Guard cell ABA and CO₂ signaling network updates and Ca²⁺ sensor priming hypothesis

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Stomatal pores in the epidermis of plants enable gas exchange between plants and the atmosphere, a process vital to plant life. Pairs of specialized guard cells surround and control stomatal apertures. Stomatal closing is induced by abscisic acid (ABA) and elevated CO₂ concentrations. Recent advances have been made in understanding ABA signaling and in characterizing CO₂ transduction mechanisms and CO₂ signaling mutants. In addition, models of Ca²⁺-dependent and Ca²⁺-independent signaling in guard cells have been developed and a new hypothesis has been formed in which physiological stimuli are proposed to prime Ca²⁺ sensors, thus enabling specificity in Ca²⁺-dependent signal transduction.

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Introduction

By regulating stomatal apertures, the rate of CO₂ diffusion into leaves is coordinated to best accommodate photosynthesis requirements while controlling transpirational water loss in plants. Guard cells are well suited for generic, cell biological, physiological and membrane-associated signal transduction studies with many investigative tools having been developed. Progress in understanding stomatal development, another rapidly moving area of research, has been recently reviewed and is not covered here [1,2]. Other reviews have recently appeared describing guard cell biology, CO₂ responses and the roles of mRNA-binding proteins in abscisic acid (ABA) signaling [3–8]. Here, we highlight new findings over the past few years, and what impact they have had on the understanding of ABA and CO₂ signaling in guard cells.

Abscisic acid signaling, mutants and mechanisms

The stress hormone ABA initiates stomatal closing and inhibits stomatal opening, thereby limiting water loss during drought conditions. Stomatal movements are induced by changes in the turgor pressure of guard cells, which are mediated by their ion and organic solute content. Interestingly, experiments utilizing an ABA-induced reporter gene system revealed that the application of drought stress to roots evoked the expression of an ABA reporter in the shoot [9**]. Christmann et al. [9**] proposed that drought stress might induce ABA synthesis in leaves rather than solely in roots. The ABA-INSSENSITIVE 1 (ABI1) and ABI2 genes encode PROTEIN PHOSPHATASE 2C (PP2C)-type proteins that negatively regulate ABA signaling [10,11]. Recently, an elegant analysis demonstrated biochemical interactions between OPEN STOMATA 1 (OST1/SnRK2E), a serine-threonine protein kinase that acts as a positive regulator in ABA signaling [12,13], and ABI1 [14**]. Yoshida et al. [14**] located the ABI1 binding site to the carboxy-terminal Domain II of OST1. ABI2 did not interact with OST1 [14**] and, in contrast to abi1-1, abi2-1 did not impair ABA activation of OST1 kinase activation [12,14**]. This led to the suggestion that ABI2 functions downstream of OST1, which correlates with previous results showing differential effects of abi1-1 and abi1-2 within the guard cell ABA signaling network [15]. The Vicia faba homolog of OST1, ABA-activated protein kinase (AAPK), interacts with the mRNA-binding protein AAPK-INTERACTING PROTEIN 1 (AKIP1) [16]. A study on the Arabidopsis homolog of AKIP1, UBPI INTERACTING PROTEIN 2a (UBA2a), showed that this protein, like AKIP, undergoes ABA-induced relocation to nuclear speckles [17]. However, this study also found no evidence for interaction of UBA2a with the OST1 kinase [17].

A pathway that integrates PHOSPHOLIPASE Dα1 (PLDα1) with ABI1 and the G-PROTEIN ALPHA SUBUNIT 1 (GPA1) in controlling stomatal aperture has been reported [18**]. In response to ABA, PLDα1 generates phosphatidic acid (PA), which is reported to bind to ABI1 [18**,19] and thereby abolish the ABI1 inhibition of ABA-induced stomatal closing [18**]. ABA-induced stomatal closure was abolished in a pldα1 knockout but this response was recovered in a pldα1 abi1
double knock-out mutant [18**], suggesting that PLDo1 is not required for the ABA response when ABI1 is knocked out. Data further suggest that PA and PLDo1 interact with GPA1 [18**] to transduce ABA inhibition of stomatal opening and that a ptds1 knock-out allele abolishes this ABA response [18**,19].

