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Guard cells: a dynamic signaling model

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The year 2003 has provided a continuing accretion of knowledge concerning the diverse ways in which guard cells sense and respond to abscisic acid. A deeper understanding of the biochemical mechanisms governing the response of guard cells to blue light has been gained, and new insights have been garnered regarding roles of the extracellular matrix in stomatal regulation.

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Abbreviations

$[Ca^{2+}]_{cyt}$	cytosolic Ca^{2+}
ABA	abscisic acid
ABC	ATP-binding cassette
AtMRP5	A. thaliana *MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN-TYPE ATP-BINDING CASSETTE TRANSPORTER5*
AtP2C-HA	A. thaliana *PROTEIN PHOSPHATASE 2C-HA*
atrbohD/F	A. thaliana *NADPH oxidase catalytic subunit D/F*
AtSTP1	A. thaliana H^+-MONOSACCHARIDE SYMPORTER1
CAS	CALCIUM SENSOR
fia	*fava bean impaired in ABA-induced stomatal closure* mutant
GCR1	*G protein-coupled receptor 1* (putative)
GORK	*GUARD CELL OUTWARD RECTIFYING K^+ CHANNEL*
gpa1	*G protein α subunit1* mutant
InsP3	inositol 1,4,5-trisphosphate
InsP6	inositol hexakisphosphate
KAT2	*Arabidopsis INWARD RECTIFIER K^+ CHANNEL2*
KZM1	*K^+ CHANNEL Zea mays1*
NO	nitric oxide
PI3P	phosphatidylinositol 3-phosphate
PLC	phopholipase C
ROS	reactive oxygen species
S1P	sphingosine-1-phosphate
WAK	wall-associated kinase

Introduction

The beautiful mosaic presented by the plant epidermis (Figure 1) has long fascinated both physiologists seeking to understand the mechanistic relationships between cell form and function, and developmental biologists intrigued by pattern formation in nature. Among the various cell types of the epidermis, the guard cell has much to satisfy researchers in both camps. Guard cells are crucially important functional elements: they regulate stomatal apertures, thereby controlling rates of CO_2 uptake and water loss and hence influencing photosynthesis and the water status of the plant. Guard cells have a non-random distribution throughout the epidermis, the governing laws of which are just beginning to be elucidated. In the following sections, recent advances in our understanding of the perception of environmental signals, particularly abscisic acid (ABA) and blue light, by guard cells are highlighted. We then discuss the emerging integrative effects of the extracellular matrix on stomatal function. This review focuses on advances in stomatal biology in 2003 and early 2004; several other recent reviews are also available [1–3]. In particular, a recent review of guard-cell development [4] has been published in this journal, and aspects covered there will not be reiterated here.

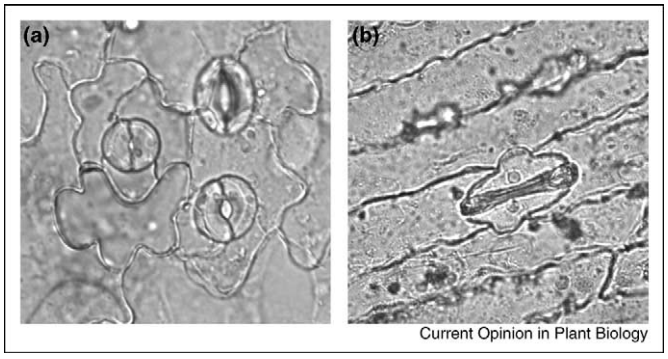
The mechanism behind the movement

Increases in guard-cell volume that are driven by the uptake of ions and other solutes, and by intracellular solute production, elevate guard-cell turgor. Because of the structural reinforcement provided by the asymmetric distribution of cellulose microfibrils throughout the guard-cell wall, the deformation of guard cells is anisotropic; thus, the two guard cells of the stomate separate when they swell, widening the stomatal aperture (Figure 1).

The above long-standing conceptual framework notwithstanding, new aspects of the mechanism behind the movement of guard cells continue to be uncovered. The development of a pressure probe that can withstand and measure guard-cell pressures, which exceed those found in a car tire, has and will continue to provide new insights into guard-cell biophysics [5]. One might assume that increases in guard-cell volume are necessarily accompanied by an increase in membrane surface area (as is the case, for example, when a balloon is inflated); however, it is also possible that guard-cell volume could increase without a concomitant increase in surface area (as is the case when air is blown into a paper bag). A recent study by Mott *et al.* [6] employed confocal imaging techniques to quantify membrane area, and showed that membrane area does indeed increase upon guard-cell inflation (see also [7] and references therein).

K^+ is one of the major osmotica that drive increases in guard-cell volume. K^+ uptake occurs passively, through inwardly rectifying K^+ channels, in response to membrane

Figure 1



Epidermis of **(a)** *Arabidopsis thaliana* and **(b)** *Zea mays*, showing typical dicot and graminaceous stomatal complexes. The left panel shows both mature stomatal complexes and recently divided guard mother cells, wherein formation of the stomatal pore by lysis of the shared cell wall is not yet complete.

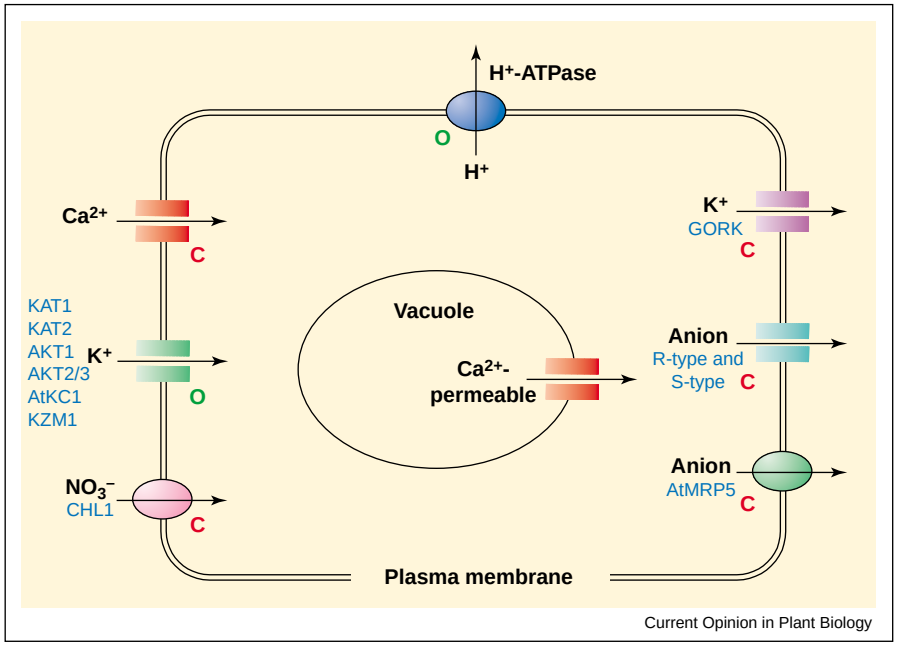
hyperpolarization that results from the activation of plasma membrane H^+-ATPases (Figure 2). Conversely, K^+ loss occurs through outwardly rectifying K^+ channels that are activated by depolarization. Genes that encode guard-cell K^+ channels [8] have previously been identified in *Arabidopsis* and in *Vicia faba*, two of the model species for guard-cell physiology. Now, Hedrich and colleagues [9] report the identification of *K⁺ CHANNEL Zea mays1* (*KZM1*), an inward K^+ channel gene expressed in the guard cells (Figure 1b) and vasculature of maize. Sequence analysis suggests that *KZM1* is likely to be orthologous to the *Arabidopsis* Shaker-type K^+ channel, *ARABIDOPSIS INWARD RECTIFIER K⁺ CHANNEL2* (*KAT2*). However, KZM1 lacks an extracellularly localized histidine, which is implicated in pH sensing, and hence KZM1 currents, unlike those of KAT2, are not stimulated by external acidification. Signals that promote stomatal opening activate both H^+ extrusion and apoplastic acidification, which presumably exert positive feedback on the inward K^+ channels in other species. Apparently, such feedback does not operate in maize, and it will be interesting to determine whether this is a general distinction between the guard cells of grasses and non-grass species.

