Tansley review

Advances and current challenges in calcium signaling

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Summary

Temporally and spatially defined changes in Ca^{2+} concentration in distinct compartments of cells represent a universal information code in plants. Recently, it has become evident that Ca^{2+} signals not only govern intracellular regulation but also appear to contribute to long distance or even organismic signal propagation and physiological response regulation. Ca^{2+} signals are shaped by an intimate interplay of channels and transporters, and during past years important contributing individual components have been identified and characterized. Ca^{2+} signals are translated by an elaborate toolkit of Ca^{2+}-binding proteins, many of which function as Ca^{2+} sensors, into defined downstream responses. Intriguing progress has been achieved in identifying specific modules that interconnect Ca^{2+} decoding proteins and protein kinases with downstream target effectors, and in characterizing molecular details of these processes. In this review, we reflect on recent major advances in our understanding of Ca^{2+} signaling and cover emerging concepts and existing open questions that should be informative also for scientists that are currently entering this field of ever-increasing breath and impact.

I. Introduction

Calcium (Ca^{2+}) serves as an ubiquitous second messenger in all eukaryotes including plants (Berridge *et al.*, 2000). For an ever increasing number of biological processes, it has been found that temporally and spatially defined changes of Ca^{2+} concentration in the cytoplasm or in defined organelles occur at one point or another (Clapham, 2007; Dodd *et al.*, 2010; Kudla *et al.*, 2010; Stael *et al.*, 2012). In this context, it is also increasingly appreciated that the formation and decoding of these Ca^{2+} signals occur in defined regions of membranes often designated as nano- or microdomains. However, we are currently only beginning to understand this emerging aspect of Ca^{2+} signaling in plants (Rizzuto & Pozzan, 2006; Demir *et al.*, 2013; Steinhorst & Kudla, 2013; Diaz *et al.*, 2016). Since the initial reports on stimulus-specific alterations of cytoplasmic $[Ca^{2+}]$ in the 1990s, there have been not only a growing number of processes described that involve Ca^{2+} signaling, but also a steadily increasing number of researchers interested in these phenomena (Fricker *et al.*, 1991; Knight *et al.*, 1991; Shacklock *et al.*, 1992). It is, therefore, only logical that our toolkit to study such processes has been constantly advanced. Also, the importance of Ca^{2+} decoding processes for the implementation of the information provided by Ca^{2+} signals has been increasingly appreciated, and several distinct families of Ca^{2+} sensing proteins have been identified and characterized (Hashimoto & Kudla, 2011). However, it has remained challenging to causally and mechanistically link the occurrence of Ca^{2+} signals to the faithful regulation of specific downstream responses. Recognizing the importance of Ca^{2+} signaling in plants, the German Science Foundation (DFG) in 2008 supported the implementation of a long-term Research Group (entitled: 'Ca^{2+} signaling via protein phosphorylation in plant model cell types during environmental stress adaptation') to foster research in this area. In this Research Group, experts in different areas related to Ca^{2+} signaling collaboratively tackled its complex research challenges. In this review, all principal investigators of the Research Group reflect on progress in elucidating Ca^{2+} regulated processes and targets, and attempt to identify the most promising and pressing challenges that need to be addressed to sustain the scientific vigor of this field.

Ca^{2+} signals steer a multitude of downstream responses (McAinsh & Hetherington, 1998; Batistič & Kudla, 2012). For its messenger function, Ca^{2+} signals need to exhibit stimulus specificity. Stimulus-specific information of cytoplasmic Ca^{2+} signals can be provided by spatio-temporal parameters such as frequency, amplitude and location (Hetherington & Brownlee, 2004; Dodd *et al.*, 2010). These observations resulted in the formulation of the Ca^{2+} 'signature' concept which implies that defined Ca^{2+} signatures elicited by distinct stimuli represent stimulus-specific Ca^{2+} pattern contribution to appropriate signal responses (Webb *et al.*, 1996). However, it is also emerging that the occurrence of Ca^{2+} signals in many different biological processes also provides a potential level for signal integration that ensures coordination of many distinct environmental responses with each other and with plant development (Edel & Kudla, 2016).

II. Ca^{2+} importer and exporter in plants

Organisms employ a set of Ca^{2+} influx as well as efflux proteins that realize the so-called 'on' and 'off' mechanisms (Berridge *et al.*, 2003). The combination of Ca^{2+} influx via Ca^{2+} channels and active energy-dependent Ca^{2+} efflux via Ca^{2+} transporters produces Ca^{2+} signatures as a net result of their combined action. In Arabidopsis, to date, five families of Ca^{2+}-permeable channels have been identified. These are cyclic nucleotide gated channels (CNGCs – 20 members), glutamate receptors (GLRs – 20 members), two pore channels (TPCs – one representative), mechanosensitive channels (MCAs – two members) and reduced hyperosmolality-induced $[Ca^{2+}]_{cyt}$ increase (OSCAs – 15 members) (Lacombe *et al.*, 2001; Price *et al.*, 2012; Zelman *et al.*, 2012; Kurusu *et al.*, 2013; Morgan & Galione, 2014; Yuan *et al.*, 2014). These channels form the influx or 'on' component of Ca^{2+} signaling and are counteracted by at least four families of Ca^{2+} transporters that shape the Ca^{2+} signature and restore basal Ca^{2+} concentrations by extruding this ion out of the cytosol. These transporters employ ATP or H^+ gradients as a source of energy to pump Ca^{2+} across a membrane against its concentration gradient. In Arabidopsis autoinhibited Ca^{2+}-ATPases (ACAs), ER-type Ca^{2+}-ATPases (ECAs), P1-ATPases (e.g. HMA1) and Ca^{2+} exchangers (CAX) represent the known efflux or 'off' components of the Ca^{2+} machinery (Moreno *et al.*, 2008; Bose *et al.*, 2011; Manohar *et al.*, 2011; Pittman, 2011; Spalding & Harper, 2011). Such a signature-shaping function of Ca^{2+} transporters was recently exemplified by studies on the function and Ca^{2+}-dependent phosphorylation mediated regulation of ACA8 (Costa *et al.*, 2017; Yang *et al.*, 2017).

However, the specific functions of individual members of these exporters and importers are only beginning to emerge. Moreover, the regulation of these proteins and, especially, the evidently required coordination of their activity represent research areas of pressing importance. Finally, the existing discrepancy between the predicted channel activities based on previous pharmacological and electrophysiological investigations and the identity of currently identified channels/transporters raises the possibility that important Ca^{2+}-permeable proteins in plants still await discovery.

III. The Ca^{2+} decoding toolkit in plants

The importance of Ca^{2+}-binding proteins in plants is reflected by their large number and diversity of for example 250 proteins in *Arabidopsis thaliana* which carry one or more EF-hand Ca^{2+}-binding motifs (Day *et al.*, 2002).

A prototypical Ca^{2+} sensor is presented by Calmodulin (CaM – seven genes in Arabidopsis), a short four EF-hand containing Ca^{2+}-binding protein present in all eukaryotic branches of life and involved in a multitude of essential responses like regulation of transcription and enzyme activity (Galon *et al.*, 2010; Perochon *et al.*, 2011; Edel & Kudla, 2015). Closely related to CaMs but with a diverse appearance diverging from the four EF-hand paradigm of CaMs, are CaM-like proteins (CMLs – 50 genes in Arabidopsis) (McCormack *et al.*, 2005; Zhu *et al.*, 2015). These Ca^{2+} sensors

vary in their composition from one to six EF-hands and analyses of available mutants have already identified various functions spanning developmental processes to stress responses. For example, Loss of CML42 was associated with aberrant trichomes, whereas CML41 was reported to regulate plasmodesmal closure during plant immune responses. Moreover, CML24 and CML9 were found to be involved in abscisic acid (ABA) responses and ionic stress, whereas CML36 was reported to regulate the activity of the Ca^{2+} ATPase ACA8 (Delk *et al.*, 2005; Magnan *et al.*, 2008; Dobney *et al.*, 2009; Astegno *et al.*, 2017; Xu *et al.*, 2017).

Calcineurin B-like proteins (CBL – 10 genes in Arabidopsis) represent a family of EF-hand containing Ca^{2+} sensors that share only little sequence similarity with CaMs and therefore appear to have diverged from CaMs very early in evolution (Batistic & Kudla, 2009; Weinl & Kudla, 2009; Edel & Kudla, 2015). Although CBL proteins also contain four EF-hand motifs, their first EF-hand provides a unique and distinguishing feature because its Ca^{2+}-binding loop consists of 14 instead of 12 amino acids but still mediates Ca^{2+} binding (Nagae *et al.*, 2003).

CBL proteins form functional complexes with CBL interacting protein kinases (CIPKs – 26 members in *A. thaliana*) (Shi *et al.*, 1999). It is noteworthy that each CBL preferentially and specifically interacts with a defined subset of CIPKs and vice versa (Albrecht *et al.*, 2001; D'Angelo *et al.*, 2006). This property of the CBL/CIPK network has been proposed to contribute to efficient signal channeling in Ca^{2+} signaling implementation (Kudla *et al.*, 2010). CIPKs display a conserved modular structure consisting of an N-terminal serine/threonine protein kinase domain, a junction domain and a CIPK-specific C-terminal regulatory domain (Shi *et al.*, 1999; Edel & Kudla, 2015). The latter includes the auto-regulatory NAF domain (after the characteristic amino acids N, A and F) and the phosphatase interaction domain (PPI) (Ohta *et al.*, 2003). Upon interaction of CBLs with CIPKs, the auto-inhibitory NAF domain is released from the kinase domain resulting in an active enzyme conformation (Chaves-Sanjuan *et al.*, 2014). Interaction with CBLs not only activates CIPKs towards target phosphorylation, but also is crucial for cellular targeting of the resulting CBL/CIPK complexes (Batistic *et al.*, 2010). Lipid modification of the N-terminus of CBLs is responsible for their association with specific membrane compartments. For instance, CBL2 and CBL3 are S-acylated and thereby targeted to the vacuolar membrane, whereas CBL1 and CBL9 are myristoylated and palmitoylated at an N-terminal MGCXXS/T motif and thereby located to the plasma membrane (Batistic *et al.*, 2008, 2012). Interaction between CBLs and CIPKs locates the CIPK at the respective membrane determined by their interacting CBL. This process allows for regulation of complex localization and enables one and the same CIPK to function at different membranes dependent on the interaction partner. This is the case for CIPK24 that regulates SOS1 in conjunction with CBL4/SOS3 at the plasma membrane and is involved in salt tolerance at the vacuole if interacting with the vacuolar targeted CBL10 (Halfter *et al.*, 2000; Ishitani *et al.*, 2000; Kim *et al.*, 2007). A rapidly growing number of physiological functions has been assigned to CBL/CIPK complexes that ranges from the regulation of transport of diverse ions, abiotic stress tolerance and plant development to plant immune responses (Kudla *et al.*, 2010; Hashimoto & Kudla, 2011; Eckert *et al.*, 2014; Steinhorst & Kudla, 2014).

An interesting emerging case of a multifunctional CIPK is provided by CIPK23, which appears to represent a key master regulator of plant nutrient sensing and acquisition. This kinase has been reported to function in potassium and nitrate as well as iron, magnesium and ammonium homeostasis (Geiger *et al.*, 2009a; Ho *et al.*, 2009; Maierhofer *et al.*, 2014; Scherzer *et al.*, 2015; Wang *et al.*, 2016a; Behera *et al.*, 2017; Straub *et al.*, 2017) (Fig. 1). CIPK23 phosphorylates and activates AKT1 (Arabidopsis K^+ transporter 1; low-affinity potassium uptake) as well as HAK5 (high-affinity K^+ transporter; high-affinity potassium uptake) but the corresponding phosphorylation sites and the activation mechanisms remain to be resolved (Ragel *et al.*, 2015). In contrast to AKT1/AtKC1 (Arabidopsis K^+ Channel 1) and HAK5 potassium uptake systems a single nitrate 'transceptor' CHL1 (NRT1.1; Nitrate transporter 1.1; NPF6.3) mediates both high-affinity and low-affinity nitrate uptake depending on its phosphorylation status at threonine residue 101 mediated by CIPK23 (Liu & Tsay, 2003; Léran *et al.*, 2015). In addition, it was recently shown that CIPK23

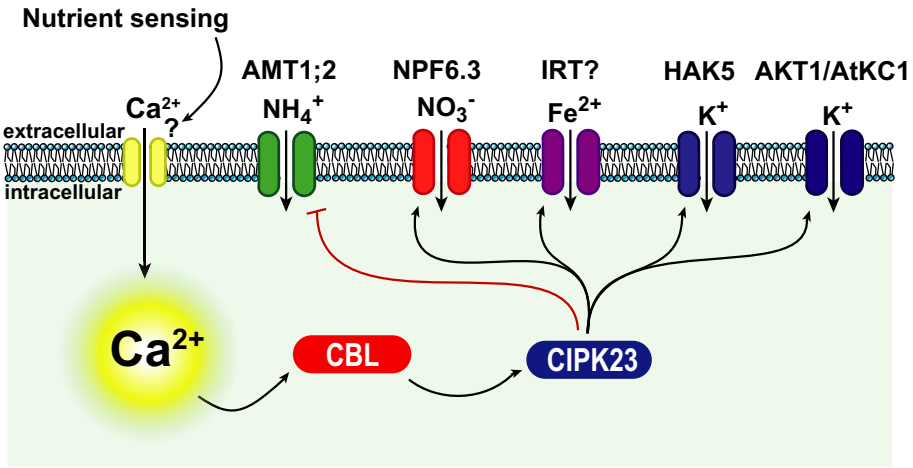

Fig. 1 Calcineurin B-like interacting protein kinase (CIPK)23 represents a 'nutritional sensor' in roots. CIPK23 activates mineral nutrient transporters/channels for potassium, nitrate and iron upon starvation. In response to high ammonium CIPK23 inhibits ammonium transport.

activates iron transport in Arabidopsis roots but inhibits AMT1;2 (ammonium transporter 1;2) mediated NH_4^+ uptake in Arabidopsis roots to avoid toxic accumulation of cytoplasmic ammonium (Tian *et al.*, 2016; Straub *et al.*, 2017) (Fig. 1). This complex but interesting situation raises questions such as how this kinase is capable of distinguishing the specific stimuli like K^+ starvation, nitrate availability or iron deficiency, and of course, also how this kinase upon one of these stimuli recognizes and regulates the appropriate target protein. In the future, high resolution *in planta* studies are needed to advance our understanding of this most interesting aspect of plant Ca^{2+} signaling.

Ca^{2+}-CaM regulated kinases (CCaMKs – absent in Arabidopsis, one member in *Lotus japonicas*) are special plant-specific protein kinases that are regulated by the concerted action of Ca^{2+} and CaM, and function as regulators of arbuscular mycorrhiza and root nodule formation (Kistner & Parniske, 2002).

Ca^{2+}-dependent protein kinases (CDPKs or CPKs for the 34 members in *A. thaliana*) structurally combine a Ca^{2+}-sensor domain and a protein kinase effector domain within one molecule (Wernimont *et al.*, 2010). CDPKs are structurally unique kinases consisting of an N-terminal variable domain, a serine/threonine protein kinase domain, a pseudo-substrate segment and a Ca^{2+}-binding four EF-hands containing a calmodulin-like domain (CLD). In the inactive enzyme conformation, the pseudo-substrate segment binds to the kinase domain (Liese & Romeis, 2013). Ca^{2+} binding to the CLD triggers a conformational change, pulling the pseudo-substrate segment out of the kinase domain and leading to an active enzyme.

It is currently largely unknown how these Ca^{2+}-regulated kinases share their function with Ca^{2+}-independent kinases (like the MAP kinase system, receptor like kinases or SnRK2s) in diverse biological pathways or processes. However, an emerging prospect, in this regard, is provided by accumulating evidence for extensive trans-phosphorylation of various CIPKs and CDPKs (Zhu *et al.*, 2007; Zhou *et al.*, 2014). The exploration of the regulatory consequences and the underlying kinases for these protein modifications provides an interesting avenue for future research.

