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Mechanisms of abscisic acid-mediated control of stomatal aperture

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Abstract

Drought stress triggers an increase in the level of the plant hormone abscisic acid (ABA), which initiates a signaling cascade to close stomata and reduce water loss. Recent studies have revealed that guard cells control cytosolic ABA concentration through the concerted actions of biosynthesis, catabolism as well as transport across membranes. Substantial progress has been made at understanding the molecular mechanisms of how the ABA signaling core module PYR/PYL/RCAR-PP2C-SnRK2 controls the activity of anion channels and thereby stomatal aperture. In this review, we focus on our current mechanistic understanding of ABA signaling in guard cells including the role of the second messenger Ca^{2+} as well as crosstalk with biotic stress responses.

Introduction

Guard cells form stomatal pores in the leaf epidermis, which enable plants to balance CO_2 uptake for photosynthesis and water loss via transpiration. Guard cells represent a powerful single-cell model system for understanding early signal transduction mechanisms in plants. They can sense and rapidly respond to a diverse set of environmental stimuli such as light, CO_2, pathogen infection, and plant hormones in a cell-autonomous way [1,2]. In response to drought, plants synthesize the phytohormone abscisic acid (ABA) that induces stomatal closure, thereby reducing transpirational water loss. It has been shown that ABA is *de-novo* synthesized from C_{40} carotenoids and has also been proposed to be rapidly released from its

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inactive conjugate, ABA glucose ester (ABA-GE) [3,4]. Through complex signaling mechanisms ABA triggers efflux of anions and potassium via guard cell plasma membrane ion channels, resulting in decrease of turgor pressure in guard cells and stomatal closure (Figure 1). Recent *in vitro* and *in vivo* studies have revealed the molecular mechanisms of how ABA signaling is initiated and transduced into the turgor regulation response in guard cells. Here we review recent advances on ABA signaling in guard cells.

ABA biosynthesis, degradation, and transport in guard cells

A recent work showed that guard cells are capable of autonomously synthesizing ABA [5], providing evidence for classical observations [6]. The wilting phenotype of the Arabidopsis *aba3-1* mutant that lacks the final step of ABA biosynthesis (the conversion of ABA-aldehyde to ABA) [7] was complemented by guard cell-targeted expression of *ABA3* [5], suggesting that guard cell-autonomous ABA synthesis is sufficient for low humidity-induced stomatal closure. Hydrolysis of ABA-GE by β-glucosidase AtBG1 is a mechanism proposed for a rapid concentration increase of ABA [8]. Interestingly, the *aba3-1* mutant exhibited an induced expression of the ABA-GE-hydrolyzing enzyme AtBG1, indicating a putative but incomplete compensatory effect for the lack of *de-novo* ABA synthesis [5]. ABA is inactivated either through hydroxylation and subsequent catabolic degradation pathways or by conjugation with glucose. The hydroxylation of ABA in Arabidopsis guard cells is catalyzed by CYP707A1, which encodes the key ABA 8'-hydroxylase [9]. There are also indications for ABA-glucosylating enzyme activities in guard cells [10,11].

Transport of ABA across membranes can be passive as described by the 'ionic trap model' [12]. Active ABA uptake into Arabidopsis guard cells has been reported through the ABC transporter ABCG40 [13]. Another ABCG gene, *ABCG22*, which is also highly expressed in guard cells, is required for proper regulation of stomatal movements [14]. However, ABA transport activity of ABCG22 has not been proven yet. Four members of the NRT/PTR family have been characterized as ABA-IMPORTING TRANSPORTERS (AIT), of which AIT1 was implicated to mediate ABA uptake into guard cells of inflorescence stems [15]. The ABA efflux transporter DETOXIFICATION EFFLUX CARRIER 50 (DTX50) is expressed in guard cells and a T-DNA insertion mutant of the *DTX50* gene exhibits a reduced water loss and ABA-hypersensitive stomatal closure [16]. FRET-based reporters for ABA (ABACUS, ABAleon) enable direct *in vivo* monitoring of ABA transport and the visualization of cytosolic ABA concentration ($[\mathrm{ABA}]_{\mathrm{cyt}}$) changes in real-time [17,18]. It was observed that guard cell $[\mathrm{ABA}]_{\mathrm{cyt}}$ increases in response to NaCl treatment or a humidity drop, but not in response to sorbitol [17]. Compared to other cells or tissues, guard cells exhibit increased $[\mathrm{ABA}]_{\mathrm{cyt}}$ [17]. Taken together with the guard cell autonomous ABA synthesis [5,6] and the expression of an ABA efflux transporter DTX50 [16] in guard cells, current data indicate that guard cells could also function as an ABA source.

Core ABA signal components in Guard Cells

The perception of ABA is achieved by members of the START protein family of ABA receptors, PYRABACTIN RESISTANCE 1 (PYR)/PYR1-LIKE (PYL)/REGULATORY COMPONENT OF ABA RECEPTOR (RCAR). In the presence of ABA, the PYR/PYL/

RCAR proteins bind to and inhibit clade A protein phosphatases type 2Cs (PP2Cs) [19,20], followed by activation of the Ca^{2+}-independent protein kinases SNF1-RELATED KINASE 2s (SnRK2s), most importantly OPEN STOMATA 1 (OST1/SnRK2.6), which phosphorylate multiple downstream targets (reviewed in [21]). In guard cells, ABA causes activation of two types of plasma membrane anion channels, called slow-sustained (S-type) and rapid-transient (R-type) anion channels, which drives plasma membrane depolarization and subsequent K^+ efflux through voltage-dependent K^+ channels (reviewed in [22–24]) (Figure 1). The release of anions and K^+ causes a reduction in the turgor pressure of guard cells, resulting in stomatal closure. In *Arabidopsis* guard cells, S-type and R-type anion channels are mainly encoded by *SLOW ANION CHANNEL-ACCOCIATED 1* (*SLAC1*) [25,26] and *ALUMINUM-ACTIVATED MALATE TRANSPORTER 12/QUICKLY ACTIVATING ANION CHANNEL 1* (*ALMT12/QUAC1*) [27,28], respectively. Several *in vitro* studies using *Xenopus laevis* oocytes show how the core ABA signaling module PYR/PYL/RCAR-PP2C-SnRK2 complex regulates SLAC1 and ALMT12/QUAC1 activity (Figure 1). In Arabidopsis guard cells, the GUARD CELL OUTWARD RECTIFYING K^+ CHANNEL (GORK) accounts for the voltage-dependent K^+ efflux channel (K^+_{out}) activity [29,30]. Involvement of K^+ UPTAKE TRANSPORTERs (KUPs) in guard cell K^+ efflux during stomatal closure was also recently reported [31].