Biochemical evidence that a putative G-PROTEIN-COUPLED RECEPTOR 1 (GCR1) interacts with GPA1 in Arabidopsis was reported using an elegant combination of in vitro pull-down assays, yeast split-ubiquitin assays, and co-immunoprecipitation from plant tissue [20]. T-DNA insertional mutations in GPA1 disrupt ABA’s inhibition of both stomatal opening and guard cell inward potassium channel regulation, traits that are associated with an ABA-insensitive phenotype [21]. By contrast, ger1 mutants showed an ABA-hypersensitive phenotype, which included increased drought tolerance [20]. In guard cells, ABA induces production of the lipid metabolite sphingosine-1-phosphate [22], which might transduce the ABA signal by interacting with a receptor upstream of GCR1 (see model proposed in [20]). Although loss-of-function mutations that affect the GCR1 receptor cause ABA-hypersensitive responses in guard cells, these ger1 mutants show ABA insensitivity in seed germination and post-germination development [23]. Further analyses will be of interest to determine the underlying mechanisms of the opposing roles of GPA1 and GCR1 in stomatal responses and also responses to ABA during seed germination.

Genetic and cell signaling evidence for a role of reactive oxygen species (ROS) as second messengers in guard cell ABA signaling [24] was obtained by research on Arabidopsis double mutants in which the guard-cell expressed membrane proteins AtrbohD (Arabidopsis thaliana RESPIRATORY BURST OXIDASE PROTEIN D) and AtrbohF were disrupted [25]. These proteins are membrane-bound NADPH oxidase catalytic subunits [25]. The guard cells of atrbohD atrbohF double mutants were impaired in ABA-induced production of ROS, ABA-induced Ca\(^{2+}\)-permeable channel activation and stomatal closing, of which the latter two were restored by H\(_2\)O\(_2\) application [25]. Furthermore, both ABA and methyl jasmonate (MJ) were shown to promote stomatal closing through a mechanism that is dependent on ROS production and cytoplasmic alkalinization [26]. Induction of stomatal closing by MJ was strongly impaired in atrbohD atrbohF double mutants [26]. Ozone is another stimulus that closes stomatal pores. A genetic analysis of ozone responses in guard cells showed that a strong ozone-induced oxidative burst is dependent on heterotrimeric G proteins, and that the spread of ROS production to adjacent cells is impaired in atrbohD atrbohF plants [27]. A recent review on ROS signaling discusses other recent studies that have analyzed the roles of ROS in guard cell signaling in greater depth [28].

Work on the nitric oxide synthesis-related 1 (nos1) mutant and isolated mitochondria in Arabidopsis revealed that NOS1 is a mitochondrial enzyme [29]. NOS1 is involved in nitric oxide (NO) signaling and protects against oxidative damage. NO promotes the release of Ca\(^{2+}\) from intracellular stores. This, in turn, downregulates cytoplasmic Ca\(^{2+}\)-regulated potassium influx channels and activates S-type anion channels, but fails to regulate the Ca\(^{2+}\)-insensitive potassium efflux channels [30]. Thus, in accordance with previous data on NO–ABA interactions [31], NO modulates a subset of ABA-induced signaling pathways. Recently, ABA-induced NO generation was reported to depend on the induction of ROS production by ABA [32**]. Bright et al. [32**] found that NO synthesis and ABA-induced stomatal closing were severely impaired in the atrbohD atrbohF double mutant, but they also emphasized the likelihood of an ABA-H\(_2\)O\(_2\) signaling branch that is divergent from NO signaling. It has been proposed that the ordering of events in linear pathways, though helpful for models, might be a simplification because many studies suggest that ABA signal transduction is mediated by a signaling network [3,33,34].