K^+ uptake is balanced by a counter-ion, typically Cl^- obtained from the apoplast or malate^{2-} derived from starch breakdown. Recently another anion, NO_3^-, has also been found to contribute significantly to stomatal opening under some conditions. Disruption of the NO_3^- transporter gene A. thaliana *NITRATE TRANSPORTER1.1* (*AtNRT1.1*)/*CHLORATE TRANSPORTER1* (*CHL1*) by γ-irradiation reduces light-stimulated nitrate accumulation in guard-cell protoplasts, and significantly impairs stomatal opening when NO_3^- is the only anion provided in the incubation buffer [10•]. Such mutation also results in stomatal apertures that are smaller than those of wildtype plants when the plants are continuously supplied with nitrate. Furthermore, the *chl1* mutation also confers reduced water loss and improved drought tolerance in intact plants.

The extent to which photosynthates that are derived from rubisco activity also contribute to guard-cell osmotic build-up has been controversial (reviewed in [11]). Recent high-resolution measurements of chlorophyll *a* fluorescence from the guard cells of a variety of species imply significant Calvin-cycle activity within guard cells in intact leaves [12]. Guard cells can also import glucose from the apoplast [13]. Two sucrose transporters are apparently expressed in guard cells, and now a H^+-monosaccharide symporter, A. thaliana H^+-MONOSACCHARIDE SYMPORTER1 (AtSTP1), has also been localized to these cells by Sauer and colleagues [14]. These authors speculate that malate or sucrose may be formed from the imported monosaccharides, and that AtSTP1 might function at night to provide energy to sustain dark metabolism and/or to scavenge glucose released during night-time starch breakdown. An *atstp1* T-DNA knockout line showed no alterations in the diurnal course of transpiration or photosynthesis, however, and so it is not yet clear whether AtSTP1 is directly involved in the osmoregulatory changes that govern stomatal apertures.

During reductions in stomatal aperture, 'slow' and 'rapid' anion channels account for Cl^-, malate^{2-}, and NO_3^- efflux from guard cells [15–17]. The consequent membrane depolarization, which is enhanced by H^+-ATPase inhibition and Ca^{2+} influx via Ca^{2+}-permeable channels, drives water efflux and guard-cell deflation. The ATP-binding cassette (ABC) superfamily is a large family of proteins that mediate the ATP-dependent transport of solutes. Glibenclamide, a potent inhibitor of ABC transporters, inhibits the ABA-stimulated slow anion channels of plants and induces stomatal opening, indicating an involvement of ABC transporters in guard-cell responses

Figure 2



Ion channels and transporters in guard cells. The terms in blue correspond to ion channels and transporters that function in guard cells; some of these are the names of gene products whereas others describe currents that have not yet been attributed to specific gene products. Those elements marked with an 'O' contribute, when activated, to stomatal opening, whereas those elements marked with a 'C' contribute, when activated, to stomatal closure. AKT1, *Arabidopsis* K^+ TRANSPORTER1; AtKC1, *Arabidopsis* K^+ RECTIFYING CHANNEL1.

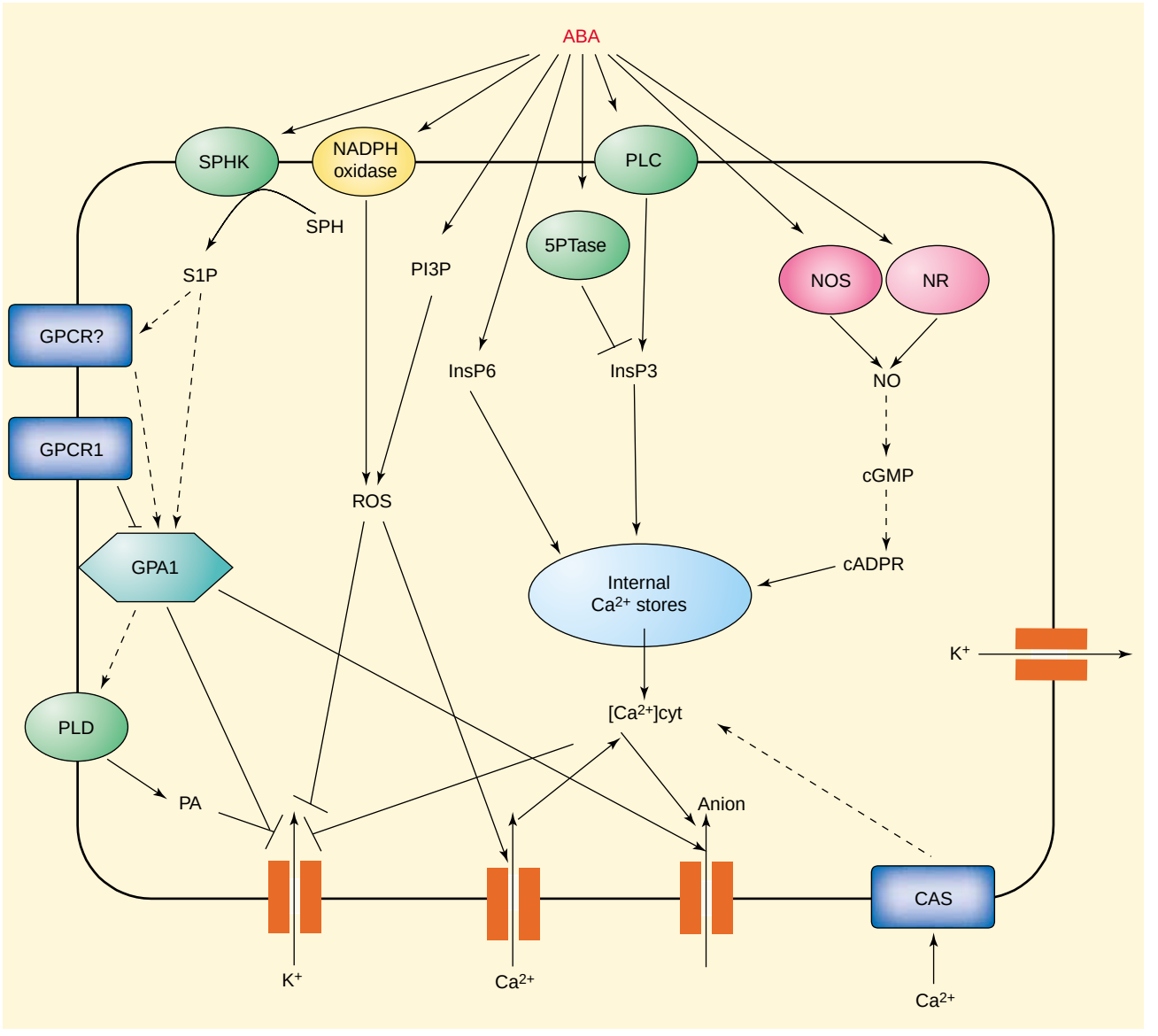
[18]. Disruption of the A. thaliana *MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN-TYPE ATP-BINDING CASSETTE TRANSPORTER5* (*AtMRP5*) gene, which encodes a MRP-type ABC transporter, reduces light-stimulated transpiration and increases drought tolerance [19]. Measurements of anion currents from *AtMRP5* knockout mutants are awaited.