ABA regulation of Ca^{2+} Signaling in Guard Cells

It has been demonstrated in several plant species that cytosolic Ca^{2+} functions as a second messenger in guard cell ABA signaling. ABA-induced S-type anion channel activation and stomatal closure are suppressed by cytoplasmic loading of a Ca^{2+} chelator, 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA) in intact guard cells [32,33]. Interestingly, it has been also shown that guard cells exhibit spontaneous cytosolic free Ca^{2+} ($[Ca^{2+}]_{cyt}$) elevations and ABA does not cause $[Ca^{2+}]_{cyt}$ elevations in all guard cells [34,35]. Furthermore, ABA enables $[Ca^{2+}]_{cyt}$ activation of S-type anion channels [33]. These observations suggest that ABA turns on guard cell Ca^{2+} signaling by enhancing (priming) $[Ca^{2+}]_{cyt}$ sensitivity of the downstream targets as well as by inducing $[Ca^{2+}]_{cyt}$ elevations [22,34,35]. It has been reported that Ca^{2+}-DEPENDENT PROTEIN KINASES (CPKs) function as Ca^{2+} sensors that mediate the Ca^{2+}-dependent regulation of S-type anion channels [36–39]. Recent *in vitro* and *in vivo* studies identified a mechanism of how the Ca^{2+}-CPK-dependent pathway is integrated with PYR/PYL/RCAR-PP2C-SnRK2 in guard cells (Figure 1).

ABA causes guard cell $[Ca^{2+}]_{cyt}$ elevations through activation of plasma membrane Ca^{2+}-permeable cation (I_{Ca}) channel and Ca^{2+} release from intracellular Ca^{2+} stores (reviewed in [22,35]). ABA activation of I_{Ca} channels requires PYR/PYL/RCAR ABA receptors [39,40], CPKs [36], and a receptor-like kinase GUARD CELL HYDROGEN PEROXIDE-RESISTANT1 (GHR1) [41]. The molecular mechanism of how ABA regulates Ca^{2+} release from intracellular Ca^{2+} stores remains to be clarified in detail.

Activation of S-type anion channel SLAC1 by protein kinases

Activation of S-type anion channels has been considered as a key step in stomatal closure. Several studies investigated the regulation of SLAC1 in *Xenopus* oocytes. When SLAC1 is expressed alone in *Xenopus* oocytes, no significant anion currents can be detected while co-expression of the Ca^{2+}-independent OST1 or several CPKs evokes large anion currents [37,39,42–44]. The importance of this central drought stress signaling mechanism is highlighted by the conservation of the SLAC1 activation mechanism by OST1 in several land plant species [45,46]. OST1 and CPKs phosphorylate the N-terminus of SLAC1 [37,42–44]. Mass spectroscopy approaches revealed that OST1 could phosphorylate SLAC1 at Serine (S) 59, S86, S113, and S120 *in vitro* [47]. S59 is also phosphorylated by CPK6 [44]. When S120 of the SLAC1 N-terminus is mutated to non-phosphorylatable Alanine (A), SLAC1 cannot be activated by OST1 anymore, but activation by CPKs is still intact in *Xenopus* oocytes [37,39,43,44,48]. A second key amino acid for the SLAC1 activation in oocytes, S59, is required for the activation by CPK5, CPK6, and CPK23 but not OST1 [39,44,48]. Research in *Arabidopsis* guard cells showed that for *in planta* ABA activation of S-type anion channels, either S59 or S120 of SLAC1 is sufficient for complete guard cell ABA responses and only mutating both of the S59 and S120 impairs guard cell ABA-responses [39]. The SLAC1 S120F mutation impairs stomatal closing in response to ozone, elevated CO_2, and low humidity [47,49], which might be due to the effect of the bulky phenylalanine residue.

Although functional reconstitution of ABA activation of SLAC1 in *Xenopus* oocytes has been achieved by co-expression of either the Ca^{2+}-dependent CPK6 or the Ca^{2+} independent OST1 protein kinase [44], it was recently found that disruption of either multiple CPKs or Ca^{2+}-independent SnRK2s causes impairment of ABA activation of S-type anion channels. Use of these higher order *cpk* and *snrk2* mutants suggests that *in planta* both Ca^{2+}-dependent and the Ca^{2+}-independent branches are required for intact stomatal ABA responses [39]. The molecular mechanism of this interdependence is still unknown and subject of future research.

The role of the cytosolic C-terminal region of SLAC1 in the regulation of SLAC1 activity is still under investigation. Reports show that the SLAC1 C-terminus can be phosphorylated by OST1 [42,43], but not by CPK6 and CPK23 [37,44]. Replacement of Threonine (T) 513 in the SLAC1 C-terminus by Aspartate, which mimics phosphorylation, results in constitutive current activation, indicating a regulatory role of the SLAC1 C-terminus T513 [48]. A SLAC1 T513A mutant channel is still activated by OST1 and CPKs [48], which suggests that T513 is not strictly required for phosphorylation dependent activation of SLAC1. The Aspartate mutation of SLAC1 T513D may also have a structural impact on the channel rendering it constitutively active. The determination of the function of the SLAC1 C-terminus for SLAC1 regulation requires further research.

SLAC1 activation and phosphorylation by all of the above-mentioned protein kinases are inhibited by PP2Cs, for example by ABA-INSENSITIVE 1 (ABI1) and PROTEIN PHOSPHATASE 2CA (PP2CA) [37,42–44,48]. Recent research revealed the mechanism by which PP2Cs inhibit SLAC1 activation by protein kinases: While OST1 kinase activity is directly regulated by PP2Cs [50,51], detailed biochemical analyses showed no evidence for a direct regulation of CPK protein kinase activity by ABI1 and PP2CA [39]. *In vitro* data showed that the PP2Cs could inhibit the activation of SLAC1 by CPKs via direct dephosphorylation of the channel [39,44]. It was initially reported that ABI1 is not able to dephosphorylate OST1-added phospho-groups at SLAC1 [43]. However, recent research shows that the OST1-phosphorylated SLAC1 N-terminus could be de-phosphorylated by PP2Cs [39,48]. The dual action of PP2Cs in directly down-regulating SnRK2.6/OST1 and SLAC1 ensures the tight and robust negative regulation of SLAC1 activity, a crucial mechanism ensuring plant stress adaption and resistance (Figure 1). *In planta* analyses revealed that GHR1, which encodes a receptor-like kinase localized on the plasma membrane, is involved in ABA- and hydrogen peroxide (H_2O_2)-induced stomatal closure [41]. GHR1 directly phosphorylates the SLAC1 N-terminus *in vitro* and activates the channel current in *Xenopus* oocytes. Although OST1, CPK6, and CPK23 activation of SLAC1 in *Xenopus* oocytes is inhibited by both PP2Cs, ABI1 and ABI2 [37,43,44], GHR1 activation of SLAC1 is inhibited by ABI2, but not ABI1 [41]. Together with a previous study [52], these results suggest different roles of each PP2C in guard cell ABA signaling. However, more detailed biochemical analyses are required to understand how PP2Cs down-regulate GHR1 activation of the SLAC1 channel.