Support for the guard cell signaling network model also comes from an elegant genetic screen that isolated two Arabidopsis mutants that are impaired in leaf temperature increases and concomitant stomatal conductance decreases in response to humidity reduction [35**]. Interestingly, mutations in genes that function in ABA biosynthesis (e.g. aba2) or ABA signaling (e.g. ost1) were isolated because they conferred the inability to reduce stomatal conductance in response to low humidity transitions [35**]. The participation of ABA signaling in the response of stomata to low humidity suggests that drought and humidity can affect the same signaling proteins in the guard cell drought response. Results from this genetic screen [35**] are in contrast to those of a previous report in which ABA-deficient and insensitive mutants showed a wildtype stomatal conductance response to both increased and decreased humidity [36].

The development of cell-type-specific microarray data for guard cells and mesophyll cells has enabled the selection of genes that are expressed in guard cells from among large gene families, easing the problem of gene redundancy during mutant analysis in Arabidopsis [37]. For example, mutation of one of the most strongly ABA-induced PP2C genes in guard cells, AtPP2C-HA (also named HAB1 [HOMOLOGY TO ABI1/ABI2]), revealed a recessive ABA-hypersensitive phenotype in stomatal closing and seed germination [37,38]. Two forward genetic screens led to the identification of mutations in a different ABA-regulated PP2CA gene, AtPP2CA, which also cause an ABA hypersensitivity during seed germination [10,39] that is stronger than that caused by T-DNA insertion mutations in the ABI1 and ABI2 PP2C genes [10]. Overexpression of PP2CA leads to a strong ABA
ABA regulation of stomatal movements includes regulation of K⁺ channels. A knockout study of the GUARD CELL ABA regulation of stomatal movements includes regulation of photosystem II during light stress, thereby mediating ABA-induced photo-protection in Arabidopsis. In abi1-1 but not in abi2-1, fibrillin transcript levels are smaller than those of wildtype plants. On the other hand, abi2-1 showed enhanced fibrillin levels, suggesting a posttranscriptional control mechanism. Such a mechanism is supported by protein–protein interaction studies which revealed that ABI2 specifically recognizes the signal peptide of the prefibrillin. ABI1 and ABI2 have also been shown to bind preferentially to different members of the SALT OVERLY SENSITIVE 2 (SOS2)-like protein kinases in Arabidopsis [41].

ABA regulation of guard cell K⁺ channel activity (IK,in) involves the interaction of a number of putative regulatory proteins with the membrane-anchored K⁺ channel(s). Two recent studies [42,43] found that KAT1 resides within microdomain clusters at the plasma membrane of guard cells that showed IK⁺ sensing and the disappearance of IK⁺ channel activity [44]. Analyses in tobacco leaf cells have revealed that the inward-rectifying Arabidopsis K⁺ channel (KAT1) is dependent on SNAREs for delivery to the plasma membrane [43**]. Furthermore, Sutter et al. [43**] found that KAT1 resides within microdomain clusters at the plasma membrane.

A recent study showed that the heterologously expressed IK⁺ channel in Arabidopsis 'senses' extracellular K⁺ such that it becomes inactive at very low K⁺ concentrations (K_D ≈ 20 μM K⁺), causing the disappearance of IK⁺ channel activity [45]. This is an important property of IK⁺ channels, which could otherwise allow high rates of K⁺ efflux leakage when extracellular K⁺ levels are depleted. These findings correlate with research on Vicia faba guard cells that showed IK⁺ sensing and the disappearance of IK⁺ channel activity when extracellular K⁺ was removed, and small inward and outward K⁺ currents close to the K⁺ equilibrium potential at 300 μM external K⁺ [46]. The findings that IK⁺ channels can sense extracellular K⁺ contradict other experiments that appear to have been done without added extracellular Ca²⁺ [47], a blocker/interactor of IK⁺ channels. It is possible, therefore, that extracellular Ca²⁺ has a role in IK⁺ channel sensing of low external K⁺ concentrations that might be worth analyzing, although other experimental parameters might also explain these interesting conflicting reports of K⁺ sensing [45,46] and non-K⁺ sensing [47]. A recent study has identified TWO PORE CHANNEL 1 (TPC1) as a gene that encodes an essential subunit of the ubiquitous slow vacuolar channels [48**]. TPC1 functions in Ca²⁺-induced stomatal closing and in the inhibition of seed germination by ABA.