IV. Mechanisms of Ca^{2+} signal decoding

Despite the conserved modular structure within the CDPK family and the deduced consensus model of Ca^{2+}-dependent CDPK activation, an increasing number of enzymes are identified which display low or no detectable Ca^{2+}-dependent changes in their *in vitro* kinase activity (Boudsocq *et al.*, 2012). Future research will have to address whether these 'apparent' Ca^{2+}-independent CDPKs indeed adopt a constitutive active enzymatic conformation and how the activity of these kinases is modulated *in vivo*.

Recently resolved crystal structures of protist CDPKs suggest that the CLD and pseudo-substrate segment form a functional structural unit which controls the Ca^{2+}-binding induced conformational change (Wernimont *et al.*, 2010, 2011). Deduced from that model, the variability of CDPK Ca^{2+}-dependency appears to be determined either by the amino acid sequence of the EF-hand containing CLD or by the pseudo-substrate segment. In this regard, Ca^{2+}-independency of CDPK activity often coincides with the occurrence of noncanonical EF-hand motifs (Liese & Romeis, 2013). CDPK EF-hands function pairwise, and a high-affinity C- and a low-affinity N-terminal EF-hand pair can be differentiated (Franz *et al.*, 2011). Notably, mutations in EF-hand motifs are usually observed in only one out of the two paired EF-hands. The understanding of how single EF-hand-dependent Ca^{2+} affinities contribute to the overall CDPK conformational change will facilitate our understanding of the postulated link between EF-hand amino acid sequence and the variability of CDPK Ca^{2+}-dependency.

Despite the presence of four consensus EF-hand motifs, CPK30 displays Ca^{2+}-independent kinase activity, at least *in vitro,* indicating the necessity and contribution of additional CDPK regions for Ca^{2+}-dependency (Boudsocq *et al.*, 2012). Current research addresses molecular determinants in the pseudo-substrate region, considering its Ca^{2+}- (and/or phosphorylation-) dependent interplay with the CLD in the context of kinase activation.

In the case of CBL/CIPKs the mode of action differs from CDPKs because here the sensor and effector functions are divided into two proteins. This feature provides an enormous versatility and flexibility in creating and evolving sensor–responder combinations. The information of the Ca^{2+} signal is transmitted from the CBL to the CIPK upon complex formation and translated into phosphorylation responses. Increasing evidence points to a contribution of both the identity of the Ca^{2+} sensor as well as of the interacting kinase to the determination of the substrate specificity of the resulting CBL/CIPK complex. CBL/CIPK complex assembly involves the formation of a hydrophobic pocket by CBLs upon structural rearrangements induced by Ca^{2+} binding that can accommodate the NAF-domain of CIPKs and thereby release the inhibitory effect, and enables CIPK activity (Sánchez-Barrena *et al.*, 2007; Chaves-Sanjuan *et al.*, 2014). Although our current knowledge about the distinct Ca^{2+}-binding affinities of individual CBLs is limited, it appears reasonable to postulate that varying Ca^{2+} affinities may convey response specificity. A first study providing a plausible affinity value of CBLs was recently published and indicates that a protist CBL can bind Ca^{2+} with an affinity (K_d) of *c.* 90 nM (Beckmann *et al.*, 2016). This would indicate a high sensitivity and argue for the capability of this CBL to react to small Ca^{2+} influxes. However, such data are still mostly missing but urgently needed for plant CBL proteins.

Phosphorylation has been described in the process of Ca^{2+} decoding for all three classes of Ca^{2+}-regulated protein kinases. In the CBL/CIPK dual component system CIPK-dependent CBL phosphorylation appears to be required for full activity of CBL/CIPK complexes (Du *et al.*, 2011; Hashimoto *et al.*, 2012). Additionally, phosphorylation within the activation loop was identified to be an important regulatory mechanism influencing CIPK activity. However, CIPKs display varying dependency on this phosphorylation as, for example, CIPK24 already shows basal activity without phosphorylation (Chaves-Sanjuan *et al.*, 2014). CCaMKs auto-phosphorylation in the CaM binding site has been shown to inhibit CCaMK activation (Routray *et al.*, 2013). For the class of CDPKs, phosphorylation of the enzyme (catalyzed by itself or by upstream kinases) has been well documented (Liese & Romeis, 2013). However, the impact of phosphorylation on

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changes in structure and activity is still not understood. It is tempting to speculate that phosphorylation is an additional (or an alternative) mechanism to control CDPK kinase activity, in particular for Ca^{2+}-independent enzymes (Zhao *et al.*, 2013).

For some enzymes, such as CDPKs, the Ca^{2+}-dependency of *in vitro* kinase activity was shown to be dependent of the nature of the substrate (Lee *et al.*, 1998; Boudsocq *et al.*, 2012). This suggests that the apparent EC_{50} values for Ca^{2+} are influenced by the substrate, and the interaction between kinase and substrate may modify the stability of the active CDPK conformation.

Major current objectives in research on Ca^{2+}-decoding mechanisms involve the quantitative determination of the Ca^{2+} binding to specific proteins, the detailed structural elucidation of the subsequently induced conformational changes, and linking these events to the consequently triggered kinase activation towards its substrates. This approach requires (1) knowledge about *in vivo* substrates of respective enzymes, (2) the determination of isoform-specific Ca^{2+}-binding affinities and dependencies for kinase activity, and (3) establishment of conformational change measurements – possibly with ratiometric reporter proteins – within an enzyme over a Ca^{2+}-range – and all of these ideally in an experimental *in vivo* system.

Significant progress has been made toward these objectives. In guard cells the anion channels SLAC1 and SLAH3 have been identified as *in vivo* phosphorylation targets of several CPKs (CPK21, CPK23, CPK3 and CPK6), and different isoform-specific Ca^{2+} affinities have been determined (Geiger *et al.*, 2010, 2011; Brandt *et al.*, 2012, 2015; Scherzer *et al.*, 2012). In addition, CIPK23 in combination with either CBL1 or CBL9 activates SLAC1 and SLAH3 (Maierhofer *et al.*, 2014) (Fig. 2). In this way, the anion channel SLAC1 provides a model case to elucidate the interplay of Ca^{2+}-dependent phosphorylation by multiple kinases with differential Ca^{2+} affinities with Ca^{2+}-independent kinases (and phosphatases) (Edel & Kudla, 2016). However, the limitations to quantitatively adjust the Ca^{2+} concentration in the Xenopus oocyte system currently hampers a further detailed quantitative study of this processes and make the development of alternative *in vivo* systems highly desirable.

V. Immediate Ca^{2+} signaling in the regulation of ion transport

When challenged with abiotic stress stimuli, such as drought or salinity, plants respond with adjustments in gene expression for mid- and long-term adaptation (Kilian *et al.*, 2007). However, ABA and cytosolic free Ca^{2+} control the activity of transport proteins involved in fast signaling events. To study this fast membrane-delimited response, guard cells represent an ideal single cell model system that largely acts autonomously from the surrounding tissues. Importantly, guard cell-expressed anion channel SLAC1 was found to act as a convergence point of multitude stimuli, including light, CO_2, pathogens, relative humidity and the drought stress hormone ABA (Levchenko *et al.*, 2005; Jeworutzki *et al.*, 2010; Kim *et al.*, 2010; Krol *et al.*, 2010; Bauer *et al.*, 2013; Guzel Deger *et al.*, 2015). Guard cells of SLAC1 loss-of-function mutants appeared largely ABA- and CO_2/O_3-insensitive and lacked S-type anion currents (Negi *et al.*, 2008; Vahisalu *et al.*, 2008). Consequently, the multifaceted regulation of SLAC1 activity provides an ideal model system to study the interrelations between the different ABA- and Ca^{2+}-responsive signaling systems.

The kinase SnRK2.6/OST1 also plays a central role in the regulation of guard cells (Mustilli *et al.*, 2002; Yoshida *et al.*, 2006). In OST1 loss-of-function mutants, ABA-dependent stomatal closure is impaired and guard cells display a lower activity of S-type anion channels (Geiger *et al.*, 2009b). In line with this phenotype, OST1 was found to activate the S-type anion channel SLAC1 in Xenopus oocytes (Geiger *et al.*, 2009b; Lee *et al.*, 2009; Vahisalu *et al.*, 2010).

The initial steps in stress signal transduction have been shown to activate guard cell anion channels in a Ca^{2+}-independent as well as a Ca^{2+}-dependent manner (Schroeder & Hagiwara, 1990; Hetherington & Brownlee, 2004; Levchenko *et al.*, 2005; Stange *et al.*, 2010). Both branches appear to share the same ABA receptors and PP2C phosphatase co-receptors such as ABI1, ABI2 and HAB1 (Fujii *et al.*, 2009; Geiger *et al.*, 2009b; Ma *et al.*, 2009; Park *et al.*, 2009; Melcher *et al.*, 2010; Hua *et al.*, 2012; Edel & Kudla, 2016).

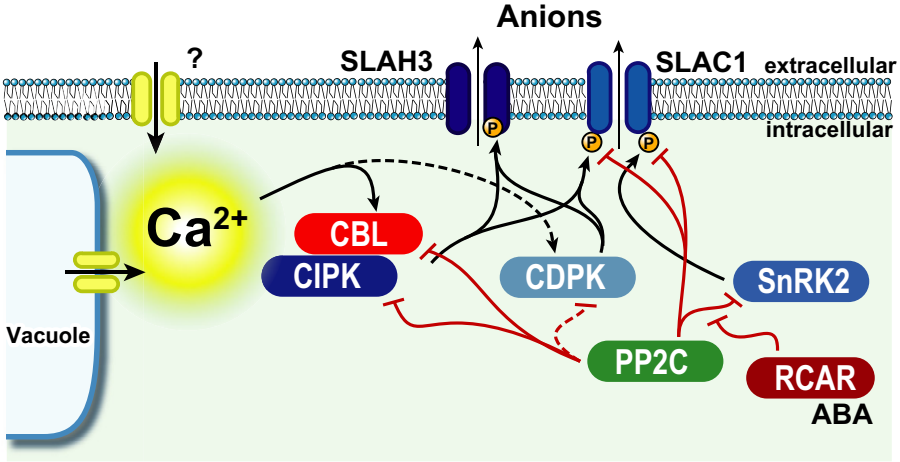

Fig. 2 Ion channels and transporters represent targets of Ca^{2+}-dependent signaling networks. Abscisic acid (ABA) signaling in guard cells builds on Ca^{2+}-dependent and -independent networks, represented by Ca^{2+}-dependent protein kinases (CDPKs) or calcineurin B-like interacting protein kinases (CIPKs), on the one hand, and sucrose nonfermenting (SNF)-related kinase (SnRK)2, on the other (for details see Section V). Kinase-mediated activation of SLAC1/SLAH3 anion channels results in plasma membrane depolarization. Please note that PP2C-like phosphatases inhibit kinase activity and at the same time are capable of dephosphorylating ion channels.

Recent work identified CPK21 and CPK23 as well as CBL1-9/CIPK23 complexes as interacting partners of SLAC1 and upon coexpression of SLAC1 with these kinases, SLAC1-derived anion currents document anion channel stimulation (Cheong *et al.*, 2007; Ma & Wu, 2007; Geiger *et al.*, 2010; Maierhofer *et al.*, 2014) (Fig. 2). In addition to CPK21 and CPK23, CPK3 and CPK6 also were reported to activate SLAC1 (Mori *et al.*, 2006; Brandt *et al.*, 2012; Scherzer *et al.*, 2012). Similar to OST1, CPK and CBL1-9/CIPK23 activation of the guard cell anion channel was counteracted by ABI1. *In vitro* kinase assays revealed that CPK3, CPK6, CPK21 and CPK23 phosphorylate the cytosolic N-terminus of SLAC1 and possess distinct Ca^{2+}-sensitivities. Although CPK3 and CPK21 display high Ca^{2+}-binding affinity, CPK6 and CPK23 exhibit a composite Ca^{2+} regulation with a Ca^{2+}-independent (up to 50%) core activity and a Ca^{2+}-inducible component. These results assign CPK6 and CPK23 to the weak Ca^{2+}-dependent ABA-signaling branch. Consequently, a strictly Ca^{2+}-dependent (CPK3, CPK21 and CBL1-9/CIPK23) and an independent branch (OST1, CPK6 and CPK23) of ABA triggered signal transduction in guard cells appear to converge on SLAC1 and this multifaceted activation is counteracted by PP2Cs such as ABI1 (Geiger *et al.*, 2010; Scherzer *et al.*, 2012; Brandt *et al.*, 2015).

Mechanistically, Serine120 in the N-terminus of SLAC1 is only phosphorylated by OST1 (resulting in channel activation) but not modified by CPKs or CIPK23 (Geiger *et al.*, 2009b; Brandt *et al.*, 2012, 2015; Maierhofer *et al.*, 2014). By contrast, serine 59 represents a major target site for CPK6 that, however, is also crucial for SLAC1 activation by all three different types of SLAC1-activating kinase families (Brandt *et al.*, 2012; Maierhofer *et al.*, 2014). These findings suggest that full SLAC1 activation is very likely a result of concerted phosphorylation by different kinases that – not mutually exclusively – either target an overlapping pattern of p-sites or produce an alternative p-site pattern that may result in similar strong activation. The combined function of Ca^{2+}-dependent kinases and Ca^{2+}-independent OST1 very likely underlie the ABA- and Ca^{2+}-responsiveness of SLAC1.

In addition to the activation of SLAC1, both CPKs and CBL1-9/CIPK23 complexes (but not OST1) address SLAH3, a second plasma membrane anion channel expressed in guard cells (Geiger *et al.*, 2011; Maierhofer *et al.*, 2014). Hence, ABA seems to activate SLAC1 through direct interaction with OST1 and CPKs as well as CIPK23, whereas SLAH3 is only stimulated by CPKs and CIPK23 as well as by heteromerization with the silent subunit SLAH1 (Cubero-Font *et al.*, 2016).

Despite this tremendous progress, many important questions remain to be addressed. These involve for example, whether and how Ca^{2+}-dependent kinases and OST1 influence each other directly to fine-tune the activation of the anion channels or if phosphorylation of SLAC1 by one kinase *in vivo* influences its phosphorylatability by other kinases. Moreover, it will be important to investigate how the function of these kinases in ion channel regulation correlates with their potential role in adjusting gene regulation. In addition, our current knowledge about the regulation of SLAC/SLAH-type anion channels is predominantly derived from work on Arabidopsis guard cells. Whether and how S-type anion channels are regulated in other tissues and/or plant species is poorly understood and demands greater attention in future. The kinase-independent activation of SLAH3 via heteromerization with the silent subunit SLAH1 in the root stele of Arabidopsis is one example that suggests that the control of anion channel activity might be multifaceted and tissue/cell-specific (Cubero-Font *et al.*, 2016).

VI. Ca^{2+} signal integration into long-term ABA responses

Ca^{2+} and ABA serve as important mediators in plant stress responses including integration of environmental cues such as drought, cold and salinity (Cutler *et al.*, 2010; Raghavendra *et al.*, 2010). Nevertheless, with the notable exception of guard cells for most tissues it is still uncertain if and how these two signaling molecules influence each other. However, Ca^{2+}-responsive gene expression depends to a large extent on ABA-responsive cis elements including ABREs and its coupling element C-Repeat/Drought-Responsive Element (Kaplan *et al.*, 2006; Whalley *et al.*, 2011) pointing to a potential upstream interconnection or convergence of both signaling systems. ABREs belong to G-box promoter elements and have the consensus motif C/TACGT which is targeted by the ABA-responsive bZIP transcription factors AREBs/ABFs (Yoshida *et al.*, 2015).