The CBL-INTERACTING PROTEIN KINASE 23 (CIPK23) was also reported to activate SLAC1 in oocytes when the Ca^{2+} sensing CALCINEURIN-B-LIKE 1 and 9 (CBL1 and CBL9) are co-expressed [48], but the role of CIPK23 in guard cell ABA signaling was not confirmed *in planta*.

PP2Cs-based Ca^{2+} sensitivity priming mechanism in guard cells

As intracellular Ca^{2+} represents a universal second messenger, a key question is how Ca^{2+} signaling specificity is achieved in plant cells. Stomatal closing stimuli, such as ABA and CO_2 prime $[Ca^{2+}]_{cyt}$ sensitivity in guard cells, thus enabling $[Ca^{2+}]_{cyt}$ activation of S-type anion channels [33,53,54]. A recent *in planta* electrophysiological study found that disruption of PP2Cs causes non-specific $[Ca^{2+}]_{cyt}$ activation of S-type anion channels [39]. These findings reveal a first mutant in guard cells that causes constitutively primed $[Ca^{2+}]_{cyt}$ signaling. Thus PP2Cs function as a master regulator that ensures Ca^{2+} signal specificity in guard cells. PP2Cs have also been shown to down-regulate ABA activation of I_{Ca} channels [52] (Figure 1).

Evolution and conservation of guard cell ABA signaling

Molecular biological and genomic analyses provide evidence that the core ABA signaling pathway consisting of PYR/PYL/RCAR, PP2Cs and OST1-like kinases and its target genes was established during the transition from an aquatic to a terrestrial environment over 400 million years ago [45,55,56]. Functional analysis in *Xenopus* oocytes using the homologs from organisms lacking stomata such as liverwort or alga supports this model [46]. An alternate view of ABA-mediated control of stomatal aperture has also been proposed [57–59]. This view is based on data obtained in evolutionary younger fern and lycophytes which show that stomata in these organisms respond poorly to endogenous ABA with the leaf water potential being proposed as the major factor determining stomatal aperture in a passive-hydraulic mechanism [57,58]. Data on the functional role and evolution of stomata in bryophytes [60] appear to be more complex in particular since stomata are not present in all sub clades and current evolutionary models implicate the existence of three origins of stomatal emergence in tracheophytes [61]. Another layer of complexity is added by the dependence of ABA responsiveness on the developmental stage as stomata are relevant for desiccation of the spore capsule [62] and in hornworts stomata never close once they open [61]. CO_2 control of stomatal development has been observed in the fossil record and is considered to be an ancient trait in plants [63]. A recent study provides evidence that this CO_2 response requires intact ABA signaling, pointing to the hypothesis that ABA signaling itself evolved at the time of or before the developmental CO_2 response [63]. For stomatal closing responses, amplification of CO_2-induced stomatal closing by ABA was identified in classical studies [64] and studies have shown that partial CO_2 responses prevail in intact leaves of strongly ABA-insensitive mutants (e.g. [49]). The study of [63] also points to the open question whether or not CO_2 causes a rapid increase in the guard cell ABA concentration (within ca. 3 min of CO_2 exposure) or whether basal ABA signaling synergistically amplifies the CO_2 response as CO_2 and ABA target the same stomatal closing mechanisms. Further evidence for establishing the evolutionary timeline when functional ABA signaling gene expression appeared in guard cells could be helpful for further refinement of evolutionary models.

Regulation of stomatal movements by pathogens and interaction with ABA signaling

Open stomata represent main gateways for pathogen entry. Therefore, closing of stomata in response to pathogens and pathogen-associated molecular patterns (PAMPs) serves as the first line of defense against pathogen invasion [65]. Many studies have investigated the interplay of PAMP-mediated and ABA-mediated stomatal closure. Exposure of guard cells to pathogens and PAMPs activates S-type anion channels [66–69]. It has been shown that OST1 is required for stomatal closure induced by PAMPs including lipopolysaccharides [70], the flagellin peptide flg22 [69,70], and yeast elicitor (YEL) [68]. However it was also reported that flg22-induced stomatal closure is partially dependent on OST1 [71], and OST1 is not activated by flg22 [71] and YEL [68]. The ABA receptors PYL8/RCAR3 and PYL7/RCAR2 and the PP2C PP2CA are not involved in *Pseudomonas syringae* pv *tomato* (*Pst)*- and flg22-triggered stomata closing [72]. The PP2C ABI1 was reported as not involved in flg22-triggered stomatal closure [69], while an independent study shows ABI1 plays a role in YEL-mediated closure [68]. It is evident that further investigation is required for the role of early ABA signaling components including OST1 in biotic signaling in guard cells. The roles of CPKs in biotic signaling will also be of interest [67]. Roles of ABA biosynthesis in pathogen/MAMP-triggered stomatal closing are controversially discussed in Arabidopsis and tomato [69–71,73].

Reactive oxygen species (ROS) function as a second messenger in both guard cell ABA and biotic signaling. The NAD(P)H oxidases RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD) and RBOHF function in ABA-triggered ROS production and Ca^{2+} channel activation in guard cells [74]. Recent biochemical analyses revealed that phosphorylation of RBOHD at distinct sites by CPKs and BOTRYTIS-INDUCED KINASE 1 (BIK1) is required for stomatal immunity to bacteria [75,76], indicating that RBOH and the downstream ROS production are key for the signal interaction between ABA and biotic signaling in guard cells. The detailed mechanisms were recently reviewed by [2].

Chemical genetics identified a novel small molecule 5-(3,4-dichlorophenyl)furan-2-yl]-piperidine-1-yl-methanethione (DFPM) that interferes with ABA signaling, including in guard cells [77]. DFPM inhibits ABA-induced stomatal closure and S-type anion channel activation. DFPM does not inhibit ABA-dependent interaction of PYR1 and ABI1 and ABA activation of SnRK2 protein kinases [77]. DFPM suppresses imposed Ca^{2+} oscillation-induced stomatal closure, suggesting that DFPM targets the Ca^{2+}-dependent branch of ABA signaling. DFPM signaling requires *PHYTOALEXIN DEFICIENT4* (*PAD4*), and *ENHANCED DISEASE SUSCEPTIBILITY1* (*EDS1*). PAD4/EDS1 are known as central regulators of basal and effector-triggered immunity [77,78]. Furthermore, natural variation in the DFPM response among *Arabidopsis* accessions identified the immune-receptor-like VICTR (VARIATION IN COMPOUND TRIGGERED ROOT growth response) further establishing a link of DFPM to effector-triggered immune signaling [78]. Thus, DFPM provides a small molecule that enables specific dissection of crosstalk between an R-protein mediated-effector triggered immune responses and ABA signaling. Together these studies imply that multiple biotic signal inputs crosstalk with ABA signaling in guard cells.