The mechanisms of ABA perception in classical stress-related ABA responses remain unknown. At present the only characterized Arabidopsis protein that has ABA-binding activity is the RNA-binding protein FLOWERING TIME CONTROL PROTEIN (FCA) [49]. Interestingly, ABA binding to FCA was reported to mediate the inhibition of flowering [49], whereas drought stress usually causes early flowering in Arabidopsis [50]. FCA does not regulate stomatal movements nor does it operate through the ABI1-1 and ABI2-1 proteins [49]. The transmembrane leucine-rich repeat protein RECEPTOR-LIKE KINASE 1 (RPK1) has been implicated in early ABA signaling. rpk1 T-DNA mutants show reduced ABA sensitivity in seed germination, root growth and stomatal closure, indicating that RPK1 acts as a positive regulator of ABA signal transduction [51]. Further research can show where RPK1 functions relative to other components of early signal transduction. Evidence for extracellular perception of ABA has been reported by several laboratories (for detailed review [52]), and a recent study reported ABA binding at the guard cell plasma membrane [53]. To date, however, four laboratories analyzing guard cell responses have reported evidence for intracellular perception of ABA ([54**]; reviewed in [34,52]). Further research will be needed to sort out the underlying biochemical and genetic mechanisms of ABA perception. As for ABA degradation, the P450 CYP707A family of cytochromes has been identified as the enzymes that are responsible for the first step in ABA degradation [55,56], and CYP707A has been implicated specifically in dehydration–dehydration responses [57].

**Drought avoidance and stomatal ABA responses**

Previous research has shown that disruption of the Arabidopsis gene that encodes the farnesyl transferase β subunit, ENHANCED RESPONSE TO ABSCISIC ACID1 (ERA1), causes ABA hypersensitive anion and Ca²⁺ channel regulation, stomatal closing and reduced transpirational water loss (i.e. drought avoidance) during drought stress [58,59]. Antisense repression of the α- or β-subunit of farnesyl transferases in Brassica napus, driven by the drought-inducible promoter rd29A, was shown to reduce stomatal conductance under stress conditions and to enhance yields [60]. Stress-inducible inhibition of farnesylation therefore provides an approach for engineering drought avoidance in field-grown plants. In other research, double knockout mutants in which the negative regulators PP2CA [10,39] and HAB1 [37,38] are
impaired show a substantial increase in drought avoidance [11]. By contrast, single mutants in these PP2Cs show no clear or only weak drought avoidance phenotypes, indicating that drought avoidance can be ‘titrated’ by down-regulating multiple PP2Cs under drought conditions [11]. Several ABA-hypersensitive mutants not only amplify the response to a given ABA concentration but also respond to lower ABA concentrations, which correlates with reduced water loss from mutant plants during drought stress [3,61]. By contrast, many of the ABA-insensitive mutants characterized to date show partial ABA insensitivities in stomatal closing, with no clearly reported or dramatic shift in the ABA dose–response curve. Also, ion channel activities in guard cells are roughly five times (or more) greater than the physiologically required ion transport activities (e.g. [62]), and many mutants show significant residual ion-channel currents in the presence of ABA. Given the slow onset of drought and of ABA biosynthesis in whole intact plants, the residual ABA responses of mutants that are partially insensitive to ABA might be expected not to affect water loss and wilting of intact plants dramatically.