ABA perception and ion-channel regulation

The stress hormone ABA restrains water loss from plants by controlling stomatal apertures. Microarrays provide a powerful tool to identify stress-regulated genes. Using oligomer-based Affymetrix chips representing 8100 genes, Schroeder and colleagues [20••] recently identified 1309 genes that are expressed in the guard cells of *Arabidopsis*, of which 64 are preferentially expressed in guard cells compared with mesophyll cells. Genes that are induced by ABA in guard cells include the dehydrin genes *EARLY RESPONSIVE TO DEHYDRATION14* (*ERD14*) and *COLD REGULATED47* (*COR47*); several genes that encode signal transduction components, such as the *PROTEIN PHOSPHATASE2C* (*PP2C*) genes, A. thaliana *PROTEIN PHOSPHATASE 2C-HA* (*AtP2C-HA*) and A. thaliana *PROTEIN PHOSPHATASE 2CA* (*AtPP2CA*); and several transcription factors. Sixty-four genes, including *KAT1* are repressed by ABA in guard cells. These genome-scale analyses provided a useful clue by which a highly ABA-induced protein phosphatase 2C gene, *AtP2C-HA*, was identified. Stomatal closure was hypersensitive to ABA when this gene was disrupted by T-DNA-mediated mutagenesis.

The ABA signaling network (Figure 3) is activated by ABA perception. An ABA-binding protein was previously isolated from crude membrane extracts of *V. faba* epidermis by affinity chromatography [21]. This putative ABA receptor may be localized at the guard-cell plasma membrane; pre-treatment of guard-cell protoplasts with a monoclonal antibody raised against the purified ABA-binding protein decreased the ABA-induced activation of phospholipase D (PLD) [22•]. This notion was supported by recent evidence that biotinylated ABA that binds to the surface of *V. faba* guard-cell protoplasts retains physiological activity in inducing stomatal closure [22•]. The first *V. faba* guard-cell signaling mutant, *fava bean impaired in ABA-induced stomatal closure* (*fia*), was found serendipitously this past year in a farmer's field [23]. The *fia* mutant lacks stomatal closure in response to ABA or elevated CO_2 concentrations, but retains sensitivity to the promotion of stomatal closure by external Ca^{2+}. Much of what we know about the responses of guard cells to ABA has been learned by taking advantage of genetic tools available in *Arabidopsis*. However, analysis of other species, such as *V. faba*, is important to assess the universality of ABA signaling mechanisms.

Figure 3



A schematic of ABA signaling in guard cells. This model is not comprehensive; for simplicity, only those components discussed in this review are depicted. ABA signaling may be initiated by ABA perception by an unknown ABA receptor and/or perceived directly by one of the components illustrated in the model. Dashed arrows indicate relationships inferred from the literature that have not yet been directly observed in guard cells. Question marks represent hypothesized signaling components. In many cases, relationships of the elements depicted still need to be elucidated. For example, based on mammalian signaling networks, PLC could plausibly function directly downstream of heterotrimeric G protein activation, and ROS could function upstream. cADPR, cyclic ADP-ribose; cGMP, cyclic guanosine 3′,5′-phosphate; GPCR, G-protein-coupled receptor; NOS, nitric oxide synthase; NR, nitrate reductase; PA, phosphatidic acid; PLD, phospholipase D; SPH, sphingosine; SPHK, sphingosine kinase.

The identification by Pei and colleagues [24••] of an *Arabidopsis* plasma membrane protein, CALCIUM SENSOR (CAS), that senses Ca^{2+} is another exciting advance in our knowledge of guard-cell surface receptors. These authors adopted a functional screening approach (see also [25]). They subdivided a leaf cDNA library into pools, and transiently transfected each pool into human embryonic kidney cells. These cells were screened for Ca^{2+}-induced Ca^{2+} increases using the ratiometric Ca^{2+} indicator Fura-2. The cDNA-encoding CAS was isolated after sequential subdivision of the positive pool. In CAS antisense plants, stomata do not close in response to external Ca^{2+} but stomatal closure in response to ABA is not altered. These phenotypes are the converse of those of

the *fia* mutants, and occur despite the fact that ABA can elicit cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) increases guard cells. This result indicates that the presence of CAS is not correlated with ABA perception. Nevertheless, the strategy used to identify this Ca^{2+} receptor might be applied to discover the identity of the ABA receptor. ABA-induced elevations in $[Ca^{2+}]_{cyt}$ are mediated by Ca^{2+} influx through Ca^{2+}-permeable channels (Ca^{2+}-channels) in the plasma membrane and by the release of Ca^{2+} from intracellular Ca^{2+} stores (Figures 2 and 3). Ca^{2+} inactivates inward K^+ channels and activates anion channels, facilitating solute efflux and stomatal closure [26]. Ca^{2+} does not appear to regulate outward K^+ currents, and there are conflicting data as to whether these channels are regulated by ABA. No effect of ABA on outward K^+ currents was observed in *Arabidopsis* guard cells impaled with double-barreled electrodes [27]. However, T-DNA disruption of *GORK*, the gene that encodes an *Arabidopsis* Shaker-like outward K^+ channel that is highly expressed in guard cells, caused a loss of the outward K^+ channel activity from guard cells, impaired stomatal closure in response to ABA or to darkness, and increased transpirational water loss under drought conditions [28•]. It is not yet clear how these two studies could be reconciled.

ABA signaling intermediates in guard cells

Lipids

The lipid metabolite sphingosine-1-phosphate (S1P) is produced from the long-chain amine alcohol sphingosine by the activity of sphingosine kinase. In 2001, S1P was shown to increase $[Ca^{2+}]_{cyt}$ and to stimulate stomatal closure in *Commelina communis* guard cells [29]. Although S1P was known at that time to signal via heterotrimeric GTP-binding (G) proteins in mammals, there was no clue as to whether these two factors were related in plants. Interestingly, it was also shown in 2001 that a G protein is involved in ABA signaling in guard cells. In *gpa1 Arabidopsis* plants (i.e. heterotrimeric G protein α subunit null mutants), stomatal opening and the regulation of inward K^+ channels are not inhibited by ABA, and these plants show altered anion-channel regulation [30]. In recent months, Coursol *et al.* [31••] demonstrated that ABA stimulates sphingosine kinase activity in guard cells, and that the inhibition of stomatal opening and the promotion of stomatal closure by S1P are also eliminated in *gpa1* mutants. They also showed, by patch-clamping *Arabidopsis* guard-cell protoplasts, that S1P can inhibit inward K^+ currents and stimulate slow anion currents in wildtype cells; however, these effects are attenuated in *gpa1* knockouts. This work provides direct evidence that S1P acts upstream of G proteins and downstream of ABA in plant cell signaling [31••].