Perspectives

Recent studies have proven that chemical control of ABA signaling is a promising potential strategy to improve drought tolerance of crop species. It was shown that synthetic ABA agonists can be utilized to induce stomatal closure and enhance drought tolerance [79,80]. In addition, a recent study elegantly proved the potential of controlling the ABA response by an engineered ABA receptor [81]. These findings also highlight the importance of basic research in this field towards addressing drought tolerance in crops. Because guard cells can synthesize ABA in a cell-autonomous manner and also take up ABA, a key question is which pathway is dominantly activated when guard cells respond to various stresses. Non-invasive single-cell imaging using ABA biosensors can answer this question in the future. Recent studies identified novel regulators that control plasma membrane localization and protein turnover of PYR/PYL/RCAR proteins [82,83], and their roles in guard cell ABA signaling remains to be determined. Single-cell metabolome and proteome analyses have identified an array of possible candidates as modulators of guard cell ABA and biotic signaling [84,85], but their function and regulation need to be investigated in detail. Electrophysiological studies using *Xenopus* oocyte systems have led to significant advances in our mechanistic understanding of guard cell ABA signaling and enable rapid testing of signaling mechanisms and models. However, observations from *in planta* analyses cannot be fully explained by these studies suggesting a more complex *in planta* network [39]. For example, *in planta* analyses demonstrate a strong dependence of Ca^{2+}-dependent signaling on the Ca^{2+}-independent ABA signaling pathway [39]. Along with oocyte electrophysiological and biochemical analyses, *in planta* analyses (e.g. higher-order mutants) will be required to understand *in vivo* guard cell ABA signaling.

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References

(* of special interest; ** of outstanding interest)

1. Kollist H, Nuhkat M, Roelfsema MRG. Closing gaps: linking elements that control stomatal movement. New Phytol. 2014; 203:44–62. [PubMed: 24800691]

2. Murata Y, Mori IC, Munemasa S. Diverse stomatal signaling and the signal integration mechanism. Annu Rev Plant Biol. 2015; 66:369–392. [PubMed: 25665132]

3. Finkelstein R. Abscisic acid synthesis and response. Arabidopsis Book. 2013; 11:e0166. http://dx.doi.org/10.1199/tab.0166. [PubMed: 24273463]

4. Nambara E, Marion-Poll A. Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol. 2005; 56:165–185. [PubMed: 15862093]

**5. Bauer H, Ache P, Lautner S, Fromm J, Hartung W, Al-Rasheid KA, Sonnewald S, Sonnewald U, Kneitz S, Lachmann N, et al. The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. Curr Biol. 2013; 23:53–57. This publication describes the ability of guard cells to autonomously synthesize ABA. Guard cells express genes for the ABA biosynthesis pathway and the authors propose the testable hypothesis that a rapid guard cell-autonomous ABA increase mediates the rapid stomatal closure initiation that occurs within 3 minutes of the low humidity stimulus. [PubMed: 23219726]

6. Cornish K, Zeevaart JAD. Abscisic acid accumulation by *in situ* and isolated guard cells of *Pisum sativum* L. and *Vicia faba* L. in relation to water stress. Plant Physiol. 1986; 81:1017–1021. [PubMed: 16664936]

7. Schwartz SH, Léon-Kloosterziel KM, Koornneef M, Zeevaart JA. Biochemical characterization of the aba2 and aba3 mutants in Arabidopsis thaliana. Plant Physiol. 1997; 114:161–166. [PubMed: 9159947]

8. Lee KH, Piao HL, Kim HY, Choi SM, Jiang F, Hartung W, Hwang I, Kwak JM, Lee IJ, et al. Activation of glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid. Cell. 2006; 126:1109–1120. [PubMed: 16990135]

9. Okamoto M, Tanaka Y, Abrams SR, Kamiya Y, Seki M, Nambara E. High humidity induces abscisic acid 8'-hydroxylase in stomata and vasculature to regulate local and systemic abscisic acid responses in Arabidopsis. Plant Physiol. 2009; 149:825–834. [PubMed: 19036833]

10. Dong T, Xu ZY, Park Y, Kim DH, Lee Y, Hwang I. Abscisic acid uridine diphosphate glucosyltransferases play a crucial role in abscisic acid homeostasis in Arabidopsis. Plant Physiol. 2014; 165:277–289. [PubMed: 24676855]

11. Liu Z, Yan JP, Li DK, Luo Q, Yan Q, Liu ZB, Ye LM, Wang JM, Li XF, Yang Y. UDP-glucosyltransferase71C5, a major glucosyltransferase, mediates abscisic acid homeostasis in Arabidopsis. Plant Physiol. 2015; 167:1659–1670. [PubMed: 25713337]

12. Boursiac Y, Léran S, Corratgé-Faillie C, Gojon A, Krouk G, Lacombe B. ABA transport and transporters. Trends Plant Sci. 2013; 18:325–333. [PubMed: 23453706]

13. Kang J, Hwang JU, Lee M, Kim YY, Assmann SM, Martinoia E, Lee Y. PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. Proc Natl Acad Sci U S A. 2010; 107:2355–2360. [PubMed: 20133880]

14. Kuromori T, Sugimoto E, Shinozaki K. Arabidopsis mutants of AtABCG22, an ABC transporter gene, increase water transpiration and drought susceptibility. Plant J. 2011; 67:885–894. [PubMed: 21575091]

15. Kanno Y, Hanada A, Chiba Y, Ichikawa T, Nakazawa M, Matsui M, Koshiba T, Kamiya Y, Seo M. Identification of an abscisic acid transporter by functional screening using the receptor complex as a sensor. Proc Natl Acad Sci U S A. 2012; 109:9653–9658. [PubMed: 22645333]

*16. Zhang H, Zhu H, Pan Y, Yu Y, Luan S, Li L. A DTX/MATE-type transporter facilitates abscisic acid efflux and modulates ABA sensitivity and drought tolerance in Arabidopsis. Mol Plant. 2014; 7:1522–1532. This publication is of special interest, as it reports a novel ABA exporter. [PubMed: 24851876]

**17. Waadt R, Hitomi K, Nishimura N, Hitomi C, Adams SR, Getzoff ED, Schroeder JI. FRET-based reporters for the direct visualization of abscisic acid concentration changes and distribution in Arabidopsis. eLife. 2014; 3:e01739. This paper developed a FRET-based ABA biosensor ABAleon and reports single-cell ABA imaging in guard cells and other cells types. The authors found that ABA concentration increases slowly in roots in response to salinity stress and upon adding exogenous ABA also observe ABA moving upwards in hypotcotyls and also from shoots to roots. [PubMed: 24737861]