ABA signaling can be considered as a three-step regulatory process involving the receptor complex, protein kinases acting as mediators, and targets such as transcription factors (Fig. 3). The heteromeric ABA receptor complex is formed by the ABA-binding regulatory component RCAR/PYR1/PYLs, the ABA receptor and the co-receptor PP2C, which is ABA-dependently inhibited in its catalytic activity by RCARs leading to the activation of ABA signaling (Ma *et al.*, 2009; Park *et al.*, 2009; Nishimura *et al.*, 2010) The integration of Ca^{2+} signals with ABA signaling could potentially occur at all three levels.

Recruitment of ABA receptors to the membrane has been found to be controlled by Ca^{2+} via the function of CAR proteins (C2-domain ABA related) a family of small proteins harboring a Ca^{2+}-binding C2 domain (Rodriguez *et al.*, 2014; Diaz *et al.*, 2016). Also, CDPKs and CBL/CIPKs provide a well-established link between Ca^{2+} signaling and ABA responses (Edel & Kudla, 2016). Members of both classes of protein kinases physically interact with ABA co-receptors and phosphorylate targets of the ABA signaling pathway (Ohta *et al.*, 2003; Zhu *et al.*, 2007; Lan *et al.*, 2011; Lynch *et al.*, 2012; Lyzenga *et al.*, 2013; Léran *et al.*, 2015). Altered expression of CDPKs and CBLs/CIPKs affect ABA sensitivity and, consequently, ABA responses. The CDPKs CPK4, CPK11 and CPK32 phosphorylate AREBs *in vitro* and may activate them *in vivo* (Choi *et al.*, 2005; Zhu *et al.*, 2007; Lynch *et al.*, 2012). CIPK11/PKS5 impinges on ABA responses by phosphorylating the bZIP transcription factor ABI5 (Zhou *et al.*, 2015). Phosphomimicking mutation of this phosphorylation site results in higher trans-activation activity of ABI5. Nevertheless, a direct effect unambiguously indicating a function of Ca^{2+} in this process is missing. Additionally, CIPK11 influences ABA signaling possibly via interaction with the homeodomain transcription factor AtHB6 that is also controlling ABA responses (Himmelbach *et al.*, 2002;

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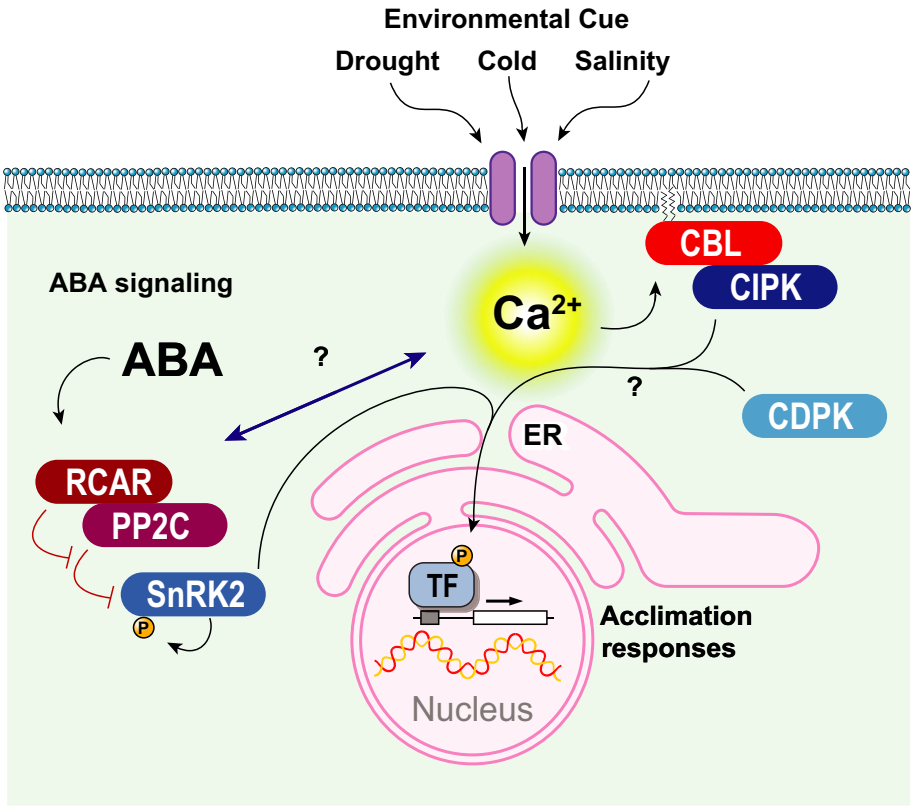

Fig. 3 Integration of environmental cues into abscisic acid (ABA) responses. Calcium ion release triggered by exogenous signals such as drought, cold and salinity is perceived by the Ca^{2+}-regulated protein kinase Ca^{2+}-dependent (CDPKs) and calcineurin B-like interacting (CIPKs) and associated CBLs. Members of CDPK and CIPK protein families may target ABA responsive bZIP transcription factors, can bind to the PP2C co-receptors and modulate ABA signaling, which make them prime candidates for integration of environmental cues into ABA signal transduction for habitat-optimized acclimation responses. The ABA receptor complex consisting of RCAR/PYR1/PYLs and the co-receptor PP2C is highlighted in red. The SNF1-related protein kinases (SnRK2) phosphorylate and activate ABA-responsive transcription factors.

Lechner *et al.*, 2011; Lumba *et al.*, 2014). However, direct *in vivo* evidence for the activation of ABA-related transcription factors via Ca^{2+}-regulated kinases is still missing. The Ca^{2+}-regulated protein kinases appear to share similarities in their PP2C interaction and target regulation with SnRK2s (Cutler *et al.*, 2010; Raghavendra *et al.*, 2010). ABA response regulatory SnRK2s such as OST1 bind to PP2Cs (Ng *et al.*, 2011; Xie *et al.*, 2012), and directly phosphorylate and activate ABA-responsive transcription factors (Kobayashi *et al.*, 2005; Ng *et al.*, 2011; Xie *et al.*, 2012; Fujita *et al.*, 2013). Recently emerging evidence suggests that SnRK2s, CDPKs and CIPKs appear to have an overlapping substrate spectrum but also specifically address distinct targets (Maierhofer *et al.*, 2014; Steinhorst & Kudla, 2014; Edel & Kudla, 2016).

Ca^{2+} signal integration by CDPKs and CIPKs might not be restricted to regulation of final targets of the ABA response cascade. It was noted early on in the era of abiotic stress research that CIPK24/SOS2 physically interacted with the ABA co-receptors ABI1 and ABI2 (Ohta *et al.*, 2003). Likewise, CPK11 associated with ABI1 and might compete with SnRK2s for co-receptor interaction (Lynch *et al.*, 2012). Analysis of a quadruple Arabidopsis mutant deficient in CPK5,6,11,23 revealed impaired ABA signaling and emphasized the interdependence of CPKs and SnRK2s for a functional ABA signal pathway (Brandt *et al.*, 2015). Although the molecular mechanisms and consequences of these interactions are still not resolved, it appears conceivable that the Ca^{2+}-responsive modulation of ABA receptor components and associated SnRK2s provide an elegant means to fine-tune sensitivity of the ABA signal pathway and consequently the extent of the ABA response.

Abiotic stress scenarios such as drought or flooding vary in different habitats forcing plant ecotypes to respond differently towards environmental cues that optimize survival and performance. CDPKs and CIPKs turn out to be prime candidates for habitat-optimized acclimation responses by integrating environmental stimuli via endogenous Ca^{2+}-release into ABA signal transduction (Fig. 3). An interesting notion in this regard is the fact that CBL/CIPKs specifically diversified during land plant evolution and now form an intricate network in extant higher plants involved in various aspects of stress signaling (Edel & Kudla, 2015; Beckmann *et al.*, 2016).

VII. Integration of Ca^{2+} and hormone signaling through dynamic complex modulation of the CCaMK/CYCLOPS complex

Plant roots engage with arbuscular mycorrhizal fungi that deliver nutrients including phosphate and with nitrogen-fixing bacteria in the root nodule symbiosis that provides independence from other nitrogen sources (Gutjahr & Parniske, 2013; Oldroyd, 2013). Ca^{2+} signaling is a hallmark of both symbioses. Microbial signaling molecules comprising N-acetyl-glucosamine tetra- or pentamers with a variety of specificity-determining chemical modifications trigger symbiotic responses of the host cells. The fastest responses detectable in root cells include Ca^{2+} influx into the cytoplasm and regular Ca^{2+} oscillations referred to as 'Ca^{2+}-spiking' in the plant nucleus (Chabaud *et al.*, 2011; Sieberer *et al.*, 2012; Sun *et al.*, 2015). In legumes, Ca^{2+}-spiking is initiated upon binding of lipo-chito-oligosaccharides by receptor complexes comprising LysM-

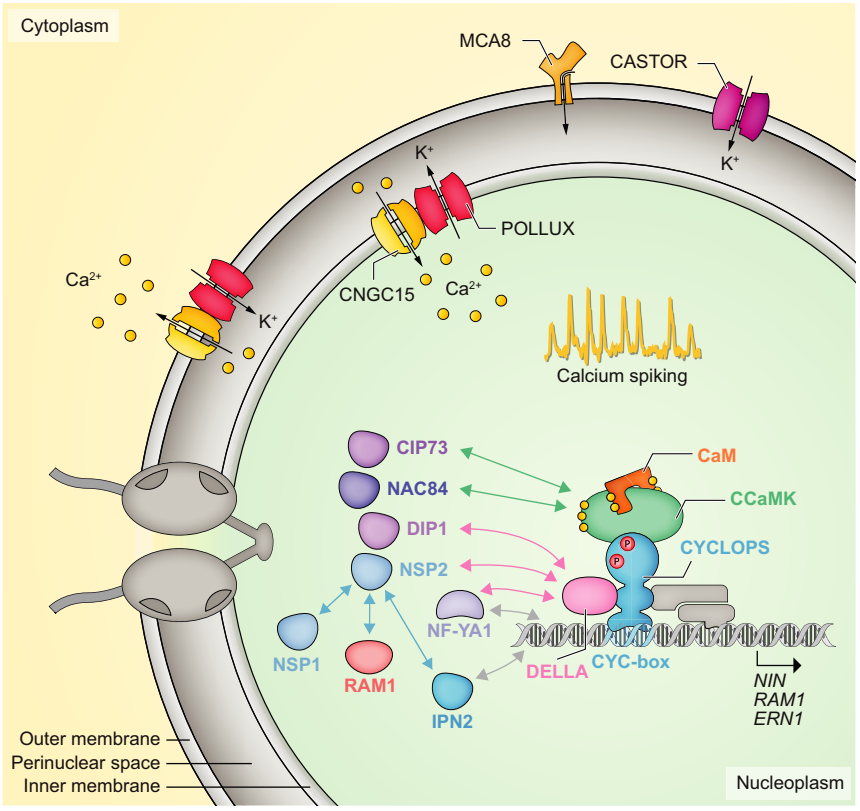

Fig. 4 The calcium-spiking machinery and the nuclear calcium signaling decoding complex. The generation of Ca^{2+} spikes in the nucleus requires the coordinated activity of a set of K^+ channels (Charpentier *et al.*, 2008), Ca^{2+} channels of the cyclic-nucleotide-gated channel 15 (CNGC15) subfamily (Charpentier *et al.*, 2016) and Ca^{2+} transporters (MCA8; Capoen *et al.*, 2011) at the inner and outer membrane of the nuclear envelope, which is thought to act as the Ca^{2+} store releasing Ca^{2+} during spiking. The interaction between CNGC15 and the K^+ channel POLLUX (DMI1) at the nuclear envelope provided clues about the molecular mechanisms involved in the regulation of the channels (Charpentier *et al.*, 2016). A decoder of the Ca^{2+} spiking signature in the nucleus comprises calmodulin (CaM), CCaMK and its phosphorylation target CYCLOPS (Singh *et al.*, 2014). This decoding complex emerges as a dynamic signaling hub. The experimental support for the interaction between CCaMK and CIP73 (Kang *et al.*, 2011), CCaMK and NAC84 (Zhu *et al.*, 2016), CYCLOPS and DELLA (Jin *et al.*, 2016; Pimprikar *et al.*, 2016), DELLA and NSP2 (Fonouni-Farde *et al.*, 2016; Jin *et al.*, 2016), DELLA and NF-YA1 (Fonouni-Farde *et al.*, 2016), DELLA and DIP1 (Yu *et al.*, 2014), RAM1 and NSP2 (Gobbato *et al.*, 2012), and IPN2 and NSP2 (Kang *et al.*, 2011) differs significantly. Nevertheless, the overall accumulating evidence pinpoints the CCaMK/CYCLOPS complex as a hub recruiting a variety of interaction partners which probably mediate signaling specificity thus leading to the activation of distinct target genes.

receptor like kinases and the SYMBIOSIS-RECEPTOR LIKE KINASE (SYMRK). A main function of SYMRK is probably the activation of Ca^{2+}-spiking because *symrk* mutants can be complemented with a dominant active version of CCaMK (Antolín-Llovera *et al.*, 2014). This chapter focuses on the molecular mechanisms generating and interpreting repetitive Ca^{2+} signatures.

The generation of Ca^{2+} spikes in the nucleus requires the coordinated activity of a set of K^+ channels, Ca^{2+} channels and Ca^{2+} transporters at the inner and outer membrane of the nuclear envelope, which is thought to act as the Ca^{2+} store releasing Ca^{2+} during spiking (Charpentier *et al.*, 2008, 2016; Capoen *et al.*, 2011). Members of the cyclic nucleotide gated channel 15 (CNGC15) subfamily recently emerged as Ca^{2+} channels mediating this release (Charpentier *et al.*, 2016). The interaction between CNGC15 and the K^+ channel DMI1 at the nuclear envelope provided clues about the molecular mechanisms involved in the regulation of the channels (Charpentier *et al.*, 2016). However, the mechanism by which Ca^{2+}-spiking in the nucleus is activated upon ligand-binding by receptors at the plasma-membrane is still obscure, despite the identification of a number of interaction partners of their kinase domains (Antolín-Llovera *et al.*, 2014).

A nuclear localized complex comprising the CCaMK and the DNA-binding transcription factor CYCLOPS represents the central signaling hub for both nodule organogenesis and microbial infection (Fig. 4). CCaMK is a central regulator of symbiotic development as deregulated versions lead to spontaneous nodule organogenesis in the absence of rhizobia and fully restore symbiosis in mutants impaired in Ca^{2+} spiking (Gleason *et al.*, 2006; Tirichine *et al.*, 2006; Madsen *et al.*, 2010; Hayashi *et al.*, 2014). A major turning point in our understanding of the mechanism of CCaMK activity by Ca^{2+} is based on (1) a refined structural model, which revealed a stabilizing role of phosphate residue of threonine 271 of the autoinhibited state of *Medicago truncatula* CCaMK, and (2) the observation that the EF-hands of CCaMK bind Ca^{2+} even at basal concentrations in the cell (Miller *et al.*, 2013). According to the resulting model, basal concentrations of Ca^{2+} activate autophosphorylation of CCaMK at T271 and this leads to autoinhibition. Calmodulin binding not only disrupts the helical

structure of the inhibition domain thus activating the kinase, but also inhibits the phosphorylation of T271 (Miller *et al.*, 2013). Autophosphorylation at positions 343 and 344 leads to sterical hindrance of calmodulin binding and thus an inhibited protein (Liao *et al.*, 2012; Routray *et al.*, 2013). CYCLOPS interacts with CCaMK also in the absence of Ca^{2+} and is a phosphorylation target of CCaMK (Yano *et al.*, 2008). CYCLOPS versions carrying phosphomimetic serine to aspartate replacements at two of the sites phosphorylated by CCaMK lead to spontaneous nodule development in the absence of rhizobia manifesting the central role of the complex in controling transcriptional regulation during symbiosis signaling (Singh *et al.*, 2014).