Recent evidence shows that GCR1, one putative G protein-coupled receptor in *Arabidopsis*, can directly interact with GPA1 in *in-vitro* pull-down assays, co-immunoprecipitation from plant tissue, and yeast split-ubiquitin assays [32]. Compared with wildtype plants, *gcr1* T-DNA insertional mutants are more tolerant of drought stress and their excised leaves show reduced rates of water loss. Consistent with these results, the stomatal movements of *gcr1* plants, including opening and closure, were found to be more sensitive to ABA and S1P than those of wildtype plants [32]. GCR1 may therefore function as a negative regulator of ABA-signaling in guard cells.

Inositol phosphates are important signaling factors in eukaryotic cells. For example, inositol 1,4,5-trisphosphate (InsP3) is involved in many pathways, including ABA signaling in guard cells. Gillaspy and colleagues [33] found that ABA-induced InsP3 accumulation was suppressed in transgenic plants in which inositol 5-phosphatase (an enzyme that hydrolyzes At5PTase1, InsP3 and inositol 1,3,4,5-tetrakisphosphate [InsP4]) was expressed ectopically, and that the stomata of these plants were less responsive to both ABA and light than those of wildtype plants.

Another way to regulate InsP3 levels is through phospholipase Cs (PLCs), which hydrolyze phosphatidylinositol bisphosphate (PtdIns[4,5]P_2), resulting in the production of InsP3 and diacylglycerol. Transgenic tobacco plants expressing *Nicotiana rustica PLC2* (*NrPLC2*) cDNA driven by a guard-cell-specific promoter had reduced levels of PLC protein, presumably because of post-transcriptional gene silencing. Stomata from these plants showed reduced sensitivity in ABA inhibition of light-induced stomatal opening [34•,35]; however, the ability of ABA to induce stomatal closure was not altered in these plants [35]. These data could signify that PLC is only required for ABA's inhibition of stomatal opening and not for ABA's promotion of stomatal closure. Microinjection of heparin, which inhibits InsP3-dependent Ca^{2+} release, into *C. communis* guard cells did attenuate ABA-induced stomatal closure, however, consistent with the results of previous studies using pharmacological PLC inhibitors [35]. The authors of these papers [34•,35] proffer three possible explanations for these apparently contradictory data. First, species-specificity: transgenic data were from tobacco whereas heparin inhibitor results were from *C. communis*. Second, the presence of different PLC isoforms in tobacco, one of which was not suppressed in the transgenics. And third, the occurrence of both short-term and long-term effects: the microinjected inhibitor produces responses that are rapid in comparison with the compensatory mechanisms involving gene regulation that operate in transgenic plants [35]. Additional research will be required to assess the validity of each of these hypotheses.

myo-inositol hexakisphosphate ($InsP_6$) levels are also elevated by ABA treatment in *Solanum tuberosum* guard cells, and InsP6 application can inactivate inward K^+ channels of *V. faba* guard cells via a Ca^{2+}-dependent pathway [36].

Subsequent patch-clamping of *V. faba* guard-cell protoplasts revealed that Ca^{2+}-permeable channels show minimal responsiveness to internal InsP6, and that inward K^+ currents retain sensitivity to InsP6 in the absence of external Ca^{2+} [37••]. By contrast, patch clamping of isolated vacuoles showed that Ca^{2+} permanent channels in the tonoplast are activated by InsP6. These data suggest that $[Ca^{2+}]_{cyt}$ is increased by InsP6-induced release of Ca^{2+} from endomembrane compartments such as the vacuole [37••].

Another IP that has been studied recently is phosphatidylinositol 3-phosphate (PI3P) [38,39]. When *V. faba* epidermal pieces were preincubated with phosphatidylinositol 3-kinase (PI3K) inhibitors, wortmannin (WM) and LY294002 (LY), ABA-induced production of reactive oxygen species (ROS) (was absent, and ABA-induced stomatal closure was inhibited. The inhibition of stomatal closure could be partially rescued by applying the ROS H_2O_2, indicating that PI3P may act upstream of ROS in ABA signaling.

Reactive oxygen species

The production of ROS in guard cells is promoted by ABA exposure [40,41,42••]. Exogenous H_2O_2 activates Ca^{2+}-permeable channels in the plasma membrane of *V. faba* and *Arabidopsis* guard cells [40,43,44] and inhibits inward K^+ channels [44,45]. The effects of ABA on these ion channels are compromised by catalase or diphenylene iodonium (DPI), which are a H_2O_2 scavenger and a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor, respectively [40,41]. In the A. thaliana *NADPH oxidase catalytic subunit D/F* (*atrbohD/F*) double mutant, which lacks two NADPH oxidase catalytic subunits, ABA-induced production of ROS, Ca^{2+}-channel activation, $[Ca^{2+}]_{cyt}$ oscillation and stomatal closure are all impaired [42••]. These results support the notion that ROS are secondary messengers in ABA signaling in guard cells. This notion was challenged, however, by the observation that ABA and H_2O_2 trigger opposite effects on outward K^+ channels in *V. faba* guard cells. As recorded by two-electrode voltage clamp, ABA has a stimulatory effect but H_2O_2 has an inhibitory effect on these currents [26,44]. Köhler *et al.* [44] conclude that the signaling cascades for ABA and H_2O_2 share some components but are not identical. Whether ROS generated by NADPH oxidase mediate the regulation of the outward K^+ current by ABA could be further assessed by evaluation of this current in the *Arabidopsis atrbohD/F* double mutant.

Nitric oxide

Nitric oxide (NO) is a highly reactive nitrogen species that serves as a cellular signaling molecule in a wide range of organisms. This concept has just recently been extended to plants, especially stomatal guard cells. The application of exogenous NO via pharmacological NO donors induces stomatal closure and reduces transpiration in a variety of plant species, including *Arabidopsis*, *V. faba*, *Tradescantia* sp., and pea [46–49]. Application of 2-pyenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO), 2-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO), NO scavengers, or N-nitro L-arginine methyl ester (L-NAME; an inhibitor of NO synthase) suppresses ABA-induced stomatal closure in *Arabidopsis*, *V. faba* and pea [47–49], indicating the involvement of endogenous NO in this process. This view has been confirmed by reverse genetic approaches. Two types of candidate enzymes for NO synthesis have been implicated for plants, nitrate reductase and nitric oxide synthase. Double mutation of the *Arabidopsis NITRATE REDUCTASE DEFECTIVE1* (*NIA1*) and *NIA2* genes blocks nitrite- and ABA-induced stomatal closure and NO synthesis, but inhibition of stomatal opening by ABA is retained in the double mutants [48]. Mutation of the nitric oxide synthase gene, A. thaliana *NITRIC OXIDE SYNTHASE1* (*AtNOS1*), decreases NO production, and reduces the induction of stomatal closure in response to ABA [50••]. Thus, these enzymes are implicated in transmitting the ABA signal to downstream effectors. A recent finding that the NO donor *S*-nitroso-*N*-acetyl-penacillamine (SNAP) inhibits inward K^+ channels and activates anion channels by increasing $[Ca^{2+}]_{cyt}$ has provided further evidence of a NO-based ABA signaling pathway in guard cells [51••]. NO actually inhibits plasma membrane Ca^{2+} currents but nevertheless increases $[Ca^{2+}]_{cyt}$, presumably by stimulating Ca^{2+} release from internal stores. Ca^{2+} elevation is inhibited by pre-treatment with either ryanodine or 1H-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one (ODQ), which are antagonists of cyclic ADP-ribose (cADPR)-mediated Ca^{2+} release and of NO-sensitive guanylate cyclase, respectively [51••].