**18. Jones AM, Danielson JA, Manojkumar SN, Lanquar V, Grossmann G, Frommer WB. Abscisic acid dynamics in roots detected with genetically encoded FRET sensors. eLife. 2014; 3:e01741. Like [16], this work also developed a FRET-based ABA biosensor called ABACUS. Using the biosensor, the authors found that transient applications of ABA to roots resulted in transient increases of ABA in the cytosol, indicating that imported ABA is rapidly sequestered, exported or metabolized in plant cells. [PubMed: 24737862]

19. Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E. Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science. 2009; 324:1064–1068. [PubMed: 19407143]

20. Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TF, et al. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science. 2009; 324:1068–1071. [PubMed: 19407142]

21. Weiner JJ, Peterson FC, Volkman BF, Cutler SR. Structural and functional insights into core ABA signaling. Curr Opin Plant Biol. 2010; 13:495–502. [PubMed: 20934900]

22. Kim TH, Böhmer M, Hu H, Nishimura N, Schroeder JI. Guard cell signal^{2+} transduction network: Advances in understanding abscisic acid, CO_2, and Ca signaling. Annu Rev Plant Biol. 2010; 61:561–591. [PubMed: 20192751]

23. Roelfsema MR, Hedrich R, Geiger D. Anion channels: master switches of stress responses. Trends Plant Sci. 2012; 17:221–229. [PubMed: 22381565]

24. Hedrich R. Ion channels in plants. Physiol Rev. 2012; 92:1777–1811. [PubMed: 23073631]

25. Negi J, Matsuda O, Nagasawa T, Oba Y, Takahashi H, Kawai-Yamada M, Uchimiya H, Hashimoto M, Iba K. CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. Nature. 2008; 452:483–486. [PubMed: 18305482]

26. Vahisalu T, Kollist H, Wang YF, Nishimura N, Chan WY, Valerio G, Lamminmäki A, Brosché M, Moldau H, Desikan R, et al. SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling. Nature. 2008; 452:487–491. [PubMed: 18305484]

27. Meyer S, Mumm P, Imes D, Endler A, Weder B, Al-Rasheid KA, Geiger D, Marten I, Martinoia E, Hedrich R. *At*ALMT12 represents an R-type anion channel required for stomatal movement in Arabidopsis guard cells. Plant J. 2010; 63:1054–1062. [PubMed: 20626656]

28. Sasaki T, Mori IC, Furuichi T, Munemasa S, Toyooka K, Matsuoka K, Murata Y, Yamamoto Y. Closing plant stomata requires a homolog of an aluminum-activated malate transporter. Plant Cell Physiol. 2010; 51:354–365. [PubMed: 20154005]

29. Ache P, Becker D, Ivashikina N, Dietrich P, Roelfsema MR, Hedrich R. GORK, a delayed outward rectifier expressed in guard cells of *Arabidopsis thaliana*, is a K^+-selective, K^+-sensing ion channel. FEBS Lett. 2000; 486:93–98. [PubMed: 11113445]

30. Hosy E, Vavasseur A, Mouline K, Dreyer I, Gaymard F, Porée F, Boucherez J, Lebaudy A, Bouchez D, Véry AA, et al. The *Arabidopsis* outward K^+ channel *GORK* is involved in regulation of stomatal movements and plant transpiration. Proc Natl Acad Sci USA. 2003; 100:5549–5554. [PubMed: 12671068]

31. Osakabe Y, Arinaga N, Umezawa T, Katsura S, Nagamachi K, Tanaka H, Ohiraki H, Yamada K, Seo SU, Abo M, et al. Osmotic stress responses and plant growth controlled by potassium transporters in. Arabidopsis Plant Cell. 2013; 25:609–624.

32. Levchenko V, Konrad KR, Dietrich P, Roelfsema MRG, Hedrich R. Cytosolic abscisic acid activates guard cell anion channels without preceding Ca^{2+} signals. Proc Natl Acad Sci U S A. 2005; 102:4203–4208. [PubMed: 15753314]

33. Siegel RS, Xue S, Murata Y, Yang Y, Nishimura N, Wang A, Schroeder JI. Calcium elevation-dependent and attenuated resting calcium-dependent abscisic acid induction of stomatal closure and abscisic acid-induced enhancement of calcium sensitivities of S-type anion and inward-rectifying K^+ channels in Arabidopsis guard cells. Plant J. 2009; 59:207–220. [PubMed: 19302418]

34. Laanemets K, Brandt B, Li J, Merilo E, Wang YF, Keshwani MM, Taylor SS, Kollist H, Schroeder JI. Calcium-Dependent and -Independent Stomatal Signaling Network and Compensatory Feedback Control of Stomatal Opening via Ca^{2+} Sensitivity Priming. Plant Physiol. 2013; 163:504–513. [PubMed: 23766366]

35. Hubbard KE, Siegel RS, Valerio G, Brandt B, Schroeder JI. Abscisic acid and CO_2 signalling via calcium sensitivity priming in guard cells, new CDPK mutant phenotypes and a method for improved resolution of stomatal stimulus-response analyses. Ann Bot. 2012; 109:5–17. [PubMed: 21994053]

36. Mori IC, Murata Y, Yang Y, Munemasa S, Wang YF, Andreoli S, Tiriac H, Alonso JM, Harper JF, Ecker JR, et al. CDPKs CPK6 and CPK3 function in ABA regulation of guard cell S-type anion- and Ca^{2+}- permeable channels and stomatal closure. PLoS Biol. 2006; 4:e327. [PubMed: 17032064]

37. Geiger D, Scherzer S, Mumm P, Marten I, Ache P, Matschi S, Liese A, Wellmann C, Al-Rasheid KAS, Grill E, et al. Guard cell anion channel SLAC1 is regulated by CDPK protein kinases with distinct Ca^{2+} affinities. Proc Natl Acad Sci U S A. 2010; 107:8023–8028. [PubMed: 20385816]

38. Munemasa S, Hossain MA, Nakamura Y, Mori IC, Murata Y. The Arabidopsis calcium-dependent protein kinase, CPK6, functions as a positive regulator of methyl jasmonate signaling in guard cells. Plant Physiol. 2011; 155:553–561. [PubMed: 20978156]

**39. Brandt B, Munemasa S, Wang C, Nguyen D, Yong T, Yang GP, Poretsky E, Belknap FT, Waadt R, Aleman F, et al. Calcium specificity signaling mechanisms in abscisic acid signal transduction in Arabidopsis guard cells. eLife. 2015; 4:e03599. This work identifies a first plant mutant that disrupts specificity in Ca^{2+} signal transduction and causes constitutive cytosolic Ca^{2+} signaling demonstrating that type 2C protein phosphatases control specificity in ABA-induced Ca^{2+} signaling in guard cells. Furthermore, using higher order mutants, this study reveals that Ca^{2+}-dependent signal transduction is dependent on the Ca^{2+}-independent SnRK2 protein kinase branch of guard cells during ABA signaling. Results suggest that these two pathways are not as clearly independent of one another as previously thought.