CCaMK/CYCLOPS activates the transcription of at least three genes with a central role in root symbiosis: *RAM1* is indispensable for arbuscule development, *ERN1* is required for bacterial infection thread formation and *NIN* has a dual role in nodule organogenesis and bacterial infection thread formation (Singh *et al.*, 2014; Pimprikar *et al.*, 2016; Cerri *et al.*, 2017).

The negative interference of GA signaling with nodulation and AM development recently could be mechanistically explained by the physical interaction between CYCLOPS with DELLA (Maekawa *et al.*, 2009; Floss *et al.*, 2013; Takeda *et al.*, 2015; Fonouni-Farde *et al.*, 2016; Jin *et al.*, 2016; Pimprikar *et al.*, 2016). DELLA is degraded upon GA binding and it is hypothetically possible that this recruitment of E3 ligases by DELLA leads to degradation of the entire CCaMK/CYLCOPS complex. In the context of complex degradation, the interaction of CCaMK with a protein containing a scythe-N ubiquitin-like domain may deserve further investigation (Davière *et al.*, 2008; Kang *et al.*, 2011). Recently, the number of GRAS proteins involved in the regulation of symbiotic responses of the root has increased (Gobbato *et al.*, 2012; Yu *et al.*, 2014; Fonouni-Farde *et al.*, 2016; Pimprikar *et al.*, 2016). Because GRAS proteins have been shown to interact with DELLA which is itself a GRAS protein, it will be interesting to investigate which of these are, at least transiently, components of a larger CCaMK/CYCLOPS complex.

Genetic and physiological evidence indicates that ABA interferes at at least two independent steps in symbiotic development. Application of high ABA concentrations instantly inhibits Ca^{2+} spiking in *Medicago* root hairs but low concentrations quantitatively promote arbuscular mycorrhiza (Ding *et al.*, 2008; Charpentier *et al.*, 2014). In addition an ABA-insensitive mutant of *Lotus japonicus* showed reduced nodulation despite an unaltered spiking behavior, thus revealing an additional ABA-sensitive step (Ding *et al.*, 2008). The recent description of the phosphorylation of a NAC transcription factor by CCaMK that regulates ABA-induced antioxidant defense in maize opens not only a new physiological role of CCaMK outside symbiosis, but also the possibility that NAC transcription factors may also work at the interface of ABA and symbiosis signaling (Zhu *et al.*, 2016).

Ca^{2+} spiking and the CCaMK/CYCLOPS complex regulate multiple distinct cellular responses, including lateral organ development (cell division) and the formation of intracellular accommodation structures for bacteria and fungi (Fig. 4). This imposes a major open question: How is transcriptional specificity of the CCaMK/CYCLOPS complex achieved? Such a mechanism may involve dynamic modular interactions with yet unknown proteins that dictate the DNA-binding sequence-specificity of the complex. However, the molecular players and the mechanisms that determine the complex composition are still only vaguely understood.

VIII. Ca^{2+} signaling in mitochondria and chloroplasts

The chloroplast is intimately linked to the cellular Ca^{2+} network. Ca^{2+} uptake into the chloroplast occurs in the light whereas Ca^{2+} is released into the cytosol in the dark (Stael *et al.*, 2012). Within the chloroplast, Ca^{2+} accumulation in the stroma was reported to occur during the transition from light to dark (Johnson *et al.*, 1995; Sai & Johnson, 2002; Sello *et al.*, 2016). Recent analyses also revealed that chloroplast displayed autonomous Ca^{2+} signatures in the stroma (Loro *et al.*, 2016). Moreover, emerging evidence indicates that chloroplasts contribute to cellular Ca^{2+}-homeostasis and signaling and identified the chloroplast Ca^{2+} sensor protein (CAS) as a central component in these processes (Nomura *et al.*, 2007, 2012; Weinl *et al.*, 2008; Petroutsos *et al.*, 2011; Hochmal *et al.*, 2015). In Arabidopsis, CAS crucially functions in the generation of cytosolic and stromal Ca^{2+} transients as well as in the modulation of MAPK signaling (Guo *et al.*, 2016). Moreover, it is involved in photo-acclimation in *Chlamydomonas reinhardtii* by contributing to the control of expression of LHCSR3 and to the expression of proteins related to the carbon-concentrating mechanism (Petroutsos *et al.*, 2011; Wang *et al.*, 2016b). Also the activity of the cyclic electron flow (CEF) is modulated via CAS and the availability of Ca^{2+} in *C. reinhardtii* and in Arabidopsis (Terashima *et al.*, 2012; Takahashi *et al.*, 2013; Strand *et al.*, 2015). Recently, a calredoxin (CRX) that contains four EF-hands and functions as a Ca^{2+}-dependent thioredoxin has been found to crucially function in the modulation of electron transfer and as potential electron donor for chloroplast 2-Cys peroxiredoxins in *C. reinhardtii* (Hochmal *et al.*, 2016). CRX represents the first calmodulin-like protein identified in the chloroplast. However, various potential calmodulin-dependent processes have been described already for chloroplasts (Hochmal *et al.*, 2015). AtTPK3 is another EF-hand-containing protein: this transmembrane thylakoid protein is a two-pore potassium channel that contributes to the regulation of photosynthetic electron transfer by modulating the composition of the proton motive force through ion counterbalancing (Carraretto *et al.*, 2013). Ca^{2+} is required for AtTPK3 function, facilitating K^+ transport out of the lumen into the stroma. Notably Ca^{2+} is also involved in regulation of metabolic enzymes, such as those of the Calvin cycle (Hochmal *et al.*, 2015). Besides, regulatory functions, Ca^{2+} is a structural component of photosystem II (PSII) and needed for oxygen evolution. PSII possesses a metal cluster containing four mixed valence manganese ions, a Ca^{2+} ion, and five oxo ligands (Mn_4CaO_5 cluster) for efficient oxidation of H_2O. In conclusion, chloroplast Ca^{2+} modulates metabolic reactions in the organelle, is involved in the regulation of the oxygen-evolving capacity of PSII and governs properties of photosynthetic electron transfer as well as the photo-protection mechanism (Ferreira *et al.*, 2004; Guskov *et al.*, 2009; Umena *et al.*, 2011). Moreover, it appears to be a central element in retrograde signaling and in the modulation of cytosolic Ca^{2+} transients.

Besides chloroplasts, also mitochondria have recently emerged as new contributors to plant Ca^{2+} signaling (Rizzuto *et al.*, 2012; Stael *et al.*, 2012). Most research in this field, however, has been conducted in animals in which mitochondria have been found to influence cytosolic Ca^{2+} signals (Wagner *et al.*, 2016). Phylogenetic analyses of the contributing proteins indicated noticeable similarities between the animal and plant Ca^{2+} uptake machinery. Because the outer mitochondrial membrane is permeable for Ca^{2+}, a similar $[Ca^{2+}]$ is shared by the cytosol and the mitochondrial intermembrane space. However, for import into the matrix, specialized Ca^{2+} uniporter complexes (MCUC) have been identified in animals and homologs were found in plants (Foskett & Philipson, 2015; Teardo *et al.*, 2017). In particular, the pore-forming MCU (mitochondrial Ca^{2+} uniporter) is well conserved and shows a similar regulatory mechanism involving additional complex proteins (Baughman *et al.*, 2011; Foskett & Philipson, 2015). One of this regulatory proteins is provided by the mitochondrial Ca^{2+} uptake 1 protein (MICU) (Wagner *et al.*, 2015; Teardo *et al.*, 2017). MICUs harbor EF-hand domains and could sense matrix $[Ca^{2+}]$ as a Ca^{2+} sensor, restricting its uptake upon Ca^{2+} saturation. MICU knock-out plants show a higher mitochondrial Ca^{2+} content indicating that MICU is involved in restricting Ca^{2+} uptake.

This added complexity, emerging from the contribution of endosymbiotic organelles towards cytosolic Ca^{2+} signals, has to be considered for future models of cellular Ca^{2+} signaling.

IX. A view beyond recent advances in Ca^{2+} imaging

In order to decipher how Ca^{2+} mediates the above-mentioned signaling processes, Ca^{2+} decoder and responder expression levels and their biochemical activities need to be correlated with cellular Ca^{2+} signals in a spatio-temporal fashion. Although stimulus-induced $[Ca^{2+}]$ changes are well described (Evans *et al.*, 2001; Scrase-Field & Knight, 2003; White & Broadley, 2003; Batistič & Kudla, 2012), quantitative data at high spatio-temporal resolution are still limited. However, during the past years tools for *in vivo* Ca^{2+} quantification have improved dramatically.

Ca^{2+} signal analyses can be performed using fluorescent dyes or genetically encoded Ca^{2+} indicators (GECIs). GECIs were categorized into bioluminescent and fluorescent indicators (Pérez Koldenkova & Nagai, 2013). Bioluminescent GECIs use either aequorin or aequorin fused to a fluorescent protein (FP) (Shimomura *et al.*, 1962). Such indicators, for example BRAC or Nano-lantern, enable Ca^{2+} analyses via bioluminescence resonance energy transfer (BRET; Saito *et al.*, 2010, 2012). Fluorescent GECIs consist either of a single circular permutated FP with GCaMP and Pericam as the prototypes or of a dual-FP fluorescence resonance energy transfer (FRET)-pair with cameleon as the founder GECI (Miyawaki *et al.*, 1997; Nagai *et al.*, 2001; Nakai *et al.*, 2001). All of these GECIs, except of aequorin, use the Ca^{2+}-dependent CaM-M13 interaction as Ca^{2+} sensory module (Pérez Koldenkova & Nagai, 2013). FRET-based GECIs, based on the Ca^{2+} binding of troponin C (TN-L15 and Twitch variants) are also available (Heim & Griesbeck, 2004; Thestrup *et al.*, 2014). An overview about GECIs has been published previously (Pérez Koldenkova & Nagai, 2013). More recent developments include GECIs based on dimerization-dependent FP (ddFP) exchange, single-FP GECIs normalized by fusion to a reference FP (Waadt *et al.*, 2017), and photoactivatable and photoconvertible GECIs (Hoi *et al.*, 2013; Ai *et al.*, 2015; Berlin *et al.*, 2015; Ding *et al.*, 2015).

In plants, the majority of studies used the FRET-based yellow cameleon YC3.6 as fluorescent GECI, including subcellular targeted variants (Nagai *et al.*, 2004; Costa & Kudla, 2015). However, simultaneous comparison of YC3.6 with the red emitting single-FP R-GECO1 revealed an increased sensitivity and signal resolution of R-GECO1 in Arabidopsis (Zhao *et al.*, 2011; Keinath *et al.*, 2015). Single-FP GECIs are to a certain degree sensitive to pH changes (Zhao *et al.*, 2011; Pérez Koldenkova & Nagai, 2013; Keinath *et al.*, 2015). Therefore, in experiments where dramatic pH changes appear, the less pH-sensitive FRET-based GECIs are preferred. Moreover, due to the increasing number of distinct Ca^{2+} reporter proteins, it is now possible to consider other biochemical properties such as Ca^{2+} affinity and Hill coefficient when choosing the most suitable reporter protein for the desired experiment (Costa & Kudla, 2015; Waadt *et al.*, 2017). The major advantage of single-FP GECIs is that they occupy a smaller range of the light spectrum, enabling multicolor analyses, for example to analyze Ca^{2+} in different compartments and cell-types, or to image multiple parameters at the same time (Zhao *et al.*, 2011; Rose *et al.*, 2014). For multiparameter imaging, red GECIs (variants of R-GECO1 and R-CaMP1; (Zhao *et al.*, 2011; Akerboom *et al.*, 2013) are best suited, as they can be combined with FRET-based reporters and other indicators that emit fluorescence at blue and green wavelengths.

A most exciting example for multicolor Ca^{2+} imaging was the simultaneous analysis of Ca^{2+} transients during double fertilization. Here, Ca^{2+} signals in pollen tubes were visualized using R-GECO1, whereas synergid Ca^{2+} concentrations were monitored using YC3.6 (Ngo *et al.*, 2014). During double fertilization synergids responded with $[Ca^{2+}]_{cyt}$ transients upon pollen tube arrival, which also exhibited tip-focused $[Ca^{2+}]_{cyt}$ transients. Pollen tube bursting and discharge resulted in a short $[Ca^{2+}]$ burst in the pollen tube tip and induced a $[Ca^{2+}]_{cyt}$ transient in egg and central cells. A final $[Ca^{2+}]_{cyt}$ transient was observed after successful fertilization (Iwano *et al.*, 2012; Denninger *et al.*, 2014; Hamamura *et al.*, 2014; Ngo *et al.*, 2014). Ca^{2+} signals, likely mediated by glutamate receptor-like channels appeared to be important for the self-incompatibility response (Iwano *et al.*, 2015). The sperm delivery process during double fertilization appeared to be coordinated by the FERONIA signaling pathway (Ngo *et al.*, 2014). Mechanical forces most likely trigger Ca^{2+} signals during pollen tube bursting, and FERONIA is also involved in Ca^{2+} responses to mechanical forces in roots (Shih *et al.*, 2014). Interestingly, it was recently reported that Ca^{2+} signaling processes at the vacuolar membrane are crucial for pollen tube growth and plant fertility, pointing to a so far not appreciated additional role of Ca^{2+} in these processes (Steinhorst *et al.*, 2015). The first example for multiparameter analyses was the simultaneous visualization of Ca^{2+} and ABA (Waadt *et al.*, 2017). ABA-induced Ca^{2+} transients are well known in guard cells (Allen *et al.*, 1999; Islam *et al.*, 2010).

In roots, however, ABA did not trigger rapid Ca^{2+} responses. Models for the interdependence of Ca^{2+} and ABA, reactive oxygen species (ROS) and electrical signals are a focus of current discussion (Steinhorst & Kudla, 2013, 2014; Gilroy *et al.*, 2014; Romeis & Herde, 2014; Choi *et al.*, 2016; Edel & Kudla, 2016). Ca^{2+} signals are also coordinated with changes in cytoplasmic and apoplastic pH (Monshausen *et al.*, 2009). The right choice of fluorescent indicator combinations to visualize these molecules will advance our view on their coordinated action in plants.

Fluorescent GECIs have the potential to not only resolve [Ca^{2+}] changes at high spatio-temporal resolution, but also, in combination with genetics, to deliver mechanistic insights into Ca^{2+} signaling processes. Knowledge about the identity of Ca^{2+} channels that mediate certain Ca^{2+} responses is still limited, and GECIs can be used as tools for their identification and characterization. An exciting new avenue of research is established by the combination of Ca^{2+} reporter proteins with reporter proteins for other second messengers (like ROS) or cellular parameters (like pH) that most likely will enable multiparameter lifetime imaging.