**New mechanisms in guard cell CO₂ signaling**

Stomata in the leaf surface open at low CO₂ concentrations ([CO₂]) and close at high [CO₂] [63]. Recently, experiments have suggested that an aquaporin can mediate the transport of CO₂ into guard cells [64]. ABA and CO₂ signaling share many ion-transport mechanisms, suggesting interaction between the two pathways (see [8,63] for reviews). Total convergence of ABA and CO₂ signaling is not supported, however, because the ABA-insensitive mutant ostl shows CO₂-induced stomatal movements [12] and no strong CO₂-signaling deficient mutants have been identified in plants.

A genetic screen for Arabidopsis mutants that have altered CO₂ responses was conducted by analyzing leaf temperature changes that were monitored by leaf thermography [65**]. This screen isolated two allelic mutations in HIGH LEAF TEMPERATURE 1, hlt-1 and hlt-2, that alter the mutants’ ability to control stomatal movements in response to CO₂. The strong hlt-2 allele causes marked impairments in stomatal movements, resulting in a constitutive high-[CO₂] response and suggesting that the HT1 kinase functions as a central negative regulator of high-[CO₂] signaling (Figure 1). Interestingly, hlt alleles show functional responses to blue light, fusicoccin and ABA, indicating an upstream role for HT1 in stomatal CO₂ signaling (Figure 1). The constitutive high-[CO₂] response in hlt-2 is reminiscent of the phenotype of constitutive triple response 1 (ctr1) mutant, which is affected in kinase function in ethylene signaling: loss of CTR1 function causes constitutive ethylene signaling.

The ABA-insensitive mutant growth control by aba2 (gca2) has recently been identified as a mutant that is strongly insensitive to high [CO₂] [66**]. gca2 mutants have a
strong CO₂ signaling phenotype: regulation of the cytosolic calcium ([Ca²⁺]₉₅) transient pattern by high [CO₂] is impaired in the guard cells of gca2 mutants, as is the induction of stomatal closure and decreased stomatal conductance by high [CO₂] in intact gca2 plants. These data suggest that the ABA and CO₂ signaling networks converge at the level of GCA2 and downstream ion channel (reviewed in [8,63]) regulation (Figure 1).

Photosynthesis has been suggested to partake in red-light-induced stomatal opening via the photosynthesis-derived decrease in intercellular leaf space [CO₂] [67,68]. A recent study suggests a role for photosynthesis in the regulation of stomatal movements by CO₂, but also suggests an additional CO₂-independent pathway that mediates red-light-induced stomatal opening [69]. The stomata of norflurazon-treated albino Vicia faba leaves that lack chlorophyll did not, however, open in response to red light but responded to blue light and changes in [CO₂] [70]. Chlorophyll-deficient leaves can sense changes in [CO₂], and so it can be concluded that CO₂ perception is not, at least not exclusively, linked to photosynthetic processes.

Abscisic acid: Ca²⁺-dependent and Ca²⁺-independent signaling pathways

ABA has been shown to shift the voltage dependence of plasma membrane Ca²⁺ permeable ‘IC₉₅’ cation channels to more positive potentials, thus enhancing Ca²⁺ influx [71]. Nevertheless, studies suggest that the ABA-activated IC₉₅ channels mainly come into play when the membrane potentials of guard cells are at more negative voltages [15,24,25,71,72] (but see also voltage dependencies in Vicia faba in [73]). Klüsener et al. [74] showed that when spontaneous [Ca²⁺]₉₅ transients [75] were present in guard cells, subsequent addition of ABA damped the transients in 60% of the cells. Studies have shown that ABA and high [CO₂] depolarize guard cells [34,76–78] and, in turn, that depolarization dampens the cytosolic Ca²⁺ transient rate in guard cells ([66**,74,79,80]; Figure 1). Therefore, ABA and high [CO₂]-induced damping of [Ca²⁺]₉₅ transients [66**,74,79] is consistent with a strong stimulus-induced depolarization of guard cells caused by anion channel activation and reduced proton pumping in response to ABA or high [CO₂] (Figure 1; [34,76,81]).