Blue light and other environmental signals

Stomatal opening occurs in response to both red and blue light, light of wavelengths that also drive photosynthesis. Perception of red light is mediated by guard-cell chlorophyll, with a possible contribution from phytochrome under specialized circumstances [11,52]. The blue-light response is mediated to a great extent by phototropins, with possible contributions from zeaxanthin or phytochrome under specialized conditions [52,53]. Phototropins are flavin mononucleotide (FMN)-binding kinases that contain light, oxygen, and voltage (LOV) domains. These blue-light photoreceptors mediate the phototrophic bending of shoots, chloroplast movement, and the negative phototropism of roots [54]. *non-phototropic hypocotyl1* (*phot1*) *phot2* double mutants of *Arabidopsis* lack blue-light-specific stomatal opening and blue-light-stimulated H^+-extrusion from guard cells [53]. *Arabidopsis* plants in which the NON-PHOTOCHEMICAL QUENCHING1 (NPQ1) enzyme that converts violaxanthin to zeaxanthin is mutated lack a specific green-light-reversible blue-light response, suggesting that

carotenoid metabolism may modulate the sensitivity of guard cells to blue light [52].

Shimazaki and colleagues [55] studied the biochemistry of the blue-light response in *V. faba* guard-cell protoplasts. *In-vivo* labeling with [^{32}P]orthophosphate has provided evidence that both Vfphot1a and Vfphot1b of guard cells, like *Arabidopsis* phototropins, become phosphorylated in response to blue light [55]. Far-Western analysis demonstrated that this phosphorylation is required for the binding of a 14-3-3 protein to phototropin. The H^+-ATPase also becomes phosphorylated and binds a 14-3-3 protein in response to blue light [56,57]. However, the phosphorylation of Vfphot precedes that of the H^+ ATPase, as would be anticipated given the upstream location of the photoreceptors in the signal transduction cascade.

In the preceding section, we described an intimate connection between ABA and $[Ca^{2+}]_{cyt}$. ABA closes stomata and blue light opens them. Yet earlier research showed that blue-light-induced stomatal opening was inhibited by the Ca^{2+}-channel blocker verapamil, and by inhibitors of calmodulin (CaM) and of CaM-dependent myosin light-chain kinase (MLCK)-type kinases ([58]; reviewed in [59]). Indeed, in *Arabidopsis* mesophyll cells, direct patch-clamp assay of Ca^{2+}-permeable cation channels reveals that these channels are activated by blue light via a phototropin-based pathway. Blue light elevates aequorin-reported $[Ca^{2+}]_{cyt}$ through both influx and PLC-based internal release [60,61]. Thus, despite the facts that Ca^{2+} inhibits the guard-cell H^+ ATPase [62] and exogenous Ca^{2+} reduces stomatal apertures in *in-vitro* assays, a positive role for this ubiquitous signaling molecule in modulating the blue-light responses of guard cells should not be discounted.

In the natural environment, carbon dioxide concentrations and atmospheric humidity are also potent regulators of stomata [63]. Elevated intercellular CO_2 concentrations stimulate stomatal closure, whereas elevated ambient humidity promotes stomatal opening. A recent study has revealed that these responses are intertwined, such that elevated relative humidity sensitizes *V. faba* guard cells to the elevated-CO_2 signal [64]. The increased CO_2 sensitivity is reversed within 2–3 days when plants are transferred from high to low humidity, and is observed in fully expanded leaves, indicating that the response is truly acclimatory rather than developmental. Little is known about the mechanism by which guard cells sense humidity, but electrophysiological assays have shown that CO_2 activates slow anion channels, inhibits inward K^+ currents and promotes outward K^+ currents (reviewed in [65,66]).

Role of the extracellular matrix in stomatal function

The effects of mobile apoplastic messengers, including ABA, H^+, and Ca^{2+}, on transmembrane ion fluxes of guard cells have received considerable attention ([24••], reviewed in [67]). Now, several new reports have hinted that the carbohydrate extracellular matrix itself may be important in the dynamic control of stomatal apertures. In cell walls, cellulose (β-[1→4]glucan chains) that are aggregated into microfibrils play the primary scaffolding role, whereas callose (β-[1→3]-glucan chains) is deposited in certain cell types during development or in response to environmental insults. Hemicelluloses, such as xyloglucans, are hypothesized to form cross-linkages between cellulose microfibrils; whereas pectins, such as homogalacturonan and rhamnogalacturonans I and II, are found in the middle lamella and cell-wall matrix.

The immunolocalization of carbohydrate moieties in the leaves of sugar beet showed that a xyloglucan or rhmnogalucturonan I epitope consisting of a terminal fucosyl residue was more common in the ventral and lateral walls of guard cells than in the walls of pavement cells [68]. Callose is deposited transiently in the newly formed wall that divides the guard mother cell into two daughter cells (see Figure 1a), and is also found sporadically in mature guard-cell walls [68]. Since H_2O_2 and Ca^{2+} both activate callose synthase and are produced in response to ABA, Majewska-Sawka *et al.* [68] speculate that the callose deposits found in the walls of mature guard cells may result from exposure to ABA.

Epitopes corresponding to highly esterified pectins are found in guard-cell walls [68,69,70••]; such pectins are more flexible than their non-esterified counterparts. In apple, overexpression of endopolygalacturonase, an enzyme that cleaves stretches of homogalacturonan in the pectin backbone, resulted in reduced levels of pectins in the epidermis [69]. Furthermore, the mature stomata of these plants were bordered at each end by holes in the epidermis, indicating a reduction in cell-to-cell adhesivity. Such stomata did not close in response to darkness or ABA; and Atkinson *et al.* [69] speculate that, with holes present in the epidermis, it is impossible for neighboring epidermal cells to exert enough back-pressure to push stomata closed. It is also possible, however, that either the altered structure of the guard-cell wall itself or cell wall degradation products contribute to this guard-cell phenotype by acting as signaling components (see below).

Recent work from the McQueen-Mason's group [70••] suggests that the specific structure of pectins within the guard-cell wall can either enhance or retard stomatal movements. Direct treatment of *C. communis* epidermal peels with a combination of pectin methylesterase and endopolygalacturonase results in stomata that open more widely in response to fusicoccin than is normal, although stomatal closure in response to ABA or hyperosmotic conditions remains normal [70••]. By contrast, treatment with an arabinase, which hydrolyses arabinosyl sidechains on the pectin rhamnogalacturonan I, inhibits both

stomatal opening and closure. These effects are blocked by pre-treatment with an arabinan monoclonal antibody, indicating the specificity of the arabinase treatment. Hydrolysis of feruloyl esters, which are found on side branches of rhamnogalacturonan I where they can oxidatively dimerize to cross-link polymers, also inhibits stomatal movements to some extent. This wall-'locking' phenomenon is hypothesized to stem from a tighter packing of pectic polymers in the absence of their bulky side chains. Remarkably, treatment with cellulases and xylanases, although generally disrupting cell walls, did not impair stomatal responses to stimuli [70••].