X. Modeling approaches in Ca^{2+} signaling

In order to integrate diverse experimental findings and gain a more holistic understanding of Ca^{2+} signaling and decoding of Ca^{2+} signals in Arabidopsis, computational modeling of the respective processes in an adequate approach. For a number of years, such models have been the basis that allowed understanding the mechanisms of Ca^{2+} signals in vertebrate cells. Here, different computational models have been built that allow the investigation of different properties of the signals (Schuster *et al.*, 2002). Thus, models also investigated the encoding and decoding of the information carried by the Ca^{2+} signals – mostly oscillations – relying on models that encompassed the current knowledge with respect to molecular mechanisms within the cell. Encoding of signals in vertebrate cells has been attributed mostly to specific receptor properties such as feedback mediated phosphorylation (e.g. via PKC) which leads to a deactivation of the receptor or the receptor coupled opening of Ca^{2+} channels. The decoding of the signals has been studied by coupling very specific models, for example of CaM Kinase (Dupont & Goldbeter, 1998) to the models of Ca^{2+} oscillations or by employing more generic equations describing the activity of Ca^{2+} activated proteins, again coupled with a Ca^{2+} signaling module (Larsen *et al.*, 2004). One such study combined actual experimental recordings with a computational model describing Ca^{2+}-dependent enzymes investigating the information content of different time-series (Pahle *et al.*, 2008). However, current literature on modeling Ca^{2+} signaling in plants is still rather limited. A few groups have set up rather generic models of Ca^{2+} signaling (no biochemical details) in order to understand, for example, signal spread and the impact of physical properties like temperature. A vertebrate model by Li and Rinzel has been adapted to plant conditions to study Ca^{2+} signaling in *Nicotiana tabacum* cell culture (Martins *et al.*, 2013; Mrozek *et al.*, 2013). In addition, also using a simplified core model for Ca^{2+} signaling, first studies on encoding and decoding have been performed (Bose *et al.*, 2011). Finally, detailed and extensive modeling studies have been performed specifically for guard cells – also with respect to Ca^{2+} signaling. However, despite the highly interesting nature of the latter studies, these do not allow direct access to the model equations, because these are not given in the respective articles, but are available only as hard-wired information in the OnGuard software (Chen *et al.*, 2012; Hills *et al.*, 2012; Wang *et al.*, 2012; Blatt *et al.*, 2014; Minguet-Parramona *et al.*, 2016). So without working with this specific software, it is not possible to readily reuse kinetic information in a different cellular context or even learn from the employed kinetics.

Computational modeling is commonly based on ordinary differential equations that represent the sum of the rates of individual reactions affecting the individual chemical species. Due to the increasing relevance of such models, specific software tailored for the needs of computational biologists has been developed and is available as open source. One of these software projects is the software Copasi (Hoops *et al.*, 2006) which is platform-independent and used in many groups around the world. This software offers support for model set-up, parameter estimation based on experimental data, as well as several ways to computationally analyze the results. The resulting models can be exported as standard SBML files and thus reused or re-analyzed in other software and models (Hucka *et al.*, 2003).

In order to advance quantitative modeling, there is the urgent need that the community of Ca^{2+} signaling researchers gathers quantitative data about the Ca^{2+}-related properties of their protein(s) of interest. Filling this gap would allow application of modeling approaches across a broader scale which would, without any question, represent a timely approach to refine our understanding of Ca^{2+} signaling processes in plants.

XI. Conclusions: Ca^{2+} signaling a still young blooming field of plant research

Over the past decade, research on plant Ca^{2+} signaling has itself established as a firm research filed that is driven by a growing and enthusiastic community of researchers. Quite excitingly, we have witnessed enormous progress in the techniques to investigate the dynamics of Ca^{2+} signaling for which an impressive toolset of reporter proteins has now become available. It has already become apparent that this allows an investigation of Ca^{2+} dynamics in unpreceded spatiotemporal resolution and the (re-)discovery of Ca^{2+} waves is only one example of such achievements.

The combination of high-resolution Ca^{2+} imaging with high-throughput (reverse) genetics screens should allow identification of components which crucially contribute to Ca^{2+} dynamics. It appears safe to predict that the emerging capabilities of performing multicolor imaging of distinct 'Ca^{2+} pools' and multiparameter imaging interconnecting Ca^{2+} alterations with the dynamics of hormones or other second messengers will revolutionize plant cell biology.

Of pre-eminent importance for advancing our understanding of Ca^{2+} signaling was the identification of complete Ca^{2+} decoder-target modules. An emerging common theme here is that multiple Ca^{2+}-regulated kinases (either from one family or a combination of CDPKs and CIPKs) often in combination with Ca^{2+}-independent

kinases and their counteracting PP2Cs share common target proteins. It can be expected that the number of such 'model modules' will increase rapidly in the near future and will allow detailed investigations of the underlying molecular principles and the coordination between different 'response modules'. Important challenges to be addressed here involve the identification and causal interconnection of Ca^{2+} channels and counteracting Ca^{2+} pumps that address these response modules. Also, the quantitative elucidation of regulatory parameters (such as Ca^{2+} concentration and enzyme kinetics) needs to be advanced and should be incorporated into modeling approaches. In any case, research on Ca^{2+} signaling currently is a captivating field with rapid progress and exciting new insights that await to be explored.

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References

Ai M, Mills H, Kanai M, Lai J, Deng J, Schreiter E, Looger L, Neubert T, Suh G. 2015. Green-to-red photoconversion of GCaMP. *PLoS ONE* **10**: e0138127.

Akerboom J, Carreras Calderón N, Tian L, Wabnig S, Prigge M, Tolö J, Gordus A, Orger MB, Severi KE, Macklin JJ *et al.* 2013. Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. *Frontiers in Molecular Neuroscience* **6**: 2.

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. *The EMBO Journal* **20**: 1051–1063.

Allen GJ, Kwak JM, Chu SP, Llopis J, Tsien RY, Harper JF, Schroeder JI. 1999. Cameleon calcium indicator reports cytoplasmic calcium dynamics in Arabidopsis guard cells. *Plant Journal* **19**: 735–747.

Antolín-Llovera M, Petutsching EK, Ried MK, Lipka V, Nürnberger T, Robatzek S, Parniske M. 2014. Knowing your friends and foes – plant receptor-like kinases as initiators of symbiosis or defence. *New Phytologist* **204**: 791–802.

Astegno A, Bonza MC, Vallone R, La Verde V, D'Onofrio M, Luoni L, Molesini B, Dominici P. 2017. Arabidopsis calmodulin-like protein CML36 is a calcium (Ca^{2+}) sensor that interacts with the plasma membrane Ca^{2+}-ATPase isoform ACA8 and stimulates its activity. *Journal of Biological Chemistry* **292**: 15 049–15 061.

Batistic O, Kudla J. 2009. Plant calcineurin B-like proteins and their interacting protein kinases. *Biochimica et Biophysica Acta* **1793**: 985–992.

Batistič O, Kudla J. 2012. Analysis of calcium signaling pathways in plants. *Biochimica et Biophysica Acta* **1820**: 1283–1293.

Batistic O, Rehers M, Akerman A, Yalovsky S, Kudla J, Batistič O, Rehers M, Akerman A, Schlücking K, Steinhorst L *et al.* 2012. S-acylation-dependent association of the calcium sensor CBL2 with the vacuolar membrane is essential for proper abscisic acid responses. *Cell Research* **22**: 1155–1168.

Batistic O, Sorek N, Schültke 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.

Batistic O, Waadt R, Steinhorst L, Held K, Kudla J. 2010. CBL-mediated targeting of CIPKs facilitates the decoding of calcium signals emanating from distinct cellular stores. *Plant Journal* **61**: 211–222.

Bauer H, Ache P, Lautner S, Fromm J, Hartung W, Al-Rasheid KAS, Sonnewald S, Sonnewald U, Kneitz S, Lachmann N *et al.* 2013. The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. *Current Biology* **23**: 53–57.

Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL *et al.* 2011. Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. *Nature* **476**: 341–345.

Beckmann L, Edel KH, Batistič O, Kudla J. 2016. A calcium sensor – protein kinase signaling module diversified in plants and is retained in all lineages of Bikonta species. *Scientific Reports* **6**: 31645.

Behera S, Long Y, Schmitz-Thom I, Wang X-P, Zhang C, Li H, Steinhorst L, Manishankar P, Ren X-L, Offenborn JN *et al.* 2017. Two spatially and temporally distinct Ca^{2+} signals convey *Arabidopsis thaliana* responses to K^+ deficiency. *New Phytologist* **213**: 739–750.

Berlin S, Carroll EC, Newman ZL, Okada HO, Quinn CM, Kallman B, Rockwell NC, Martin SS, Lagarias JC, Isacoff EY. 2015. Photoactivatable genetically encoded calcium indicators for targeted neuronal imaging. *Nature Methods* **12**: 852–858.

Berridge MJ, Bootman MD, Roderick HL. 2003. Calcium signalling: dynamics, homeostasis and remodelling. *Nature Reviews. Molecular Cell Biology* **4**: 517–529.

Berridge MJ, Lipp P, Bootman MD. 2000. The versatility and universality of calcium signalling. *Nature Reviews. Molecular Cell Biology* **1**: 11–21.

Blatt MR, Wang Y, Leonhardt N, Hills A. 2014. Exploring emergent properties in cellular homeostasis using OnGuard to model K^+ and other ion transport in guard cells. *Journal of Plant Physiology* **171**: 770–788.

Bose J, Pottosin II, Shabala SS, Palmgren MG, Shabala S. 2011. Calcium efflux systems in stress signaling and adaptation in plants. *Frontiers in Plant Science* **2**: 85.

Boudsocq M, Droillard M-J, Regad L, Laurière C. 2012. Characterization of Arabidopsis calcium-dependent protein kinases: activated or not by calcium? *The Biochemical Journal* **447**: 291–299.

Brandt B, Brodsky DE, Xue S, Negi J, Iba K, Kangasjarvi J, Ghassemian M, Stephan AB, Hu H, Schroeder JI *et al.* 2012. Reconstitution of abscisic acid activation of SLAC1 anion channel by CPK6 and OST1 kinases and branched ABI1 PP2C phosphatase action. *Proceedings of the National Academy of Sciences, USA* **109**: 10593–10598.

Brandt B, Munemasa S, Wang C, Nguyen D, Yong T, Yang PG, Poretsky E, Belknap TF, Waadt R, Alemán F *et al.* 2015. Calcium specificity signaling mechanisms in abscisic acid signal transduction in Arabidopsis guard cells. *eLife* **4**: 1–25.

Capoen W, Sun J, Wysham D, Otegui MS, Venkateshwaran M, Hirsch S, Miwa H, Downie JA, Morris RJ, Ané J-M *et al.* 2011. Nuclear membranes control symbiotic calcium signaling of legumes. *Proceedings of the National Academy of Sciences, USA* **108**: 14 348–14 353.

Carraretto L, Formentin E, Teardo E, Checchetto V, Tomizioli M, Morosinotto T, Giacometti GM, Finazzi G, Szabó I. 2013. A thylakoid-located two-pore K^+ channel controls photosynthetic light utilization in plants. *Science* **342**: 114–118.

Cerri MR, Wang Q, Stolz P, Folgmann J, Frances L, Katzer K, Li X, Heckmann AB, Wang TL, Downie JA *et al.* 2017. The *ERN1* transcription factor gene is a target of the CCaMK/CYCLOPS complex and controls rhizobial infection in *Lotus japonicus*. *New Phytologist* **215**: 323–337.

Chabaud M, Genre A, Sieberer BJ, Faccio A, Fournier J, Novero M, Barker DG, Bonfante P. 2011. Arbuscular mycorrhizal hyphopodia and germinated spore exudates trigger Ca^{2+} spiking in the legume and nonlegume root epidermis. *The New Phytologist* **189**: 347–355.

Charpentier M, Bredemeier R, Wanner G, Takeda N, Schleiff E, Parniske M. 2008. *Lotus japonicus* CASTOR and POLLUX are ion channels essential for perinuclear calcium spiking in legume root endosymbiosis. *Plant Cell* **20**: 3467–3479.

Charpentier M, Sun J, Vaz Martins T, Radhakrishnan GV, Findlay K, Soumpourou E, Thouin J, Véry A-A, Sanders D, Morris RJ *et al.* 2016. Nuclear-localized cyclic nucleotide-gated channels mediate symbiotic calcium oscillations. *Science* **352**: 1102–1105.

Charpentier M, Sun J, Wen J, Mysore KS, Oldroyd GED. 2014. Abscisic acid promotion of arbuscular mycorrhizal colonization requires a component of the PROTEIN PHOSPHATASE 2A complex. *Plant Physiology* **166**: 2077–2090.

Chaves-Sanjuan A, Sanchez-Barrena MJ, Gonzalez-Rubio JM, Moreno M, Ragel P, Jimenez M, Pardo JM, Martinez-Ripoll M, Quintero FJ, Albert A. 2014. Structural basis of the regulatory mechanism of the plant CIPK family of protein kinases controlling ion homeostasis and abiotic stress. *Proceedings of the National Academy of Sciences, USA* **111**: E4532–E4541.

Chen Z-H, Hills A, Bätz U, Amtmann A, Lew VL, Blatt MR. 2012. Systems dynamic modeling of the stomatal guard cell predicts emergent behaviors in transport, signaling, and volume control. *Plant Physiology* **159**: 1235–1251.

Cheong YH, Pandey GK, Grant JJ, Batistic O, Li L, Kim B-G, Lee S-C, 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 Journal* **52**: 223–239.

Choi W-G, Hilleary R, Swanson SJ, Kim S-H, Gilroy S. 2016. Rapid, long-distance electrical and calcium signaling in plants. *Annual Review of Plant Biology* **67**: 287–307.

Choi H, Park H-J, Park JH, Kim S, Im M-Y, Seo H-H, Kim Y-W, Hwang I, Kim SY. 2005. Arabidopsis calcium-dependent protein kinase AtCPK32 interacts with ABF4, a transcriptional regulator of abscisic acid-responsive gene expression, and modulates its activity. *Plant Physiology* **139**: 1750–1761.

Clapham DE. 2007. Calcium signaling. *Cell* **131**: 1047–1058.

Costa A, Kudla J. 2015. Colorful insights: advances in imaging drive novel breakthroughs in Ca^{2+} signaling. *Molecular Plant* **8**: 352–355.

Costa A, Luoni L, Marrano CA, Hashimoto K, Köster P, Giacometti S, De Michelis MI, Kudla J, Bonza MC. 2017. Ca^{2+}-dependent phosphoregulation of the plasma membrane Ca^{2+}-ATPase ACA8 modulates stimulus-induced calcium signatures. *Journal of Experimental Botany* **68**: 3215–3230.

Cubero-Font P, Maierhofer T, Jaslan J, Rosales MA, Espartero J, Díaz-Rueda P, Müller HM, Hürter A-L, Al-Rasheid KAS, Marten I *et al.* 2016. Silent S-type anion channel subunit SLAH1 gates SLAH3 open for chloride root-to-shoot translocation. *Current Biology* **26**: 2213–2220.

Cutler SR, Rodriguez PL, Finkelstein R, Abrams SR. 2010. Abscisic acid: emergence of a core signaling network. *Annual Review of Plant Biology* **61**: 651–679.

D'Angelo C, Weinl S, Batistic O, Pandey GK, Cheong YH, Schültke S, Albrecht V, Ehlert B, Schulz B, Harter K *et al.* 2006. Alternative complex formation of the Ca-regulated protein kinase CIPK1 controls abscisic acid-dependent and independent stress responses in Arabidopsis. *Plant Journal* **48**: 857–872.

Davière J-M, de Lucas M, Prat S. 2008. Transcriptional factor interaction: a central step in DELLA function. *Current Opinion in Genetics & Development* **18**: 295–303.

Day IS, Reddy VS, Shad Ali G, Reddy ASN. 2002. Analysis of EF-hand-containing proteins in Arabidopsis. *Genome Biology* **3**: research0056.1–research0056.24..

Delk NA, Johnson KA, Chowdhury NI, Braam J. 2005. CML24, regulated in expression by diverse stimuli, encodes a potential Ca^{2+} sensor that functions in responses to abscisic acid, daylength, and ion stress. *Plant Physiology* **139**: 240–253.

Demir F, Horntrich C, Blachutzik JO, Scherzer S, Reinders Y, Kierszniowska S, Schulze WX, Harms GS, Hedrich R, Geiger D *et al.* 2013. Arabidopsis nanodomain-delimited ABA signaling pathway regulates the anion channel SLAH3. *Proceedings of the National Academy of Sciences, USA* **110**: 8296–8301.

Denninger P, Bleckmann A, Lausser A, Vogler F, Ott T, Ehrhardt DW, Frommer WB, Sprunck S, Dresselhaus T, Grossmann G. 2014. Male-female communication triggers calcium signatures during fertilization in Arabidopsis. *Nature Communications* **5**: 4645.