Results from imposed Ca²⁺ elevation patterns (Figure 2b,d; [82–84]) have in some cases been over-interpreted to propose that only certain Ca²⁺ elevation patterns will close stomatal pores [85]. Regardless of the Ca²⁺-elevation pattern, experimentally imposed cytosolic calcium transients above a threshold in Arabidopsis and Vicia faba guard cells triggered rapid Ca²⁺-reactive stomatal closing ([82–84]; [http://www.nature.com/naturejournal/v411/n6841/supplinfo/41111053a0.html]). However, the long-term inhibition of the re-opening of stomata depended on the preceding imposed [Ca²⁺]₉₅ elevation pattern rather than on Ca²⁺-induced stomatal closing itself [82–84]. Thus, the ABA- and CO₂-induced slowing of Ca²⁺ transients upon depolarization [66**,74,79,80] might function in the long-term inhibition of stomatal re-opening (also referred to as ‘long-term Ca²⁺-programmed stomatal closure’) [82–84].

Cell to cell variability in [Ca²⁺]₉₅ responses to ABA has been a persistent phenomenon in the field, dating back to the first guard cell [Ca²⁺]₉₅ measurements 16 years ago. This might, in part, reflect variation in the membrane potential of guard cells. At the same time, the lack of [Ca²⁺]₉₅ transients in guard cells recorded in many studies is an important and puzzling observation that might also indicate a Ca²⁺-independent pathway. Previous studies have supported a model that includes Ca²⁺-independent ABA and CO₂ signaling pathways ([54**,66**,86,87]; for reviews see [88,89]). To make further progress in distinguishing Ca²⁺-independent from Ca²⁺-dependent signaling, genetic mutants that function in Ca²⁺-independent ABA regulation of stomatal movements must be characterized.

Improved Ca²⁺ imaging and new Ca²⁺ sensor priming model for Ca²⁺ signaling specificity

Use of cameleon Ca²⁺ indicators allows ratiometric real-time monitoring of changes in cytosolic Ca²⁺ concentrations over long periods of time (e.g. more than 2 hours) and at indicator concentrations that are low relative to those of other ratiometric [Ca²⁺]₉₅ reporters, including the highly developed bis-(o-aminophenoxy)ethane-N,N,N’,N’-tetra-acetic acid (BAPTA)-derived indicators fura-2 and indo-1 [90]. A recent study implied that Cl⁻-sensitive cameleon isofoms have been used in plant-cell imaging [85]. However, the ‘clomeleon’ isoform used in most of the experiments in that study [85] utilizes a version of yellow fluorescent protein (YFP) that does not contain the modifications present in yellow cameleon (YC)2.1 that reduce Cl⁻ sensitivity. Furthermore, the ratio changes observed were ≈8-fold smaller [85] than the calcium-dependent ratio changes reported in other studies. YC2.1 cameleons that have reduced pH and Cl⁻ sensitivity have been used in all other studies in plants [75]. Plant YC2.1 cameleon studies included extensive control experiments to ensure that observed changes in cameleon fluorescence and physiological responses resulted from [Ca²⁺]₉₅ changes [82] and not from changes in external [Cl⁻] (http://www.nature.com/naturejournal/v411/n6841/supplinfo/41111053a0.html; Figure 2). New cameleon isofoms also make use of pH-/Cl⁻-desensitized YFP isofoms [91] and hold promise for future Ca²⁺ imaging in plant cells [92**].

Long-term [Ca²⁺]₉₅ imaging using cameleons has led to the resolution of spontaneously occurring Ca²⁺ transients in guard cells [75]. A switch from high [CO₂], which
mediates stomatal closing, to low [CO₂], which mediates stomatal opening, caused an increase of Ca²⁺ transients in Landsberg erecta guard cells [66**], which is consistent with low [CO₂]-induced hyperpolarization ([74–76,79]; Figure 1). High [CO₂] and ABA utilize Ca²⁺ to mediate stomatal closure (for reviews see [3,4,7]), so how can low [CO₂] induce stomatal opening by causing rapid [Ca²⁺]cyt transients [66**]? Could it be that the stomatal opening signal, low [CO₂], deactivates a Ca²⁺ sensor that mediates stomatal closing by reducing its Ca²⁺ sensitivity or affecting its localization (Figure 1; [66**])? In this ‘Ca²⁺ sensor priming hypothesis’, the de-primed Ca²⁺ sensor at low [CO₂] would not permit strong Ca²⁺-induced stomatal closure to proceed. Additional data in support of this hypothesis have been obtained in analyses of [Ca²⁺]cyt activation of S-type anion channels in Arabidopsis guard cells [58]. This study showed that prior incubation conditions could de-prime (turn off) or prime (turn on) the cytosolic [Ca²⁺] activation of S-type anion channels (Figure 3).