40. Wang Y, Chen ZH, Zhang B, Hills A, Blatt MR. PYR/PYL/RCAR abscisic acid receptors regulate K^+ and Cl^- channels through reactive oxygen species-mediated activation of Ca^{2+} channels at the plasma membrane of intact Arabidopsis guard cells. Plant Physiol. 2013; 163:566–577. [PubMed: 23899646]

**41. Hua D, Wang C, He J, Liao H, Duan Y, Zhu Z, Guo Y, Chen Z, Gong Z. A plasma membrane receptor kinase, GHR1, mediates abscisic acid- and hydrogen peroxide-regulated stomatal movement in *Arabidopsis*. Plant Cell. 2012; 24:2546–2561. In this research, the authors identified a plasma membrane receptor kinase, GHR1 that is required for ABA-, H_2O_2-, salicylic acid- and methyl jasmonate-induced stomatal closure. Similar to SnRKs and CDPKs, GHR1 participates in SLAC1 activation by direct phosphorylation. [PubMed: 22730405]

42. Lee SC, Lan W, Buchanan BB, Luan S. A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. Proc Natl Acad Sci U S A. 2009; 106:21419–21424. [PubMed: 19955427]

43. Geiger D, Scherzer S, Mumm P, Stange A, Marten I, Bauer H, Ache P, Matschi S, Liese A, Al-Rasheid KA, et al. Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase pair. Proc Natl Acad Sci U S A. 2009; 106:21425–21430. [PubMed: 19955405]

44. Brandt B, Brodsky DE, Xue S, Negi J, Iba K, Kangasjärvi J, Ghassemian M, Stephan AB, Hu H, Schroeder JI. Reconstitution of abscisic acid activation of SLAC1 anion channel by CPK6 and

OST1 kinases and branched ABI1 PP2C phosphatase action. Proc Natl Acad Sci U S A. 2012; 109:10593–10598. [PubMed: 22689970]

45. Hauser F, Waadt R, Schroeder JI. Evolution of abscisic acid synthesis and signaling mechanisms. Curr Biol. 2011; 21:R346–R355. [PubMed: 21549957]

*46. Lind C, Dreyer I, López-Sanjurjo EJ, von Meyer K, Ishizaki K, Kohchi T, Lang D, Zhao Y, Kreuzer I, Al-Rasheid KAS, et al. Stomatal guard cells co-opted an ancient ABA-dependent desiccation survival system to regulate stomatal closure. Curr Biol. 2015; 25:928–935. This report provides molecular evidence that SnRK2-mediated activation of SLAC1 appears first in mosses and is absent in more ancient species. In addition the data shown indicate that ABA-mediated transcriptional activation is evolutionary older and existing before the appearance of stomata and before the transition to land. [PubMed: 25802151]

47. Vahisalu T, Puzõrjova I, Brosché M, Valk E, Lepiku M, Moldau H, Pechter P, Wang YS, Lindgren O, Salojärvi J, et al. Ozone-triggered rapid stomatal response involves the production of reactive oxygen species, and is controlled by SLAC1 and OST1. Plant J. 2010; 62:442–453. [PubMed: 20128877]

48. Maierhofer T, Diekmann M, Offenborn JN, Lind C, Bauer H, Hashimoto K, Al-Rasheid KAS, Luan S, Kudla J, Geiger D, et al. Site- and kinase-specific phosphorylation-mediated activation of SLAC1, a guard cell anion channel stimulated by abscisic acid. Sci Signal. 2014; 7:ra86. [PubMed: 25205850]

*49. Merilo E, Laanemets K, Hu H, Xue S, Jakobson L, Tulva I, Gonzalez-Guzman M, Rodriguez PL, Schroeder JI, Broschè M, et al. PYR/RCAR receptors contribute to ozone-, reduced air humidity-, darkness-, and CO_2-induced stomatal regulation. Plant Physiol. 2013; 162:1652–1668. This report examines responses of many ABA signaling/metabolism mutants to environmental stimuli that cause stomatal closing including elevated CO_2, ozone and low humidity. The authors dissect differential roles of the ABA signaling components in closing stomata in response to diverse conditions. [PubMed: 23703845]

50. Umezawa T, Sugiyama N, Mizoguchi M, Hayashi S, Myouga F, Yamaguchi-Shinozaki K, Ishihama Y, Hirayama T, Shinozaki K. Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in *Arabidopsis*. Proc Natl Acad Sci U S A. 2009; 106:17588–17593. [PubMed: 19805022]

51. Vlad F, Rubio S, Rodrigues A, Sirichandra C, Belin C, Robert N, Leung J, Rodriguez PL, Laurière C, Merlot S. Protein phosphatases 2C regulate the activation of the Snf1-related kinase OST1 by abscisic acid in *Arabidopsis*. Plant Cell. 2009; 21:3170–3184. [PubMed: 19855047]

52. Murata Y, Pei ZM, Mori IC, Schroeder J. Abscisic acid activation of plasma membrane Ca^{2+} channels in guard cells requires cytosolic NAD(P)H and is differentially disrupted upstream and downstream of reactive oxygen species production in *abi1-1* and *abi2-1* protein phosphatase 2C mutants. Plant Cell. 2001; 13:2513–2523. [PubMed: 11701885]

53. Chen ZH, Hills A, Lim CK, Blatt MR. Dynamic regulation of guard cell anion channels by cytosolic free Ca^{2+} concentration and protein phosphorylation. Plant J. 2010; 61:816–825. [PubMed: 20015065]

54. Xue S, Hu H, Ries A, Merilo E, Kollist H, Schroeder JI. Central functions of bicarbonate in S-type anion channel activation and OST1 protein kinase in CO_2 signal transduction in guard cell. EMBO J. 2011; 30:1645–1658. [PubMed: 21423149]

55. Chater C, Kamisugi Y, Movahedi M, Fleming A, Cuming AC, Gray JE, Beerling DJ. Regulatory mechanism controlling stomatal behavior conserved across 400 million years of land plant evolution. Curr Biol. 2011; 21:1025–1029. [PubMed: 21658944]

56. Ruszala EM, Beerling DJ, Franks PJ, Chater C, Casson SA, Gray JE, Hetherington AM. Land Plants Acquired Active Stomatal Control Early in Their Evolutionary History. Curr Biol. 2011; 21:1030–1035. [PubMed: 21658945]

57. McAdam SAM, Brodribb TJ. Fern and lycophyte guard cells do not respond to endogenous abscisic acid. Plant Cell. 2012; 24:1510–1521. [PubMed: 22517320]

58. McAdam SAM, Brodribb TJ. Stomatal innovation and the rise of seed plants. Ecol Lett. 2012; 15:1–8. [PubMed: 22017636]

59. McAdam SAM, Brodribb TJ. The evolution of mechanisms driving the stomatal response to vapor pressure deficit. Plant Physiol. 2015; 167:833–843. [PubMed: 25637454]

60. Ligrone R, Duckett JG, Renzaglia KS. Major transitions in the evolution of early land plants: a bryological perspective. Ann Bot. 2012; 109:851–871. [PubMed: 22356739]

61. Pressel S, Goral T, Duckett JG. Stomatal differentiation and abnormal stomata in hornworts. J Bryol. 2014; 36:87–103.