Diaz M, Sanchez-Barrena MJ, Gonzalez-Rubio JM, Rodriguez L, Fernandez D, Antoni R, Yunta C, Belda-Palazon B, Gonzalez-Guzman M, Peirats-Llobet M *et al.* 2016. Calcium-dependent oligomerization of CAR proteins at cell membrane modulates ABA signaling. *Proceedings of the National Academy of Sciences, USA* **113**: E396–E405.

Ding Y, Kalo P, Yendrek C, Sun J, Liang Y, Marsh JF, Harris JM, Oldroyd GED. 2008. Abscisic acid coordinates nod factor and cytokinin signaling during the regulation of nodulation in *Medicago truncatula*. *Plant Cell* **20**: 2681–2695.

Ding Y, Li J, Enterina JR, Shen Y, Zhang I, Tewson PH, Mo GCH, Zhang J, Quinn AM, Hughes TE *et al.* 2015. Ratiometric biosensors based on dimerization-dependent fluorescent protein exchange. *Nature Methods* **12**: 195–198.

Dobney S, Chiasson D, Lam P, Smith SP, Snedden WA. 2009. The calmodulin-related calcium sensor CML42 Plays a role in trichome branching. *Journal of Biological Chemistry* **284**: 31 647–31 657.

Dodd AN, Kudla J, Sanders D. 2010. The language of calcium signaling. *Annual Review of Plant Biology* **61**: 593–620.

Du W, Lin H, Chen S, Wu Y, Zhang J, Fuglsang AT, Palmgren MG, Wu W, Guo Y. 2011. Phosphorylation of SOS3-like calcium-binding proteins by their interacting SOS2-like protein kinases is a common regulatory mechanism in Arabidopsis. *Plant Physiology* **156**: 2235–2243.

Dupont G, Goldbeter A. 1998. CaM kinase II as frequency decoder of Ca^{2+} oscillations. *BioEssays* **20**: 607–610.

Eckert C, Offenborn JN, Heinz T, Armarego-Marriott T, Schültke S, Zhang C, Hillmer S, Heilmann M, Schumacher K, Bock R *et al.* 2014. The vacuolar calcium sensors CBL2 and CBL3 affect seed size and embryonic development in *Arabidopsis thaliana*. *Plant Journal* **78**: 146–156.

Edel KH, Kudla J. 2015. Increasing complexity and versatility: how the calcium signaling toolkit was shaped during plant land colonization. *Cell Calcium* **57**: 231–246.

Edel KH, Kudla J. 2016. Integration of calcium and ABA signaling. *Current Opinion in Plant Biology* **33**: 83–91.

Evans NH, McAinsh MR, Hetherington AM. 2001. Calcium oscillations in higher plants. *Current Opinion in Plant Biology* **4**: 415–420.

Ferreira KN, Iverson TM, Maghlaoui K, Barber J, Iwata S. 2004. Architecture of the photosynthetic oxygen-evolving center. *Science* **303**: 1831–1838.

Floss DS, Levy JG, Levesque-Tremblay V, Pumplin N, Harrison MJ. 2013. DELLA proteins regulate arbuscule formation in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* **110**: E5025–E5034.

Fonouni-Farde C, Tan S, Baudin M, Brault M, Wen J, Mysore KS, Niebel A, Frugier F, Diet A. 2016. DELLA-mediated gibberellin signalling regulates Nod factor signalling and rhizobial infection. *Nature Communications* **7**: 12636.

Foskett JK, Philipson B. 2015. The mitochondrial Ca^{2+} uniporter complex. *Journal of Molecular and Cellular Cardiology* **78**: 3–8.

Franz S, Ehlert B, Liese A, Kurth J, Cazalé A-C, Romeis T. 2011. Calcium-dependent protein kinase CPK21 functions in abiotic stress response in *Arabidopsis thaliana*. *Molecular Plant* **4**: 83–96.

Fricker MD, Gilroy S, Read ND, Trewavas AJ. 1991. Visualisation and measurement of the calcium message in guard cells. *Symposia of the Society for Experimental Biology* **45**: 177–190.

Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park S-Y, Cutler SR, Sheen J, Rodriguez PL, Zhu J-K. 2009. *In vitro* reconstitution of an abscisic acid signalling pathway. *Nature* **462**: 660–664.

Fujita Y, Yoshida T, Yamaguchi-Shinozaki K. 2013. Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. *Physiologia Plantarum* **147**: 15–27.

Galon Y, Finkler A, Fromm H. 2010. Calcium-regulated transcription in plants. *Molecular Plant* **3**: 653–669.

Geiger D, Becker D, Vosloh D, Gambale F, Palme K, Rehers M, Anschuetz U, Dreyer I, Kudla J, Hedrich R. 2009a. Heteromeric AtKC1/AKT1 channels in Arabidopsis roots facilitate growth under K^+-limiting conditions. *The Journal of Biological Chemistry* **284**: 21 288–21 295.

Geiger D, Maierhofer T, Al-Rasheid KAS, Scherzer S, Mumm P, Liese A, Ache P, Wellmann C, Marten I, Grill E *et al.* 2011. Stomatal closure by fast abscisic acid signaling is mediated by the guard cell anion channel SLAH3 and the receptor RCAR1. *Science Signaling* **4**: ra32.

Geiger D, Scherzer S, Mumm P, Marten I, Ache P, Matschi S, Liese A, Wellmann C, Al-Rasheid KAS, Grill E *et al.* 2010. Guard cell anion channel SLAC1 is regulated by CDPK protein kinases with distinct Ca^{2+} affinities. *Proceedings of the National Academy of Sciences, USA* **107**: 8023–8028.

Geiger D, Scherzer S, Mumm P, Stange A, Marten I, Bauer H, Ache P, Matschi S, Liese A, Al-Rasheid KAS *et al.* 2009b. Activity of guard cell anion channel SLAC1

is controlled by drought-stress signaling kinase-phosphatase pair. *Proceedings of the National Academy of Sciences, USA* **106**: 21425–21430.

Gilroy S, Suzuki N, Miller G, Choi W-G, Toyota M, Devireddy AR, Mittler R. 2014. A tidal wave of signals: calcium and ROS at the forefront of rapid systemic signaling. *Trends in Plant Science* **19**: 623–630.

Gleason C, Chaudhuri S, Yang T, Muñoz A, Poovaiah BW, Oldroyd GED. 2006. Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. *Nature* **441**: 1149–1152.

Gobbato E, Marsh JF, Vernié T, Wang E, Maillet F, Kim J, Miller JB, Sun J, Bano SA, Ratet P *et al.* 2012. A GRAS-type transcription factor with a specific function in mycorrhizal signaling. *Current Biology* **22**: 2236–2241.

Guo H, Feng P, Chi W, Sun X, Xu X, Li Y, Ren D, Lu C, David Rochaix J, Leister D *et al.* 2016. Plastid-nucleus communication involves calcium-modulated MAPK signalling. *Nature Communications* 7: 12173.

Guskov A, Kern J, Gabdulkhakov A, Broser M, Zouni A, Saenger W. 2009. Cyanobacterial photosystem II at 2.9-Å resolution and the role of quinones, lipids, channels and chloride. *Nature Structural & Molecular Biology* **16**: 334–342.

Gutjahr C, Parniske M. 2013. Cell and developmental biology of arbuscular mycorrhiza symbiosis. *Annual Review of Cell and Developmental Biology* **29**: 593–617.

Guzel Deger A, Scherzer S, Nuhkat M, Kedzierska J, Kollist H, Brosché M, Unyayar S, Boudsocq M, Hedrich R, Roelfsema MRG. 2015. Guard cell SLAC1-type anion channels mediate flagellin-induced stomatal closure. *New Phytologist* **208**: 162–173.

Halfter U, Ishitani M, Zhu J-K. 2000. The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. *Proceedings of the National Academy of Sciences, USA* **97**: 3735–3740.

Hamamura Y, Nishimaki M, Takeuchi H, Geitmann A, Kurihara D, Higashiyama T. 2014. Live imaging of calcium spikes during double fertilization in Arabidopsis. *Nature Communications* **5**: 4722.

Hashimoto K, Eckert C, Anschuetz U, Scholz M, Held K, Waadt R, Reyer A, Hippler M, Becker D, Kudla J. 2012. Phosphorylation of calcineurin B-like (CBL) calcium sensor proteins by their CBL-interacting protein kinases (CIPKs) is required for full activity of CBL-CIPK complexes towards their target proteins. *The Journal of Biological Chemistry* **287**: 7956–7968.

Hashimoto K, Kudla J. 2011. Calcium decoding mechanisms in plants. *Biochimie* **93**: 2054–2059.

Hayashi T, Shimoda Y, Sato S, Tabata S, Imaizumi-Anraku H, Hayashi M. 2014. Rhizobial infection does not require cortical expression of upstream common symbiosis genes responsible for the induction of Ca^{2+} spiking. *Plant Journal* **77**: 146–159.

Heim N, Griesbeck O. 2004. Genetically encoded indicators of cellular calcium dynamics based on troponin c and green fluorescent protein. *Journal of Biological Chemistry* **279**: 14 280–14 286.

Hetherington AM, Brownlee C. 2004. The generation of Ca^{2+} signals in plants. *Annual Review of Plant Biology* **55**: 401–427.

Hills A, Chen Z-H, Amtmann A, Blatt MR, Lew VL. 2012. OnGuard, a computational platform for quantitative kinetic modeling of guard cell physiology. *Plant Physiology* **159**: 1026–1042.

Himmelbach A, Hoffmann T, Leube M, Höhener B, Grill E. 2002. Homeodomain protein ATHB6 is a target of the protein phosphatase ABI1 and regulates hormone responses in Arabidopsis. *EMBO Journal* **21**: 3029–3038.

Ho C-H, Lin S-H, Hu H-C, Tsay Y-F. 2009. CHL1 functions as a nitrate sensor in plants. *Cell* **138**: 1184–1194.

Hochmal AK, Schulze S, Trompelt K, Hippler M. 2015. Calcium-dependent regulation of photosynthesis. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1847**: 993–1003.

Hochmal AK, Zinzius K, Charoenwattanasatien R, Gäbelein P, Mutoh R, Tanaka H, Schulze S, Liu G, Scholz M, Nordhues A *et al.* 2016. Calredoxin represents a novel type of calcium-dependent sensor-responder connected to redox regulation in the chloroplast. *Nature Communications* **7**: 11 847.

Hoi H, Matsuda T, Nagai T, Campbell RE. 2013. Highlightable Ca^{2+} indicators for live cell imaging. *Journal of the American Chemical Society* **135**: 46–49.

Hoops S, Sahle S, Gauges R, Lee C, Pahle J, Simus N, Singhal M, Xu L, Mendes P, Kummer U. 2006. COPASI–a complex pathway simulator. *Bioinformatics* **22**: 3067–3074.

Hua D, Wang C, He J, Liao H, Duan Y, Zhu Z, Guo Y, Chen Z, Gong Z. 2012. A plasma membrane receptor kinase, GHR1, mediates abscisic acid- and hydrogen peroxide-regulated stomatal movement in Arabidopsis. *Plant Cell* **24**: 2546–2561.

Hucka M, Finney A, Sauro HM, Bolouri H, Doyle JC, Kitano H, Arkin AP, Bornstein BJ, Bray D, Cornish-Bowden A *et al.* 2003. The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics* **19**: 524–531.

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

Islam MM, Hossain MA, Jannat R, Munemasa S, Nakamura Y, Mori IC, Murata Y. 2010. Cytosolic alkalization and cytosolic calcium oscillation in Arabidopsis guard cells response to ABA and MeJA. *Plant & Cell Physiology* **51**: 1721–1730.

Iwano M, Ito K, Fujii S, Kakita M, Asano-Shimosato H, Igarashi M, Kaothien-Nakayama P, Entani T, Kanatani A, Takehisa M *et al.* 2015. Calcium signalling mediates self-incompatibility response in the Brassicaceae. *Nature Plants* **1**: 15128.

Iwano M, Ngo QA, Entani T, Shiba H, Nagai T, Miyawaki A, Isogai A, Grossniklaus U, Takayama S. 2012. Cytoplasmic Ca^{2+} changes dynamically during the interaction of the pollen tube with synergid cells. *Development* **139**: 4202–4209.

Jeworutzki E, Roelfsema MRG, Anschütz U, Krol E, Elzenga JTM, Felix G, Boller T, Hedrich R, Becker D. 2010. Early signaling through the Arabidopsis pattern recognition receptors FLS2 and EFR involves Ca^{2+}-associated opening of plasma membrane anion channels. *Plant Journal* **62**: 367–378.

Jin Y, Liu H, Luo D, Yu N, Dong W, Wang C, Zhang X, Dai H, Yang J, Wang E. 2016. DELLA proteins are common components of symbiotic rhizobial and mycorrhizal signalling pathways. *Nature Communications* 7: 12433.

Johnson CH, Knight MR, Kondo T, Masson P, Sedbrook J, Haley A, Trewavas A. 1995. Circadian oscillations of cytosolic and chloroplastic free calcium in plants. *Science* **269**: 1863–1865.

Kang H, Zhu H, Chu X, Yang Z, Yuan S, Yu D, Wang C, Hong Z, Zhang Z. 2011. A novel interaction between CCaMK and a protein containing the Scythe_N ubiquitin-like domain in Lotus japonicus. *Plant Physiology* **155**: 1312–1324.

Kaplan B, Davydov O, Knight H, Galon Y, Knight MR, Fluhr R, Fromm H. 2006. Rapid transcriptome changes induced by cytosolic Ca^{2+} transients reveal ABRE-related sequences as Ca^{2+}-responsive cis elements in Arabidopsis. *Plant Cell* **18**: 2733–2748.

Keinath NF, Waadt R, Brugman R, Schroeder JI, Grossmann G, Schumacher K, Krebs M. 2015. Live cell imaging with R-GECO1 sheds light on flg22- and chitin-induced transient $[Ca^{2+}]_{cyt}$ patterns in Arabidopsis. *Molecular Plant* **8**: 1188–1200.

Kilian J, Whitehead D, Horak J, Wanke D, Weinl S, Batistic O, D'Angelo C, Bornberg-Bauer E, Kudla J, Harter K. 2007. The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. *Plant Journal* **50**: 347–363.

Kim T-H, Böhmer M, Hu H, Nishimura N, Schroeder JI. 2010. Guard cell signal transduction network: advances in understanding abscisic acid, CO_2, and Ca^{2+} signaling. *Annual Review of Plant Biology* **61**: 561–591.

Kim B-G, Waadt R, Cheong YH, Pandey GK, Dominguez-Solis JR, Schültke S, Lee S-C, Kudla J, Luan S. 2007. The calcium sensor CBL10 mediates salt tolerance by regulating ion homeostasis in Arabidopsis. *Plant Journal* **52**: 473–484.

Kistner C, Parniske M. 2002. Evolution of signal transduction in intracellular symbiosis. *Trends in Plant Science* 7: 511–518.

Knight MR, Campbell AK, Smith SM, Trewavas AJ. 1991. Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. *Nature* **352**: 524–526.

Kobayashi Y, Murata M, Minami H, Yamamoto S, Kagaya Y, Hobo T, Yamamoto A, Hattori T. 2005. Abscisic acid-activated SNRK2 protein kinases function in the gene-regulation pathway of ABA signal transduction by phosphorylating ABA response element-binding factors. *Plant Journal* **44**: 939–949.

Krol E, Mentzel T, Chinchilla D, Boller T, Felix G, Kemmerling B, Postel S, Arents M, Jeworutzki E, Al-Rasheid KAS *et al.* 2010. Perception of the

Arabidopsis danger signal peptide 1 involves the pattern recognition receptor *At* PEPR1 and its close homologue *At* PEPR2. *Journal of Biological Chemistry* **285**: 13 471–13 479.

Kudla J, Batistic O, Hashimoto K. 2010. Calcium signals: the lead currency of plant information processing. *Plant Cell* **22**: 541–563.