Some studies have also suggested a role for cytosolic Ca²⁺ in stomatal opening [66**,93,94]. The proposed sensor priming hypothesis [66**] provides a new model for specificity in Ca²⁺ signaling in plants, and perhaps in other eukaryotes. According to this hypothesis, a stimulus modulates (primes) the appropriate Ca²⁺ sensors, enabling specificity and diverse responses to Ca²⁺ elevations. The large number of Ca²⁺-dependent responses in plants, and the unusually large number of Ca²⁺-binding proteins represented in plant genomes [95], also supports the proposed need for stimulus-dependent priming and de-priming of Ca²⁺ sensors. Negatively regulating Ca²⁺ sensors that are involved in ABA and

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**Figure 2**

External chloride insensitivity of YC2.1 cameleons. (a) Diagram of the classical GFP chromophore environment, showing the closely associated and interacting binding sites for H⁺ and Cl⁻ before the YFP modifications V68L and Q69K, which render YC2.1 less sensitive to H⁺ and Cl⁻ [75,80]. The chromophore is circled. (Reprinted with permission from [101]. Copyright 1996, American Association for the Advancement of Science [AAAS].) (b) Upper panel: cameleon fluorescence changes are induced by alternating extracellular perfusion of a low Ca²⁺ + high KCl buffer (black bars) or a high Ca²⁺ + low KCl buffer (white bars). Lower panel: cameleon fluorescence ratio changes are inhibited when the same chloride-containing buffers are used but with 10 mM EGTA (ethyleneglycol-bis[2-aminoethyl] N,N,N',N'-tetraacetic acid) added to chelate most of the residual external calcium. Removal of the EGTA at the end of trace shows Ca²⁺-responsiveness of the cell. (b,c) Reprinted from supplementary data from http://www.nature.com/nature/journal/v411/n6841/suppinfo/4111053a0.html with permission from Macmillan Publishers Ltd: Nature [82], copyright 2001. (c) Calcium chelation inhibits stomatal movements induced by the buffer exchanges shown in (b). Bars labeled as (i) and (ii) in (c) correspond to experiments as labeled (i) and (ii) in (b). (d) Repetitive, transient increases in cameleon fluorescence are also induced when solutions used in upper panel of (b) are altered such that Cl⁻ is replaced by the impermeant anion iminodiacetate [102], further demonstrating that fluorescence ratio changes are not due to Cl⁻ ions but to Ca²⁺ changes.
stress signaling have been characterized [96–98]. In guard cells, however, we need to determine the molecular identities of positively transducing Ca2+ sensors that function as Ca2+ switches for the different known stomatal Ca2+ responses, including Ca2+-reactive stomatal closure, long-term Ca2+-programmed stomatal closure [82–84] and stomatal opening [66**,93,94]. (Note that a distinction has been discussed between ‘Ca2+ switches’ and ‘Ca2+ signature-mediated’ responses [85], but this appears to be semantic because even Ca2+ ’signature’-dependent responses are mediated by biochemical Ca2+ sensors [i.e. ’switches’] [99,100]. We have therefore avoided the otherwise attractive term ’signature’ in previous research reports to avoid such ambiguities). A recent study has identified Ca2+ sensors that function as positively transducing switches of ABA signaling in guard cells [92**]. Double mutant alleles in the Ca2+-dependent protein kinases (CDPKs) CPK6 and CKP3 impair ABA- and calcium-activation of S-type anion channels. Unexpectedly, they also impair ABA activation of Ca2+-permeable channels. These CDPK mutants show partial impairment in ABA-induced and Ca2+-reactive stomatal closing [92**]. Further research in guard cells could be useful to test the Ca2+-sensor priming hypothesis [66**] for plant cell Ca2+ signaling.

