li, B.G. Kim, S.C. Lee, J. Kudla, S. Luan, Two calcineurin B-like calcium sensors, interacting with protein kinase CIPK23, regulate leaf transpiration and root potassium uptake in *Arabidopsis*, Plant J. 52 (2007) 223–239.
[43] L.M. Godsel, D.M. Engman, Flagellar protein localization mediated by a calcium-myristoyl/palmitoyl switch mechanism, EMBO J. 18 (1999) 2057–2065.
[44] T. Rowe, M. Aridor, J.M. McCaffery, H. Plutner, C. Nuoffer, W.E. Balch, COPII vesicles derived from mammalian endoplasmic reticulum microsomes recruit COPI, J. Cell Biol. 135 (1996) 895–911.
[45] A.V. Andreeva, H. Zheng, C.M. Saint-Jore, M.A. Kutuzov, D.E. Evans, C.R. Hawes, Organization of transport from endoplasmic reticulum to Golgi in higher plants, Biochem. Soc. Trans. 28 (2000) 505–512.
[46] B. Hasdemir, D.J. Fitzgerald, I.A. Prior, A.V. Tepikin, R.D. Burgoyne, Traffic of Kv4 K^+ channels mediated by KChIP1 is via a novel post-ER vesicular pathway, J. Cell Biol. 171 (2005) 459–469.
[47] R. Waadt, L.K. Schmidt, M. Lohse, K. Hashimoto, R. Bock, J. Kudla, Multicolor bimolecular fluorescence complementation (mcBiFC) reveals simultaneous formation of alternative CBL/CIPK complexes in planta, Plant J. 56 (2008) 505–516.
[48] R. Hedrich, J. Kudla, Calcium signaling networks channel plant K^+ uptake, Cell 125 (2006) 1221–1223.
[49] J.K. Zhu, J. Liu, L. Xiong, Genetic analysis of salt tolerance in *Arabidopsis*. Evidence for a critical role of potassium nutrition, Plant Cell 10 (1998) 1181–1191.
[50] U. Halfter, M. Ishitani, J.K. Zhu, The *Arabidopsis* SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 3735–3740.
[51] Q.S. Qiu, Y. Guo, M.A. Dietrich, K.S. Schumaker, J.K. Zhu, Regulation of SOS1, a plasma membrane Na+/H+ exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 8436–8441.
[52] F.J. Quintero, M. Ohta, H. Shi, J.K. Zhu, J.M. Pardo, Reconstitution in yeast of the *Arabidopsis* SOS signaling pathway for Na^+ homeostasis, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 9061–9066.
[53] J. Xu, H.D. Li, L.Q. Chen, Y. Wang, L.L. Liu, L. He, W.H. Wu, A protein kinase, interacting with two calcineurin B-like proteins, regulates K^+ transporter AKT1 in *Arabidopsis*, Cell 125 (2006) 1347–1360.
[54] R.D. Burgoyne, Neuronal calcium sensor proteins: generating diversity in neuronal $Ca^{(2+)}$ signalling, Nat. Rev. Neurosci. 8 (2007) 182–193.
[55] N.H. Cheng, J.K. Pittman, J.K. Zhu, K.D. Hirschi, The protein kinase SOS2 activates the *Arabidopsis* $H^{(+)}/Ca^{(2+)}$ antiporter CAX1 to integrate calcium transport and salt tolerance, J. Biol. Chem. 279 (2004) 2922–2926.
[56] G. Batelli, P.E. Verslues, F. Agius, Q. Qiu, H. Fujii, S. Pan, K.S. Schumaker, S. Grillo, J.K. Zhu, SOS2 promotes salt tolerance in part by interacting with the vacuolar H^+-ATPase and upregulating its transport activity, Mol. Cell Biol. 27 (2007) 7781–7790.
[57] J. Liu, M. Ishitani, U. Halfter, C.S. Kim, J.K. Zhu, The *Arabidopsis thaliana* SOS2 gene encodes a protein kinase that is required for salt tolerance, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 3730–3734.
[58] J. Shi, K.N. Kim, O. Ritz, V. Albrecht, R. Gupta, K. Harter, S. Luan, J. Kudla, Novel protein kinases associated with calcineurin B-like calcium sensors in *Arabidopsis*, Plant Cell 11 (1999) 2393–2405.
[59] K.N. Kim, Y.H. Cheong, R. Gupta, S. Luan, Interaction specificity of *Arabidopsis* calcineurin B-like calcium sensors and their target kinases, Plant Physiol. 124 (2000) 1844–1853.
[60] V. Albrecht, S. Weinl, D. Blazevic, C. D'Angelo, O. Batistic, U. Kolukisaoglu, R. Bock, B. Schulz, K. Harter, J. Kudla, The calcium sensor CBL1 integrates plant responses to abiotic stresses, Plant J. 36 (2003) 457–470.
[61] G.K. Pandey, Y.H. Cheong, K.N. Kim, J.J. Grant, L. Li, W. Hung, C. D'Angelo, S. Weinl, J. Kudla, S. Luan, The calcium sensor calcineurin B-like 9 modulates abscisic acid sensitivity and biosynthesis in *Arabidopsis*, Plant Cell 16 (2004) 1912–1924.