It is also possible that cell-wall-loosening enzymes lose access to their binding sites and/or that hydrolyzed wall components themselves serve as signals that regulate stomatal apertures in the experiments described above. For example, application of the elicitor oligogalacturonic acid inhibits stomatal opening and promotes stomatal closure [71]. Extracellular enzymes may also participate in the regulation of stomatal dynamics in response to pathogens and other stresses. Wall-associated kinases (WAKs), which are induced by pathogen infection, wounding, and nutritional stress, extend through the plasma membrane; the cell-wall domain of WAKs binds pectins and glycine-rich wall proteins whereas the WAK kinase domain is retained in the cytosol. Expression of WAKs in the inner guard-cell walls bordering the stomatal pore is induced by the application of excess aluminum to the roots, and stomatal closure ensues after 9 h of aluminum treatment [72]. One model of WAK activity suggests that the kinase domain may be cleaved and gain intracellular function in response to the appropriate apoplastic ligand [72]. WAKs may be an archetype of enzymatically mediated apoplast to symplast signaling. It would be of interest to assess whether each of the environmental stimuli discussed in this review might activate WAKs or apoplast-localized enzymes, resulting in the production of molecules with signaling function. Such phenomena would link apoplast and symplast, and provide yet another layer of complexity to the regulation of stomatal function.

Conclusions and future prospects

This review has discussed recent highlights in guard-cell research. Despite many significant breakthroughs, much remains to be learned. To give one example, experimental evidence indicates that *gpa1* null mutants have greatly reduced sensitivity to ABA inhibition of stomatal opening [30,31••]. Yet, according to Figure 3, genetic elimination of *GPA1* should still leave guard cells sensitive to ABA inhibition of stomatal opening via pathways mediated by NADPH oxidase and PLC. How might this apparent contradiction be reconciled? First, Figure 3 shows only known epistatic relationships; additional epistatic relationships are likely, but have not yet been put to experimental test. Therefore, pathways that appear independent on the basis of current knowledge may not be so in reality. Second, pathways may function additively or synergistically, such that if one pathway is eliminated, the other pathways do not suffice to effect the response. Third, in many cases the relative roles of secondary messengers in ABA-inhibition of stomatal opening as opposed to ABA-promotion of stomatal closure have not been explicated; Figure 3 subsumes both these ABA responses. Fourth, depending on growth and experimental conditions, guard-cell responses to other stimuli discussed in this review, i.e. blue light, CO_2, and humidity, may influence the extent of ABA sensitivity recorded in any given experiment. In conclusion, illustrations such as those of Figure 3 both highlight the need for additional experimental elucidation of the inter-relationships between guard-cell signaling elements and suggest that, in the future, the guard cell will prove a fertile ground for researchers in the emerging field of systems biology.

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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

1. Kuhn JM, Schroeder JI: **Impacts of altered RNA metabolism on abscisic acid signaling**. *Curr Opin Plant Biol* 2003, **6**:463-469.

2. Nadeau JA, Sack FD: **Stomatal development: cross talk puts mouths in place**. *Trends Plant Sci* 2003, **8**:294-299.

3. Desikan R, Cheung MK, Bright J, Henson D, Hancock JT, Neill SJ: **ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells**. *J Exp Bot* 2004, **55**:205-212.

4. Bergmann DC: **Integrating signals in stomatal development**. *Curr Opin Plant Biol* 2004, **7**:26-32.

5. Franks PJ: **Use of the pressure probe in studies of stomatal function**. *J Exp Bot* 2003, **54**:1495-1504.

6. Shope JC, DeWald DB, Mott KA: **Changes in surface area of intact guard cells are correlated with membrane internalization**. *Plant Physiol* 2003, **133**:1314-1321.

7. Hurst AC, Meckel T, Tayefeh S, Thiel G, Homann U: **Trafficking of the plant potassium inward rectifier KAT1 in guard cell protoplasts of *Vicia faba***. *Plant J* 2004, **37**:391-397.

8. Very AA, Sentenac H: **Cation channels in the *Arabidopsis* plasma membrane**. *Trends Plant Sci* 2002, **7**:168-175.

9. Philippar K, Buchsenschutz K, Abshagen M, Fuchs I, Geiger D, Lacombe B, Hedrich R: **The K^+ channel KZM1 mediates potassium uptake into the phloem and guard cells of the C4 grass *Zea mays***. *J Biol Chem* 2003, **278**:16973-16981.

10. • Guo FQ, Young J, Crawford NM: **The nitrate transporter AtNRT1.1 (CHL1) functions in stomatal opening and contributes to drought susceptibility in *Arabidopsis***. *Plant Cell* 2003, **15**:107-117.
The authors of this article demonstrate that the dual-affinity nitrate transporter gene (*AtNRT1.1*) is expressed in guard cells. Plants that lack the AtNRT1.1 enzyme show reduced stomatal opening and enhanced

drought tolerance under certain growth conditions, correlating with their reduced use of NO_3^- as a guard-cell osmoticum.

11. Zeiger E, Talbott LD, Frechilla S, Srivastava A, Zhu JX: **The guard cell chloroplast: a perspective for the twenty-first century**. *New Phytol* 2002, **153**:415-424.

12. Lawson T, Oxborough K, Morison JI, Baker NR: **The responses of guard and mesophyll cell photosynthesis to CO_2, O_2, light, and water stress in a range of species are similar**. *J Exp Bot* 2003, **54**:1743-1752.

13. Ritte G, Rosenfeld J, Rohrig K, Raschke K: **Rates of sugar uptake by guard cell protoplasts of *Pisum sativum* L. related to the solute requirement for stomatal opening**. *Plant Physiol* 1999, **121**:647-656.

14. Stadler R, Buttner M, Ache P, Hedrich R, Ivashikina N, Melzer M, Shearson SM, Smith SM, Sauer N: **Diurnal and light-regulated expression of AtSTP1 in guard cells of *Arabidopsis***. *Plant Physiol* 2003, **133**:528-537.

15. Schmidt C, Schroeder JI: **Anion selectivity of slow anion channels in the plasma membrane of guard cells (large nitrate permeability)**. *Plant Physiol* 1994, **106**:383-391.

16. Schroeder JI: **Anion channels as central mechanisms for signal transduction in guard cells and putative functions in roots for plant–soil interactions**. *Plant Mol Biol* 1995, **28**:353-361.

17. Raschke K: **Alternation of the slow with the quick anion conductance in whole guard cells effected by external malate**. *Planta* 2003, **217**:651-657.

18. Leonhardt N, Vavasseur A, Forestier C: **ATP binding cassette modulators control abscisic acid-regulated slow anion channels in guard cells**. *Plant Cell* 1999, **11**:1141-1152.

19. Klein M, Perfus-Barbeoch L, Frelet A, Gaedeke N, Reinhardt D, Mueller-Roeber B, Martinoia E, Forestier C: **The plant multidrug resistance ABC transporter AtMRP5 is involved in guard cell hormonal signalling and water use**. *Plant J* 2003, **33**:119-129.