Conclusions

Many new advances have been made in characterizing ABA and CO2 signal transduction mechanisms. Studies have shown that the guard cell signaling network has quantifiable ion-channel targets and rate-limiting hubs [5] that provide islands for signal transduction analyses. Analyses of individual guard cell signal transduction branches are elucidating important new mechanisms in ion channel regulation and signal transduction in plant cells. This research field will continue to furnish new insights and surprises, and promises to continue to flourish.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

This study suggests that drought stress in roots causes ABA synthesis in shoots rather than in roots. Thus, an unknown signal is proposed to signal drought stress from roots to shoots.


An elegant genetic screen using leaf thermography identified mutated genes that are affected in ABA biosynthesis (ABA2) and ABA signaling (OST1) as rate-limiting genetic components in stomatal humidity responses. The identification of ABA-related genes in mediating humidity responses supports the existence of a signal transduction network to control stomatal closing and shows that ABA synthesis and ABA signaling mechanisms are required for the humidity response.


On the basis of previous findings that SNAREs are important for ABA-induced K+-channel regulation, the authors used a photoactivatable green fluorescent protein (GFP) system to analyze how the inward K+-channel KAT1 is delivered to the plasma membrane. Impairment of SNAREs by expression of a dominant-negative truncated form of the SNARE SYNTAXIN121 disrupts KAT1 delivery. Furthermore, elegant cell biology work demonstrates how KAT1 is confined to positionally stable microdomains at the plasma membrane.


49. The TPC1 gene is shown to encode an essential subunit of plant Ca2+-activated Ca2+-permeable SV channels. As SV channels have been described in most plant vacuoles, this study opens the door to analyses of the functions of the ubiquitous SV channels in many tissues and responses.


A function of RPK1 in early ABA signalling is reported on the basis of RPK1 T-DNA insertion, anti-sense and overexpression mutants of Arabidopsis. The authors show that RPK1 impairment disrupts ABA signalling, as detected among several ABA responses and during ABA-induced transcription changes.


Experiments using external application or direct injection of ABA into guard cells show an ABA response on inward current activation within seconds of ABA injection, but there is a 2 min lag in the response to external ABA. Vicia faba guard cells that are injected with the BAPTA analog fura2 show no ABA-induced Ca2+ elevations during signalling, but microinjection of the Ca2+ chelator BAPTA inhibits ABA responses, indicating a requirement for cytosolic Ca2+ in mediating ABA signalling.


66. A genetic screen based on leaf thermography that monitors differences in leaf temperature at low [CO2] identified the HT1 kinase as a major molecular component of CO2 regulated stomatal movements. The HT1 kinase was shown to negatively regulate high [CO2]-induced stomatal closing. ht1 mutant plants exhibited functional responses to blue light, fusocinoc and ABA.


The gca2 mutant is shown to be strongly insensitive to CO2. This paper further shows that repetitive cytosolic calcium transients occur in guard cells that are exposed to either elevated [CO2] or low [CO2]. A Ca2+-dependent and an early Ca2+-independent phase of the CO2 response are resolved in this study. Elevation of [CO2] causes a slowing of the cytosolic Ca2+ transient rate, and the authors propose that this is caused by CO2-induced depolarization. The Ca2+ dependence of the CO2 responses...
together with the cytosolic Ca\(^{2+}\) elevations at low [CO\(_2\)] and high [CO\(_2\)] lead the authors to propose a hypothesis in which the physiological stimulus CO\(_2\) primes the appropriate Ca\(^{2+}\) sensors, thus allowing specific downstream responses.