Kurusu T, Kuchitsu K, Nakano M, Nakayama Y, Iida H. 2013. Plant mechanosensing and Ca^{2+} transport. *Trends in Plant Science* **18**: 227–233.

Lacombe B, Becker D, Hedrich R, DeSalle R, Hollmann M, Kwak JM, Schroeder JI, Le Novère N, Nam HG, Spalding EP *et al.* 2001. The identity of plant glutamate receptors. *Science* **292**: 1486–1487.

Lan W-Z, Lee S-C, Che Y-F, Jiang Y-Q, Luan S. 2011. Mechanistic analysis of AKT1 regulation by the CBL-CIPK-PP2CA interactions. *Molecular Plant* **4**: 527–536.

Larsen AZ, Olsen LF, Kummer U. 2004. On the encoding and decoding of calcium signals in hepatocytes. *Biophysical Chemistry* **107**: 83–99.

Lechner E, Leonhardt N, Eisler H, Parmentier Y, Alioua M, Jacquet H, Leung J, Genschik P. 2011. MATH/BTB CRL3 receptors target the homeodomain-leucine zipper ATHB6 to modulate abscisic acid signaling. *Developmental Cell* **21**: 1116–1128.

Lee S-C, Lan W, Buchanan BB, Luan S. 2009. A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. *Proceedings of the National Academy of Sciences, USA* **106**: 21419–21424.

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.

Léran S, Edel KH, Pervent M, Hashimoto K, Corratgé-Faillie C, Offenborn JN, Tillard P, Gojon A, Kudla J, Lacombe B. 2015. Nitrate sensing and uptake in Arabidopsis are enhanced by ABI2, a phosphatase inactivated by the stress hormone abscisic acid. *Science Signaling* **8**: ra43.

Levchenko V, Konrad KR, Dietrich P, Roelfsema MRG, Hedrich R. 2005. Cytosolic abscisic acid activates guard cell anion channels without preceding Ca^{2+} signals. *Proceedings of the National Academy of Sciences, USA* **102**: 4203–4208.

Liao J, Singh S, Hossain MS, Andersen SU, Ross L, Bonetta D, Zhou Y, Sato S, Tabata S, Stougaard J *et al.* 2012. Negative regulation of CCaMK is essential for symbiotic infection. *Plant Journal* **72**: 572–584.

Liese A, Romeis T. 2013. Biochemical regulation of *in vivo* function of plant calcium-dependent protein kinases (CDPK). *Biochimica et Biophysica Acta* **1833**: 1582–1589.

Liu K-H, Tsay Y-F. 2003. Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation. *EMBO Journal* **22**: 1005–1013.

Loro G, Wagner S, Doccula FG, Behera S, Weinl S, Kudla J, Schwarzländer M, Costa A, Zottini M. 2016. Chloroplast-specific *in vivo* Ca^{2+} imaging using Yellow Cameleon fluorescent protein sensors reveals organelle-autonomous Ca^{2+} signatures in the stroma. *Plant Physiology* **171**: 2317–2330.

Lumba S, Toh S, Handfield L-F, Swan M, Liu R, Youn J-Y, Cutler SR, Subramaniam R, Provart N, Moses A *et al.* 2014. A mesoscale abscisic acid hormone interactome reveals a dynamic signaling landscape in Arabidopsis. *Developmental Cell* **29**: 360–372.

Lynch T, Erickson BJ, Finkelstein RR. 2012. Direct interactions of ABA-insensitive (ABI)-clade protein phosphatase(PP)2Cs with calcium-dependent protein kinases and ABA response element-binding bZIPs may contribute to turning off ABA response. *Plant Molecular Biology* **80**: 647–658.

Lyzenga WJ, Liu H, Schofield A, Muise-Hennessey A, Stone SL. 2013. Arabidopsis CIPK26 interacts with KEG, components of the ABA signalling network and is degraded by the ubiquitin-proteasome system. *Journal of Experimental Botany* **64**: 2779–2791.

Ma S-Y, Wu W-H. 2007. AtCPK23 functions in Arabidopsis responses to drought and salt stresses. *Plant Molecular Biology* **65**: 511–518.

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E. 2009. Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* **324**: 1064–1068.

Madsen LH, Tirichine L, Jurkiewicz A, Sullivan JT, Heckmann AB, Bek AS, Ronson CW, James EK, Stougaard J. 2010. The molecular network governing nodule organogenesis and infection in the model legume *Lotus japonicus*. *Nature Communications* **1**: 10.

Maekawa T, Maekawa-Yoshikawa M, Takeda N, Imaizumi-Anraku H, Murooka Y, Hayashi M. 2009. Gibberellin controls the nodulation signaling pathway in *Lotus japonicus*. *Plant Journal* **58**: 183–194.

Magnan F, Ranty B, Charpenteau M, Sotta B, Galaud J-P, Aldon D. 2008. Mutations in AtCML9, a calmodulin-like protein from *Arabidopsis thaliana*, alter plant responses to abiotic stress and abscisic acid. *Plant Journal* **56**: 575–589.

Maierhofer T, Diekmann M, Offenborn JN, Lind C, Bauer H, Hashimoto K, Al-Rasheid KAS, Luan S, Kudla J, Geiger D *et al.* 2014. Site- and kinase-specific phosphorylation-mediated activation of SLAC1, a guard cell anion channel stimulated by abscisic acid. *Science Signaling* **7**: ra86.

Manohar M, Shigaki T, Hirschi KD. 2011. Plant cation/H^+ exchangers (CAXs): biological functions and genetic manipulations. *Plant Biology* **13**: 561–569.

Martins TV, Evans MJ, Woolfenden HC, Morris RJ. 2013. Towards the physics of calcium signalling in plants. *Plants* **2**: 541–588.

McAinsh MR, Hetherington AM. 1998. Encoding specificity in Ca^{2+} signalling systems. *Trends in Plant Science* **3**: 32–36.

McCormack E, Tsai Y-C, Braam J. 2005. Handling calcium signaling: Arabidopsis CaMs and CMLs. *Trends in Plant Science* **10**: 383–389.

Melcher K, Xu Y, Ng L-M, Zhou XE, Soon F-F, Chinnusamy V, Suino-Powell KM, Kovach A, Tham FS, Cutler SR *et al.* 2010. Identification and mechanism of ABA receptor antagonism. *Nature Structural & Molecular Biology* **17**: 1102–1108.

Miller JB, Pratap A, Miyahara A, Zhou L, Bornemann S, Morris RJ, Oldroyd GED. 2013. Calcium/Calmodulin-dependent protein kinase is negatively and positively regulated by calcium, providing a mechanism for decoding calcium responses during symbiosis signaling. *Plant Cell* **25**: 5053–5066.

Minguet-Parramona C, Wang Y, Hills A, Vialet-Chabrand S, Griffiths H, Rogers S, Lawson T, Lew VL, Blatt MR. 2016. An optimal frequency in Ca^{2+} oscillations for stomatal closure is an emergent property of ion transport in guard cells. *Plant Physiology* **170**: 33–42.

Miyawaki A, Llopis J, Heim R, Mccaffery JM, Adams JA, Ikurak M, Tsien RY. 1997. Fluorescent indicators for Ca^{2+} based on green fluorescent proteins and calmodulin. *Nature* **388**: 882–887.

Monshausen GB, Bibikova TN, Weisenseel MH, Gilroy S. 2009. Ca^{2+} regulates reactive oxygen species production and pH during mechanosensing in Arabidopsis roots. *Plant Cell* **21**: 2341–2356.

Moreno I, Norambuena L, Maturana D, Toro M, Vergara C, Orellana A, Zurita-Silva A, Ordenes VR. 2008. AtHMA1 is a thapsigargin-sensitive Ca2+/heavy metal pump. *Journal of Biological Chemistry* **283**: 9633–9641.

Morgan AJ, Galione A. 2014. Two-pore channels (TPCs): current controversies. *BioEssays* **36**: 173–183.

Mori IC, Murata Y, Yang Y, Munemasa S, Wang YF, Andreoli S, Tiriac H, Alonso JM, Harper JF, Ecker JR *et al.* 2006. CDPKs CPK6 and CPK3 function in ABA regulation of guard cell S-type anion- and Ca^{2+}- permeable channels and stomatal closure. *PLoS Biology* **4**: 1749–1762.

Mrozek K, Niehaus K, Lutter P. 2013. Experimental measurements and mathematical modeling of cytosolic Ca^{2+} signatures upon elicitation by penta-N-acetylchitopentaose oligosaccharides in *Nicotiana tabacum* cell cultures. *Plants* **2**: 750–768.

Mustilli A-C, Merlot S, Vavasseur A, Fenzi F, Giraudat J. 2002. Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. *Plant Cell* **14**: 3089–3099.

Nagae M, Nozawa A, Koizumi N, Sano H, Hashimoto H, Sato M, Shimizu T. 2003. The crystal structure of the novel calcium-binding protein AtCBL2 from *Arabidopsis thaliana*. *The Journal of Biological Chemistry* **278**: 42240–42246.

Nagai T, Sawano A, Park ES, Miyawaki A. 2001. Circularly permuted green fluorescent proteins engineered to sense Ca^{2+}. *Proceedings of the National Academy of Sciences, USA* **98**: 3197–3202.

Nagai T, Yamada S, Tominaga T, Ichikawa M, Miyawaki A. 2004. Expanded dynamic range of fluorescent indicators for Ca^{2+} by circularly permuted yellow. *Proceedings of the National Academy of Sciences, USA* **101**: 10554–10559.

Nakai J, Ohkura M, Imoto K. 2001. A high signal-to-noise Ca^{2+} probe composed of a single green fluorescent protein. *Nature Biotechnology* **19**: 137–141.

Negi J, Matsuda O, Nagasawa T, Oba Y, Takahashi H, Kawai-Yamada M, Uchimiya H, Hashimoto M, Iba K. 2008. CO_2 regulator SLAC1 and its

homologues are essential for anion homeostasis in plant cells. *Nature* **452**: 483–486.

Ng L-M, Soon F-F, Zhou XE, West GM, Kovach A, Suino-Powell KM, Chalmers MJ, Li J, Yong E-L, Zhu J-K *et al.* 2011. Structural basis for basal activity and autoactivation of abscisic acid (ABA) signaling SnRK2 kinases. *Proceedings of the National Academy of Sciences, USA* **108**: 21 259–21 264.

Ngo QA, Vogler H, Lituiev DS, Nestorova A, Grossniklaus U. 2014. A calcium dialog mediated by the FERONIA signal transduction pathway controls plant sperm delivery. *Developmental Cell* **29**: 491–500.

Nishimura N, Sarkeshik A, Nito K, Park S-Y, Wang A, Carvalho PC, Lee S, Caddell DF, Cutler SR, Chory J *et al.* 2010. PYR/PYL/RCAR family members are major in-vivo ABI1 protein phosphatase 2C-interacting proteins in Arabidopsis. *Plant Journal* **61**: 290–299.

Nomura H, Komori T, Kobori M, Nakahira Y, Shiina T. 2007. Evidence for chloroplast control of external Ca^{2+}-induced cytosolic Ca^{2+} transients and stomatal closure. *Plant Journal* **53**: 988–998.

Nomura H, Komori T, Uemura S, Kanda Y, Shimotani K, Nakai K, Furuichi T, Takebayashi K, Sugimoto T, Sano S *et al.* 2012. Chloroplast-mediated activation of plant immune signalling in Arabidopsis. *Nature Communications* **3**: 926.

Ohta M, Guo Y, Halfter U, Zhu J-K. 2003. A novel domain in the protein kinase SOS2 mediates interaction with the protein phosphatase 2C ABI2. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 11 771–11 776.

Oldroyd GED. 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Reviews. Microbiology* **11**: 252–263.

Pahle J, Green AK, Dixon CJ, Kummer U. 2008. Information transfer in signaling pathways: a study using coupled simulated and experimental data. *BMC Bioinformatics* **9**: 139.

Park S-Y, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow T-FF *et al.* 2009. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* **324**: 1068–1071.

Pérez Koldenkova V, Nagai T. 2013. Genetically encoded Ca^{2+} indicators: properties and evaluation. *Biochimica et Biophysica Acta* **1833**: 1787–1797.

Perochon A, Aldon D, Galaud J-P, Ranty B. 2011. Calmodulin and calmodulin-like proteins in plant calcium signaling. *Biochimie* **93**: 2048–2053.

Petroutsos D, Busch A, Janßen I, Trompelt K, Bergner SV, Weinl S, Holtkamp M, Karst U, Kudla J, Hippler M. 2011. The chloroplast calcium sensor CAS is required for photoacclimation in *Chlamydomonas reinhardtii*. *Plant Cell* **23**: 2950–2963.

Pimprikar P, Carbonnel S, Paries M, Katzer K, Klingl V, Bohmer MJ, Karl L, Floss DS, Harrison MJ, Parniske M *et al.* 2016. A CCaMK-CYCLOPS-DELLA complex activates transcription of RAM1 to regulate arbuscule branching. *Current Biology* **26**: 1126.

Pittman JK. 2011. Vacuolar Ca^{2+} uptake. *Cell Calcium* **50**: 139–146.

Price MB, Jelesko J, Okumoto S. 2012. Glutamate receptor homologs in plants: functions and evolutionary origins. *Frontiers in Plant Science* **3**: 235.

Ragel P, Ródenas R, García-Martín E, Andrés Z, Villalta I, Nieves-Cordones M, Rivero RM, Martínez V, Pardo JM, Quintero FJ *et al.* 2015. CIPK23 regulates HAK5-mediated high-affinity K^+ uptake in Arabidopsis roots. *Plant Physiology* **169**: 2863–2873.

Raghavendra AS, Gonugunta VK, Christmann A, Grill E. 2010. ABA perception and signalling. *Trends in Plant Science* **15**: 395–401.

Rizzuto R, De Stefani D, Raffaello A, Mammucari C. 2012. Mitochondria as sensors and regulators of calcium signalling. *Nature Reviews Molecular Cell Biology* **13**: 566–578.

Rizzuto R, Pozzan T. 2006. Microdomains of intracellular Ca^{2+}: molecular determinants and functional consequences. *Physiological Reviews* **86**: 369–408.

Rodriguez L, Gonzalez-Guzman M, Diaz M, Rodrigues A, Izquierdo-Garcia AC, Peirats-Llobet M, Fernandez MA, Antoni R, Fernandez D, Marquez JA *et al.* 2014. C2-domain abscisic acid-related proteins mediate the interaction of PYR/PYL/RCAR abscisic acid receptors with the plasma membrane and regulate abscisic acid sensitivity in Arabidopsis. *Plant Cell* **26**: 4802–4820.

Romeis T, Herde M. 2014. From local to global: CDPKs in systemic defense signaling upon microbial and herbivore attack. *Current Opinion in Plant Biology* **20C**: 1–10.

Rose T, Goltstein PM, Portugues R, Griesbeck O. 2014. Putting a finishing touch on GECIs. *Frontiers in Molecular Neuroscience* **7**: 88.

Routray P, Miller JB, Du L, Oldroyd G, Poovaiah BW. 2013. Phosphorylation of S344 in the calmodulin-binding domain negatively affects CCaMK function during bacterial and fungal symbioses. *Plant Journal* **76**: 287–296.

Sai J, Johnson CH. 2002. Dark-stimulated calcium ion fluxes in the chloroplast stroma and cytosol. *Plant Cell* **14**: 1279–1291.

Saito K, Chang Y-F, Horikawa K, Hatsugai N, Higuchi Y, Hashida M, Yoshida Y, Matsuda T, Arai Y, Nagai T. 2012. Luminescent proteins for high-speed single-cell and whole-body imaging. *Nature Communications* **3**: 1262.

Saito K, Hatsugai N, Horikawa K, Kobayashi K, Matsu-Ura T, Mikoshiba K, Nagai T. 2010. Auto-luminescent genetically-encoded ratiometric indicator for real-time Ca^{2+} imaging at the single cell level. *PLoS ONE* **5**: e9935.