20. •• Leonhardt N, Kwak JM, Robert N, Waner D, Leonhardt G, Schroeder JI: **Microarray expression analyses of *Arabidopsis* guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant**. *Plant Cell* 2004, **16**:596-615.

The microarray analyses reported in this paper provide an overview of ABA-induced and repressed genes in guard-cell and mesophyll-cell protoplasts. A highly ABA-induced protein phosphatase 2C gene, *AtP2C-HA*, was identified in these experiments. T-DNA disruption of this gene confers ABA hypersensitivity during stomatal closure.

21. Zhang DP, Wu ZY, Li XY, Zhao ZX: **Purification and identification of a 42-kilodalton abscisic acid-specific-binding protein from epidermis of broad bean leaves**. *Plant Physiol* 2002, **128**:714-725.

22. • Yamazaki D, Yoshida S, Asami T, Kuchitsu K: **Visualization of abscisic acid-perception sites on the plasma membrane of stomatal guard cells**. *Plant J* 2003, **35**:129-139.

The authors use a fluorescently tagged avidin probe to show that biotinylated ABA binds to the surface of guard-cell protoplasts, and that biotinylated ABA is physiologically functional in inducing stomatal closure in *Vicia faba*. This is good evidence for the presence of an ABA receptor at the plasma membrane of this cell type.

23. Iwai S, Shimomura N, Nakashima A, Etoh T: **New fava bean guard cell signaling mutant impaired in ABA-induced stomatal closure**. *Plant Cell Physiol* 2003, **44**:909-913.

24. •• Han S, Tang R, Anderson LK, Woerner TE, Pei ZM: **A cell surface receptor mediates extracellular Ca^{2+} sensing in guard cells**. *Nature* 2003, **425**:196-200.

A cDNA clone that encodes a plasma-membrane protein named CAS is identified. Using antisense techniques, the authors demonstrate that CAS functions as a Ca^{2+} receptor that senses external Ca^{2+}.

25. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D: **The capsaicin receptor: a heat-activated ion channel in the pain pathway**. *Nature* 1997, **389**:816-824.

26. Blatt MR: **Ca^{2+} signalling and control of guard-cell volume in stomatal movements**. *Curr Opin Plant Biol* 2000, **3**:196-204.

27. Becker D, Hoth S, Ache P, Wenkel S, Roelfsema MR, Meyerhoff O, Hartung W, Hedrich R: **Regulation of the ABA-sensitive *Arabidopsis* potassium channel gene *GORK* in response to water stress**. *FEBS Lett* 2003, **554**:119-126.

28. • Hosy E, Vavasseur A, Mouline K, Dreyer I, Gaymard F, Poree F, Boucherez J, Lebaudy A, Bouchez D, Very 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.

Outward K^+ channels participate in the regulation of stomatal apertures. This concept was extended by the authors of this paper, who used reverse genetics approaches to identify the role of a particular outward K^+ channel, GORK, in stomatal movements.

29. Ng CK, Carr K, McAinsh MR, Powell B, Hetherington AM: **Drought-induced guard cell signal transduction involves sphingosine-1-phosphate**. *Nature* 2001, **410**:596-599.

30. Wang XQ, Ullah H, Jones AM, Assmann SM: **G protein regulation of ion channels and abscisic acid signaling in *Arabidopsis* guard cells**. *Science* 2001, **292**:2070-2072.

31. •• Coursol S, Fan LM, Le Stunff H, Spiegel S, Gilroy S, Assmann SM: **Sphingolipid signalling in *Arabidopsis* guard cells involves heterotrimeric G proteins**. *Nature* 2003, **423**:651-654.

This paper makes an important contribution to the field of heterotrimeric G protein signaling in plant cells. It provides biochemical and electrophysiological evidence that S1P acts as a secondary messenger upstream of the G protein α subunit in ABA signaling in guard cells.

32. Pandey S, Assmann SM: **The *Arabidopsis* putative G protein-coupled receptor GCR1 interacts with the G protein α subunit GPA1 and regulates abscisic acid signaling**. *Plant Cell* 2004, **16**:1616-1632.

33. Burnette RN, Gunesekera BM, Gillaspy GE: **An *Arabidopsis* inositol 5-phosphatase gain-of-function alters abscisic acid signaling**. *Plant Physiol* 2003, **132**:1011-1019.

34. • Hunt L, Mills LN, Pical C, Leckie CP, Aitken FL, Kopka J, Mueller-Roeber B, McAinsh MR, Hetherington AM, Gray JE: **Phospholipase C is required for the control of stomatal aperture by ABA**. *Plant J* 2003, **34**:47-55.

The authors show that transgenic plants that have low levels of PLC protein are less sensitive to ABA inhibition of light-induced stomatal opening.

35. Mills LN, Hunt L, Leckie CP, Aitken FL, Wentworth M, McAinsh MR, Gray JE, Hetherington AM: **The effects of manipulating phospholipase C on guard cell ABA-signalling**. *J Exp Bot* 2004, **55**:199-204.

36. Lemtiri-Chlieh F, MacRobbie EAC, Brearley CA: **Inositol hexakisphosphate is a physiological signal regulating the K^+-inward rectifying conductance in guard cells**. *Proc Natl Acad Sci USA* 2000, **97**:8687-8692.

37. •• Lemtiri-Chlieh F, MacRobbie EAC, Webb AAR, Manison NF, Brownlee C, Skepper JN, Chen J, Prestwich GD, Brearley CA: **Inositol hexakisphosphate mobilizes an endomembrane store of calcium in guard cells**. *Proc Natl Acad Sci USA* 2003, **100**:10091-10095.

The authors show that InsP6 does not activate Ca^{2+}-permeable channels at the guard-cell plasma membrane, but rather releases Ca^{2+} from endomembrane compartments such as the vacuole. This paper provides new insights into the complexity of inositol phosphate signaling in plant cells.

38. Jung JY, Kim YW, Kwak JM, Hwang JU, Young J, Schroeder JI, Hwang I, Lee Y: **Phosphatidylinositol 3- and 4-phosphate are required for normal stomatal movements**. *Plant Cell* 2002, **14**:2399-2412.

39. Park KY, Jung JY, Park J, Hwang JU, Kim YW, Hwang I, Lee Y: **A role for phosphatidylinositol 3-phosphate in abscisic acid-induced reactive oxygen species generation in guard cells**. *Plant Physiol* 2003, **132**:92-98.

40. Pei ZM, Murata Y, Benning G, Thomine S, Klusener B, Allen GJ, Grill E, Schroeder JI: **Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells**. *Nature* 2000, **406**:731-734.

41. Zhang X, Zhang L, Dong F, Gao J, Galbraith DW, Song CP: **Hydrogen peroxide is involved in abscisic acid-induced**

stomatal closure in *Vicia faba*. *Plant Physiol* 2001, **126**:1438-1448.

42. •• 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.
The authors of this paper used reverse genetic methods to identify the roles of two NADPH oxidase subunits in ABA-induced ROS generation and Ca^{2+}-channel stimulation in guard cells. This work extends the concept that ROS function as signaling intermediates during guard-cell ABA responses.

43. Hamilton DW, Hills A, Köhler B, Blatt MR: **Ca^{2+} channels at the plasma membrane of stomatal guard cells are activated by hyperpolarization and abscisic acid**. *Proc Natl Acad Sci USA* 2000, **97**:4967-4972.