62. Haig D. Filial mistletoes: the functional morphology of moss sporophytes. Ann Bot. 2013; 111:337–345. [PubMed: 23277472]

63. Chater C, Peng K, Movahedi M, Dunn JA, Walker HJ, Liang YK, McLachlan DH, Casson S, Isner JC, Wilson I, Neill SJ, Hedrich R, Gray JE, Hetherington AM. Elevated CO_2-induced responses in stomata require ABA and ABA signaling. Curr Biol. 2015; 25:2709–2716. [PubMed: 26455301]

64. Raschke K. Simultaneous requirement of carbon dioxide and abscisic acid for stomatal closing in Xanthium strumarium L. Planta. 1975; 125:243–259. [PubMed: 24435438]

65. McLachlan DH, Kopischke M, Robatzek S. Gate control: guard cell regulation by microbial stress. New Phytol. 2014; 203:1049–1063. [PubMed: 25040778]

66. Koers S, Guzel-Deger A, Marten I, Roelfsema MRG. Barley mildew and its elicitor chitosan promote closed stomata by stimulating guard-cell S-type anion channels. Plant J. 2011; 68:670–680. [PubMed: 21781196]

67. Ye W, Muroyama D, Munemasa S, Nakamura Y, Mori IC, Murata Y. Calcium-dependent protein kinase CPK6 positively functions in induction by yeast elicitor of stomatal closure and inhibition by yeast elicitor of light-induced stomatal opening in Arabidopsis. Plant Physiol. 2013; 163:591–599. [PubMed: 23922271]

68. Ye W, Adachi Y, Munemasa S, Nakamura Y, Mori IC, Murata Y. Open Stomata 1 Kinase is Essential for Yeast Elicitor-Induced Stomatal Closure in Arabidopsis. Plant Cell Physiol. 2015; 56:1239–1248. [PubMed: 25840086]

*69. Guzel Deger A, Scherzer S, Nuhkat M, Kedzierska J, Kollist H, Brosché M, Unyayar S, Boudsocq M, Hedrich R, Roelfsema MRG. Guard cell SLAC1 type anion channels mediate flagellin-induced stomatal closure. New Phytol. 2015; 208:162–173. This report measures the ion channel activity of intact guard cells after nano-infusing flg22 into the sub-stomatal cavity of Arabidopsis. The authors suggest an important role of OST1 in stomatal immunity since *ost1–2* fails to close stomata after nano-infusing flg22. [PubMed: 25932909]

70. Melotto M, Underwood W, Koczan J, Nomura K, He SY. Plant stomata function in innate immunity against bacterial invasion. Cell. 2006; 126:969–980. [PubMed: 16959575]

*71. Montillet JL, Leonhardt N, Mondy S, Tranchimand S, Rumeau D, Boudsocq M, Garcia AV, Douki T, Bigeard J, Laurière C, et al. An abscisic acid-independent oxylipin pathway controls stomatal closure and immune defense in Arabidopsis. PLoS Biol. 2013; 11:e1001513. This study reports a 9-lipoxygenase gene, *LOX1* and its oxylipin products play a major role in *Pst*- and flg22-triggerred stomatal closure. The authors suggest that contrasting early research on flg22 signaling, stomata immunity is independent of early ABA signaling including *OST1* and the ABA biosynthesis gene *ABA2*. [PubMed: 23526882]

72. Lim CW, Luan S, Lee SC. A prominent role for RCAR3-mediated ABA signaling in response to *Pseudomonas syringae* pv. tomato DC3000 infection in Arabidopsis. Plant Cell Physiol. 2014; 55:1691–1703. [PubMed: 25063782]

73. Du M, Zhai Q, Deng L, Li S, Li H, Yan L, Huang Z, Wang B, Jiang H, Huang T, et al. Closely related NAC transcription factors of tomato differentially regulate stomatal closure and reopening during pathogen attack. Plant Cell. 2014; 26:3167–3184. [PubMed: 25005917]

74. Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JD, Schroeder JI. NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. EMBO J. 2003; 22:2623–2633. [PubMed: 12773379]

75. Kadota Y, Sklenar J, Derbyshire P, Stransfeld L, Asai S, Ntoukakis V, Jones JD, Shirasu K, Menke F, Jones A, et al. Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. Mol Cell. 2014; 54:43–55. [PubMed: 24630626]

76. Li L, Li M, Yu L, Zhou Z, Liang X, Liu Z, Cai G, Gao L, Zhang X, Wang Y, et al. The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. Cell Host Microbe. 2014; 15:329–338. [PubMed: 24629339]

77. Kim TH, Hauser F, Ha T, Xue S, Bohmer M, Nishimura N, Munemasa S, Hubbard K, Peine N, Lee BH, et al. Chemical genetics reveals negative regulation of abscisic acid signaling by a plant immune response pathway. Curr Biol. 2011; 21:990–997. [PubMed: 21620700]

78. Kim TH, Kunz HH, Bhattacharjee S, Hauser F, Park J, Engineer C, Liu A, Ha T, Parker JE, Gassmann W, et al. Natural variation in small molecule-induced TIR-NB-LRR signaling induces root growth arrest via EDS1- and PAD4-complexed R protein VICTR in Arabidopsis. Plant Cell. 2012; 24:5177–5192. [PubMed: 23275581]

79. Okamoto M, Peterson FC, Defries A, Park SY, Endo A, Nambara E, Volkman BF, Cutler SR. Activation of dimeric ABA receptors elicits guard cell closure, ABA-regulated gene expression, and drought tolerance. Proc Natl Acad Sci USA. 2013; 110:12132–12137. [PubMed: 23818638]

80. Cao M, Liu X, Zhang Y, Xue X, Zhou XE, Melcher K, Gao P, Wang F, Zeng L, Zhao Y, et al. An ABA-mimicking ligand that reduces water loss and promotes drought resistance in plants. Cell Res. 2013; 23:1043–1054. [PubMed: 23835477]