Sánchez-Barrena MJ, Fujii H, Angulo I, Martínez-Ripoll M, Zhu J-KK, Albert A. 2007. The structure of the C-terminal domain of the protein kinase AtSOS2 bound to the calcium sensor AtSOS3. *Molecular Cell* **26**: 427–435.

Scherzer S, Böhm J, Krol E, Shabala L, Kreuzer I, Larisch C, Bemm F, Al-Rasheid KAS, Shabala S, Rennenberg H *et al.* 2015. Calcium sensor kinase activates potassium uptake systems in gland cells of Venus flytraps. *Proceedings of the National Academy of Sciences, USA* **112**: 7309–7314.

Scherzer S, Maierhofer T, Al-Rasheid KAS, Geiger D, Hedrich R. 2012. Multiple calcium-dependent kinases modulate ABA-activated guard cell anion channels. *Molecular Plant* **5**: 1409–1412.

Schroeder JI, Hagiwara S. 1990. Repetitive increases in cytosolic Ca^{2+} of guard cells by abscisic acid activation of nonselective Ca^{2+} permeable channels. *Proceedings of the National Academy of Sciences, USA* **87**: 9305–9309.

Schuster S, Marhl M, Höfer T. 2002. Modelling of simple and complex calcium oscillations. From single-cell responses to intercellular signalling. *European Journal of Biochemistry* **269**: 1333–1355.

Scrase-Field SAMG, Knight MR. 2003. Calcium: just a chemical switch? *Current Opinion in Plant Biology* **6**: 500–506.

Sello S, Perotto J, Carraretto L, Szabò I, Vothknecht UC, Navazio L. 2016. Dissecting stimulus-specific Ca^{2+} signals in amyloplasts and chloroplasts of *Arabidopsis thaliana* cell suspension cultures. *Journal of Experimental Botany* **67**: 3965–3974.

Shacklock PS, Read ND, Trewavas AJ. 1992. Cytosolic free calcium mediates red light-induced photomorphogenesis. *Nature* **358**: 753–755.

Shi J, Kim K-N, 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.

Shih H-W, Miller ND, Dai C, Spalding EP, Monshausen GB. 2014. The receptor-like kinase FERONIA is required for mechanical signal transduction in Arabidopsis seedlings. *Current Biology* **24**: 1887–1892.

Shimomura O, Johnson FH, Saiga Y. 1962. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. *Journal of Cellular and Comparative Physiology* **59**: 223–239.

Sieberer BJ, Chabaud M, Fournier J, Timmers ACJ, Barker DG. 2012. A switch in Ca^{2+} spiking signature is concomitant with endosymbiotic microbe entry into cortical root cells of *Medicago truncatula*. *Plant Journal* **69**: 822–830.

Singh S, Katzer K, Lambert J, Cerri M, Parniske M. 2014. CYCLOPS, a DNA-binding transcriptional activator, orchestrates symbiotic root nodule development. *Cell Host & Microbe* **15**: 139–152.

Spalding EP, Harper JF. 2011. The ins and outs of cellular Ca^{2+} transport. *Current Opinion in Plant Biology* **14**: 715–720.

Stael S, Wurzinger B, Mair A, Mehlmer N, Vothknecht UC, Teige M. 2012. Plant organellar calcium signalling: an emerging field. *Journal of Experimental Botany* **63**: 1525–1542.

Stange A, Hedrich R, Roelfsema MRG. 2010. Ca^{2+}-dependent activation of guard cell anion channels, triggered by hyperpolarization, is promoted by prolonged depolarization. *Plant Journal* **62**: 265–276.

Steinhorst L, Kudla J. 2013. Calcium and reactive oxygen species rule the waves of signaling. *Plant Physiology* **163**: 471–485.

Steinhorst L, Kudla J. 2014. Signaling in cells and organisms – calcium holds the line. *Current Opinion in Plant Biology* **22**: 14–21.

Steinhorst L, Mähs A, Ischebeck T, Zhang C, Zhang X, Arendt S, Schültke S, Heilmann I, Kudla J. 2015. Vacuolar CBL-CIPK12 Ca^{2+}-sensor-kinase complexes are required for polarized pollen tube growth. *Current Biology* **25**: 1475–1482.

Strand DD, Livingston AK, Satoh-Cruz M, Froehlich JE, Maurino VG, Kramer DM. 2015. Activation of cyclic electron flow by hydrogen peroxide *in vivo*. *Proceedings of the National Academy of Sciences, USA* **112**: 5539–5544.

Straub T, Ludewig U, Neuhäuser B. 2017. The kinase CIPK23 inhibits ammonium transport in *Arabidopsis thaliana*. *The Plant Cell* **29**: 409–422.

Sun J, Miller JB, Granqvist E, Wiley-Kalil A, Gobbato E, Maillet F, Cottaz S, Samain E, Venkateshwaran M, Fort S *et al.* 2015. Activation of symbiosis signaling by arbuscular mycorrhizal fungi in legumes and rice. *Plant Cell* **27**: 823–838.

Takahashi H, Clowez S, Wollman F-A, Vallon O, Rappaport F. 2013. Cyclic electron flow is redox-controlled but independent of state transition. *Nature Communications* **4**: 1954.

Takeda N, Handa Y, Tsuzuki S, Kojima M, Sakakibara H, Kawaguchi M. 2015. Gibberellins interfere with symbiosis signaling and gene expression and alter colonization by arbuscular mycorrhizal fungi in *Lotus japonicus*. *Plant Physiology* **167**: 545–557.

Teardo E, Carraretto L, Wagner S, Formentin E, Behera S, De Bortoli S, Larosa V, Fuchs P, Lo Schiavo F, Raffaello A *et al.* 2017. Physiological characterization of a plant mitochondrial calcium uniporter *in Vitro* and *in Vivo*. *Plant Physiology* **173**: 1355–1370.

Terashima M, Petroutsos D, Hudig M, Tolstygina I, Trompelt K, Gabelein P, Fufezan C, Kudla J, Weinl S, Finazzi G *et al.* 2012. Calcium-dependent regulation of cyclic photosynthetic electron transfer by a CAS, ANR1, and PGRL1 complex. *Proceedings of the National Academy of Sciences* **109**: 17717–17722.

Thestrup T, Litzlbauer J, Bartholomäus I, Mues M, Russo L, Dana H, Kovalchuk Y, Liang Y, Kalamakis G, Laukat Y *et al.* 2014. Optimized ratiometric calcium sensors for functional *in vivo* imaging of neurons and T lymphocytes. *Nature Methods* **11**: 175–182.

Tian Q, Zhang X, Yang A, Wang T, Zhang W-H. 2016. CIPK23 is involved in iron acquisition of Arabidopsis by affecting ferric chelate reductase activity. *Plant Science* **246**: 70–79.

Tirichine L, Imaizumi-Anraku H, Yoshida S, Murakami Y, Madsen LH, Miwa H, Nakagawa T, Sandal N, Albrektsen AS, Kawaguchi M *et al.* 2006. Deregulation of a Ca^{2+}/calmodulin-dependent kinase leads to spontaneous nodule development. *Nature* **441**: 1153–1156.

Umena Y, Kawakami K, Shen J-R, Kamiya N. 2011. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* **473**: 55–60.

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

Vahisalu T, Puzõrjova I, Brosché M, Valk E, Lepiku M, Moldau H, Pechter P, Wang Y-S, Lindgren O, Salojärvi J *et al.* 2010. Ozone-triggered rapid stomatal response involves the production of reactive oxygen species, and is controlled by SLAC1 and OST1. *Plant Journal* **62**: 442–453.

Waadt R, Krebs M, Kudla J, Schumacher K. 2017. Multiparameter imaging of calcium and abscisic acid and high resolution quantitative calcium measurements using R-GECO1-mTurquoise in Arabidopsis. *New Phytologist* **216**: 303–320.

Wagner S, Behera S, De Bortoli S, Logan DC, Fuchs P, Carraretto L, Teardo E, Cendron L, Nietzel T, Füßl M *et al.* 2015. The EF-Hand Ca^{2+} binding protein MICU choreographs mitochondrial Ca^{2+} dynamics in Arabidopsis. *Plant Cell* **27**: 3190–3212.

Wagner S, De Bortoli S, Schwarzländer M, Szabò I. 2016. Regulation of mitochondrial calcium in plants versus animals. *Journal of Experimental Botany* **67**: 3809–3829.

Wang X-P, Chen L-M, Liu W-X, Shen L-K, Wang F-L, Zhou Y, Zhang Z, Wu W-H, Wang Y. 2016a. AtKC1 and CIPK23 synergistically modulate AKT1-mediated low-potassium stress responses in Arabidopsis. *Plant Physiology* **170**: 2264–2277.

Wang L, Yamano T, Takane S, Niikawa Y, Toyokawa C, Ozawa S, Tokutsu R, Takahashi Y, Minagawa J, Kanesaki Y *et al.* 2016b. Chloroplast-mediated regulation of CO_2 -concentrating mechanism by Ca^{2+} -binding protein CAS in the green alga *Chlamydomonas reinhardtii*. *Proceedings of the National Academy of Sciences, USA* **113**: 12586–12591.

Wang Y, Papanatsiou M, Eisenach C, Karnik R, Williams M, Hills A, Lew VL, Blatt MR. 2012. Systems dynamic modeling of a guard cell Cl^- channel mutant uncovers an emergent homeostatic network regulating stomatal transpiration. *Plant Physiology* **160**: 1956–1967.

Webb AAR, Mcainsh MR, Taylor JE, Hetherington AM. 1996. Calcium ions as intracellular second messengers in higher plants. *Advances in Botanical Research* **22**: 45–96.

Weinl S, Held K, Schlücking K, Steinhorst L, Kuhlgert S, Hippler M, Kudla J. 2008. A plastid protein crucial for Ca^{2+}-regulated stomatal responses. *New Phytologist* **179**: 675–686.

Weinl S, Kudla J. 2009. The CBL-CIPK Ca^{2+}-decoding signaling network: function and perspectives. *New Phytologist* **184**: 517–528.

Wernimont AK, Amani M, Qiu W, Pizarro JC, Artz JD, Lin Y-H, Lew J, Hutchinson A, Hui R. 2011. Structures of parasitic CDPK domains point to a common mechanism of activation. *Proteins* **79**: 803–820.

Wernimont AK, Artz JD, Finerty P, Lin Y-H, Amani M, Allali-Hassani A, Senisterra G, Vedadi M, Tempel W, Mackenzie F *et al.* 2010. Structures of apicomplexan calcium-dependent protein kinases reveal mechanism of activation by calcium. *Nature Structural & Molecular Biology* **17**: 596–601.

Whalley H, Sargeant AW, Steele JFC, Lacoere T, Lamb R, Saunders NJ, Knight H, Knight MR. 2011. Transcriptomic analysis reveals calcium regulation of specific promoter motifs in Arabidopsis. *Plant Cell* **23**: 4079–4095.

White PJ, Broadley MR. 2003. Calcium in plants. *Annals of Botany* **92**: 487–511.

Xie T, Ren R, Zhang Y, Pang Y, Yan C, Gong X, He Y, Li W, Miao D, Hao Q *et al.* 2012. Molecular mechanism for inhibition of a critical component in the *Arabidopsis thaliana* abscisic acid signal transduction pathways, SnRK2.6, by protein phosphatase ABI1. *Journal of Biological Chemistry* **287**: 794–802.

Xu B, Cheval C, Laohavisit A, Hocking B, Chiasson D, Olsson TSG, Shirasu K, Faulkner C, Gilliham M. 2017. A calmodulin-like protein regulates plasmodesmal closure during bacterial immune responses. *New Phytologist* **215**: 77–84.

Yang DL, Shi Z, Bao Y, Yan J, Yang Z, Yu H, Li Y, Gou M, Wang S, Zou B *et al.* 2017. Calcium pumps and interacting BON1 protein modulate calcium signature, stomatal closure, and plant immunity. *Plant Physiology* **175**: 424–437.

Yano K, Yoshida S, Müller J, Singh S, Banba M, Vickers K, Markmann K, White C, Schuller B, Sato S *et al.* 2008. CYCLOPS, a mediator of symbiotic intracellular accommodation. *Proceedings of the National Academy of Sciences, USA* **105**: 20540–20545.

Yoshida T, Fujita Y, Maruyama K, Mogami J, Todaka D, Shinozaki K, Yamaguchi-Shinozaki K. 2015. Four Arabidopsis AREB/ABF transcription factors function predominantly in gene expression downstream of SnRK2 kinases in abscisic acid signalling in response to osmotic stress. *Plant, Cell & Environment* **38**: 35–49.

Yoshida R, Umezawa T, Mizoguchi T, Takahashi S, Takahashi F, Shinozaki K. 2006. The regulatory domain of SRK2E/OST1/SnRK2.6 interacts with ABI1 and integrates abscisic acid (ABA) and osmotic stress signals controlling stomatal closure in Arabidopsis. *Journal of Biological Chemistry* **281**: 5310–5318.

Yu N, Luo D, Zhang X, Liu J, Wang W, Jin Y, Dong W, Liu J, Liu H, Yang W *et al.* 2014. A DELLA protein complex controls the arbuscular mycorrhizal symbiosis in plants. *Cell Research* **24**: 130–133.

Yuan F, Yang H, Xue Y, Kong D, Ye R, Li C, Zhang J, Theprungsirikul L, Shrift T, Krichilsky B *et al.* 2014. OSCA1 mediates osmotic-stress-evoked Ca^{2+} increases vital for osmosensing in Arabidopsis. *Nature* **514**: 367–371.

Zelman AK, Dawe A, Gehring C, Berkowitz GA. 2012. Evolutionary and structural perspectives of plant cyclic nucleotide-gated cation channels. *Frontiers in Plant Science* **3**: 95.

Zhao Y, Araki S, Wu J, Teramoto T, Chang Y-F, Nakano M, Abdelfattah AS, Fujiwara M, Ishihara T, Nagai T *et al.* 2011. An expanded palette of genetically encoded Ca^{2+} indicators. *Science* **333**: 1888–1891.

Zhao L-N, Shen L-K, Zhang W-Z, Zhang W, Wang Y, Wu W-H. 2013. Ca^{2+}-dependent protein kinase11 and 24 modulate the activity of the inward rectifying K^+ channels in Arabidopsis pollen tubes. *Plant Cell* **25**: 649–661.

Zhou X, Hao H, Zhang Y, Bai Y, Zhu W, Qin Y, Yuan F, Zhao F, Wang M, Hu J *et al.* 2015. PKS5/CIPK11, a SnRK3-type protein kinase, is important for ABA responses in arabidopsis through phosphorylation of ABI5. *Plant Physiology* **168**: 659–676.

Zhou H, Lin H, Chen S, Becker K, Yang Y, Zhao J, Kudla J, Schumaker KS, Guo Y. 2014. Inhibition of the Arabidopsis salt overly sensitive pathway by 14-3-3 proteins. *Plant Cell* **26**: 1166–1182.

Zhu X, Dunand C, Snedden W, Galaud J-P. 2015. CaM and CML emergence in the green lineage. *Trends in Plant Science* **20**: 483–489.

Zhu Y, Yan J, Liu W, Liu L, Sheng Y, Sun Y, Li Y, Scheller HV, Jiang M, Hou X *et al.* 2016. Phosphorylation of a NAC transcription factor by a calcium/calmodulin-dependent protein kinase regulates abscisic acid-induced antioxidant defense in maize. *Plant Physiology* **171**: 1651–1664.

Zhu S-Y, Yu X-C, Wang X-J, Zhao R, Li Y, Fan R-C, Shang Y, Du S-Y, Wang X-F, Wu F-Q *et al.* 2007. Two calcium-dependent protein kinases, CPK4 and CPK11, regulate abscisic acid signal transduction in Arabidopsis. *Plant Cell* **19**: 3019–3036.