44. Köhler B, Hills A, Blatt MR: **Control of guard cell ion channels by hydrogen peroxide and abscisic acid indicates their action through alternate signaling pathways**. *Plant Physiol* 2003, **131**:385-388.

45. Zhang X, Miao YC, An GY, Zhou Y, Shangguan ZP, Gao JF, Song CP: **K^+ channels inhibited by hydrogen peroxide mediate abscisic acid signaling in *Vicia* guard cells**. *Cell Res* 2001, **11**:195-202.

46. Garcia-Mata CG, Lamattina L: **Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress**. *Plant Physiol* 2001, **126**:1196-1204.

47. Garcia-Mata C, Lamattina L: **Nitric oxide and abscisic acid cross talk in guard cells**. *Plant Physiol* 2002, **128**:790-792.

48. Desikan R, Griffiths R, Hancock J, Neill S: **A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana***. *Proc Natl Acad Sci USA* 2002, **99**:16314-16318.

49. Neill SJ, Desikan R, Clarke A, Hancock JT: **Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells**. *Plant Physiol* 2002, **128**:13-16.

50. •• Guo FQ, Okamoto M, Crawford NM: **Identification of a plant nitric oxide synthase gene involved in hormonal signaling**. *Science* 2003, **302**:100-103.
The authors of this paper reveal another source of nitric oxide generation via nitric oxide synthase. Mutation of the nitric oxide synthase gene *AtNOS1* results in a decrease in NO production, and in reduced induction of stomatal closure in response to ABA. This is a fundamental advance in the characterization of NO function in plants.

51. •• Garcia-Mata C, Gay R, Sokolovski S, Hills A, Lamattina L, Blatt MR: **Nitric oxide regulates K^+ and Cl^- channels in guard cells through a subset of abscisic acid-evoked signaling pathways**. *Proc Natl Acad Sci USA* 2003, **100**:11116-11121.
Nitric oxide is emerging as an important signaling molecule in plants. This work provides evidence that NO influences the inward K^+ channels and anion channels of guard cells by mediating Ca^{2+} release from internal stores during ABA signaling.

52. Talbott LD, Shmayevich IJ, Chung Y, Hammad JW, Zeiger E: **Blue light and phytochrome-mediated stomatal opening in the *npq1* and *phot1 phot2* mutants of *Arabidopsis***. *Plant Physiol* 2003, **133**:1522-1529.

53. Kinoshita T, Doi M, Suetsugu N, Kagawa T, Wada M, Shimazaki K: **phot1 and phot2 mediate blue light regulation of stomatal opening**. *Nature* 2001, **414**:656-660.

54. Doi M, Shigenaga A, Emi T, Kinoshita T, Shimazaki K: **A transgene encoding a blue light receptor, *phot1*, restores blue-light responses in the *Arabidopsis phot1 phot2* double mutant**. *J Exp Bot* 2004, **55**:517-523.

55. Kinoshita T, Emi T, Tominaga M, Sakamoto K, Shigenaga A, Doi M, Shimazaki K: **Blue-light- and phosphorylation-dependent binding of a 14-3-3 protein to phototropins in stomatal guard cells of broad bean**. *Plant Physiol* 2003, **133**:1453-1463.

56. Kinoshita T, Shimazaki K: **Blue light activates the plasma membrane H^+-ATPase by phosphorylation of the C-terminus in stomatal guard cells**. *EMBO J* 1999, **18**:5548-5558.

57. Kinoshita T, Shimazaki K: **Biochemical evidence for the requirement of 14-3-3 protein binding in activation of the guard-cell plasma membrane H^+-ATPase by blue light**. *Plant Cell Physiol* 2002, **43**:1359-1365.

58. Shimazaki K, Tominaga M, Shigenaga A: **Inhibition of the stomatal blue light response by verapamil at high concentration**. *Plant Cell Physiol* 1997, **38**:747-750.

59. Assmann SM, Shimazaki K: **The multisensory guard cell. Stomatal responses to blue light and abscisic acid**. *Plant Physiol* 1999, **119**:809-816.

60. Harada A, Sakai T, Okada K: **Phot1 and phot2 mediate blue light-induced transient increases in cytosolic Ca^{2+} differently in *Arabidopsis* leaves**. *Proc Natl Acad Sci USA* 2003, **100**:8583-8588.

61. Stoelzle S, Kagawa T, Wada M, Hedrich R, Dietrich P: **Blue light activates calcium-permeable channels in *Arabidopsis* mesophyll cells via the phototropin signaling pathway**. *Proc Natl Acad Sci USA* 2003, **100**:1456-1461.

62. Kinoshita T, Nishimura M, Shimazaki K: **Cytosolic concentration of Ca^{2+} regulates the plasma membrane H^+-ATPase in guard cells of fava bean**. *Plant Cell* 1995, **7**:1333-1342.

63. Hetherington AM, Woodward FI: **The role of stomata in sensing and driving environmental change**. *Nature* 2003, **424**:901-908.

64. Talbott LD, Rahveh E, Zeiger E: **Relative humidity is a key factor in the acclimation of the stomatal response to CO_2**. *J Exp Bot* 2003, **54**:2141-2147.

65. Assmann SM: **The cellular basis of guard cell sensing of rising CO_2**. *Plant Cell Environ* 1999, **22**:629-637.

66. Raschke K, Shabahang M, Wolf R: **The slow and the quick anion conductance in whole guard cells: their voltage-dependent alternation, and the modulation of their activities by abscisic acid and CO_2**. *Planta* 2003, **217**:639-650.

67. Bibikova TN, Assmann SM, Gilroy S: **Ca^{2+} and pH as integrating signals in transport control**. In *Membrane Transport in Plants*. Edited by Blatt MR. London: Blackwell Scientific; 2004: in press.

68. Majewska-Sawka A, Munster A, Rodriguez-Garcia MI: **Guard cell wall: immunocytochemical detection of polysaccharide components**. *J Exp Bot* 2002, **53**:1067-1079.

69. Atkinson RG, Schroder R, Hallett IC, Cohen D, MacRae EA: **Overexpression of polygalacturonase in transgenic apple trees leads to a range of novel phenotypes involving changes in cell adhesion**. *Plant Physiol* 2002, **129**:122-133.

70. •• Jones L, Milne JL, Ashford D, McQueen-Mason SJ: **Cell wall arabinan is essential for guard cell function**. *Proc Natl Acad Sci USA* 2003, **100**:11783-11788.
This report highlights the emerging concept that the composition of the guard-cell wall exerts control on stomatal responsiveness. The authors show that treatment of epidermis with enzymes that hydrolyze pectic side-chains influences the extent of stomatal response to opening and closing signals.

71. Lee S, Choi H, Suh S, Doo IS, Oh KY, Choi EJ, Schroeder Taylor AT, Low PS, Lee Y: **Oligogalacturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from guard cells of tomato and *Commelina communis***. *Plant Physiol* 1999, **121**:147-152.

72. Sivaguru M, Ezaki B, He ZH, Tong H, Osawa H, Baluska F, Volkmann D, Matsumoto H: **Aluminum-induced gene expression and protein localization of a cell wall-associated receptor kinase in *Arabidopsis***. *Plant Physiol* 2003, **132**:2256-2266.