**81. Park SY, Peterson FC, Mosquna A, Yao J, Volkman BF, Cutler SR. Agrochemical control of plant water use using engineered abscisic acid receptors. Nature. 2015; 520:545–548. In this work, an ABA receptor PYR1 was engineered to be activated by a commercial agrochemical mandipropamid, which is widely used as a fungicide. Transgenic Arabidopsis and Tomato plants that express the engineered receptor $\mathrm{PYR}^{\mathrm{MANDI}}$ exhibit ABA responses including enhanced drought tolerance when exposed to mandipropamid. This report provides a novel strategy to improve environmental stress tolerance of crop plants. [PubMed: 25652827]

*82. Bueso E, Rodriguez L, Lorenzo-Orts L, Gonzalez-Guzman M, Sayas E, Muñoz-Bertomeu J, Ibañez C, Serrano R, Rodriguez PL. The single-subunit RING-type E3 ubiquitin ligase RSL1 targets PYL4 and PYR1 ABA receptors in plasma membrane to modulate abscisic acid signaling. Plant J. 2014; 80:1057–1071. This work identified a single-subunit E3 ligase RSL1 that interacts with PYR1 and PYL4 at the plasma membrane and mediates protein turnover of the ABA receptors by ubiquitylation. [PubMed: 25330042]

**83. 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. 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. 2014; 26:4802–4820. This work identified C2-domain ABA-related (CAR) proteins that bind PYR/PYL/RCAR receptors and positively regulate ABA signaling. The C2 domain of the CAR proteins includes Ca^{2+} binding motifs that regulate Ca^{2+}-dependent targeting to cell membranes, suggesting that the CAR proteins translocate PYR/PYL/RCAR receptors to plasma membranes in response to $[\mathrm{Ca}^{2+}]_{\mathrm{cyt}}$ elevation. [PubMed: 25465408]

84. Jin X, Wang RS, Zhu M, Jeon BW, Albert R, Chen S, Assmann SM. Abscisic acid–responsive guard cell metabolomes of *Arabidopsis* wild-type and *gpa1* G-protein mutants. Plant Cell. 2013; 25:4789–4811. [PubMed: 24368793]

85. Zhu M, Zhu N, Song WY, Harmon AC, Assmann SM, Chen S. Thiol-based redox proteins in abscisic acid and methyl jasmonate signaling in *Brassica napus* guard cells. Plant J. 2014; 78:491–515. [PubMed: 24580573]

Highlight

- ABA triggers a robust signal network that controls stomatal closing
- Guard cell ABA levels are controlled by biosynthesis, catabolism, and transport
- SnRKs and CPKs are key for ABA activation of anion channels in guard cells
- PP2Cs down-regulate both Ca^{2+}-independent and Ca^{2+}-dependent ABA signaling branches
- Multiple biotic signal inputs target ABA signaling in guard cells

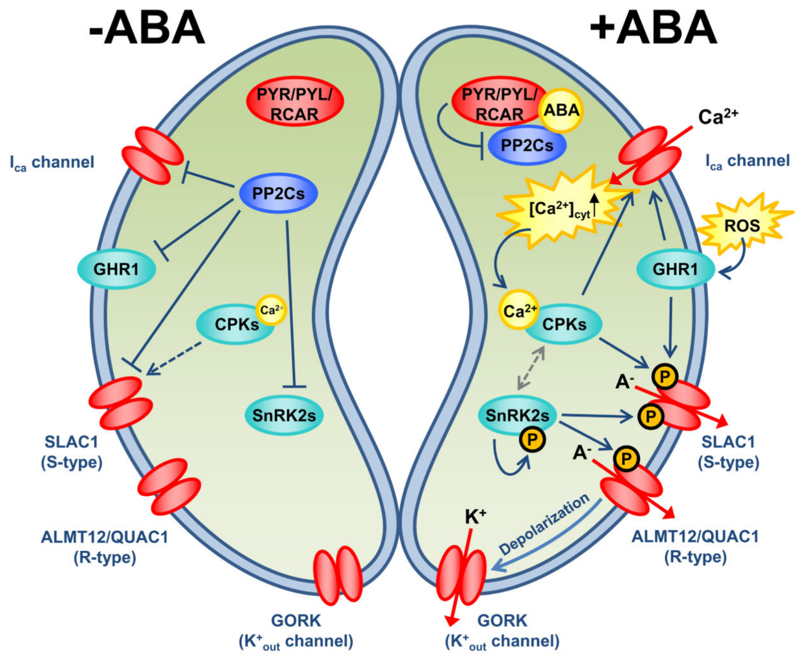

Figure 1. Schematic model of ABA signal transduction mechanisms in guard cells. (Left) In the absence of ABA, PP2Cs dephosphorylate SnRK2 protein kinases and the S-type anion channel SLAC1. Note that non-specific Ca^{2+} elevations are prohibited from activating stomatal closing mechanisms, as direct dephosphorylation of SLAC1 by PP2Cs prevents non-specific $[Ca^{2+}]_{cyt}$ activation of S-type anion channels [39]. **(Right)** In the presence of ABA, PYR/PYL/RCAR ABA receptors bind to and inhibit PP2Cs, followed by activation of the Ca^{2+}-independent protein kinases SnRK2s, including OST1, possibly by auto-phosphorylation. Hyperpolarization-dependent Ca^{2+}-permeable cation (I_{Ca}) channels are released from PP2C-dependent down regulation, resulting in ABA-responsive $[Ca^{2+}]_{cyt}$ increases that activate CPKs. CPKs also are required for activation of I_{Ca} channels. ABA-induced Ca^{2+} release from intracellular Ca^{2+} stores is not shown in this figure for simplicity and due to the need to further characterize the detailed signaling mechanisms. The active SnRK2s and CPKs phosphorylate SLAC1 with preferential affinities at different sites and activate the channel. The SnRK2 protein kinase OST1 also phosphorylates and activates the R-type anion channel ALMT12/QUAC1. *In planta* roles of CPKs and PP2Cs in ALMT12/QUAC1 regulation and possible direct cross-regulation of CPKs and SnRK2s need to be further investigated. Activation of the two types of anion channels causes sustained plasma membrane depolarization, which drives K^+ efflux through the voltage-dependent outward K^+ (K^+_{out}) channel GORK. The loss of K^+ and anion leads to guard cell turgor decrease and stomatal closure. NAD(P)H oxidase-mediated ROS production is involved in guard cell ABA signaling. The ROS possibly activate GHR1 that mediates ABA activation of I_{Ca} and S-type anion channels. Phosphorylation sites in SLAC1 mediated by GHR1 need to be identified.