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Update on Signal Transduction

The Multisensory Guard Cell. Stomatal Responses to Blue Light and Abscisic Acid[1]

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Microscopic stomatal pores in the epidermes of aerial plant organs allow the loss of water vapor to the atmosphere in a process known as transpiration and the entry of CO_2 into the plant for photosynthetic carbon fixation. Stomatal apertures are rapidly and reversibly regulated by pairs of guard cells that border and define the pores. These cells are kidney-shaped in dicots and nongraminaceous monocots and dumbbell-shaped in grasses. Fine control of stomatal aperture is essential so that the plant neither undergoes excessive water loss and desiccates nor becomes starved for CO_2. This fine control is achieved through an exquisite sensitivity of the guard cells to a multitude of environmental and endogenous signals, including light, humidity, temperature, CO_2, plant water status, and plant hormones, particularly ABA. Because of their demonstrated ability to process so many signals, and because of their vital role in plant function, guard cells have become a premier model system in modern plant cell biology. Many studies on guard cell physiology have been conducted with fava bean (*Vicia faba*) or *Commelina communis* because of the ease with which the epidermis of these plants can be stripped from the mesophyll tissue.

In general, stomatal apertures widen when an increase in the osmotic concentration of the guard cell drives water uptake and guard cell swelling. This elevation in osmoticum results from the uptake of K^+ and Cl^-, from the synthesis of malate from starch breakdown, and, under some conditions, from the accumulation of Suc. Because of the radial reinforcement of the guard cell wall, cell swelling results in a separation of the two guard cells, thus producing an increase in pore aperture. Conversely, stomatal closure occurs when the loss of solutes from the guard cells drives water loss and guard cell deflation. In this *Update*, we briefly summarize current knowledge regarding two aspects of stomatal movements: opening of stomata by blue light and closure of stomata by ABA.

STOMATAL OPENING INDUCED BY BLUE LIGHT

Both red and blue light stimulate stomatal opening. Because chlorophyll also absorbs these wavelengths, sensitivity to red and blue light is consistent with a role of guard cells in opening stomata under conditions conducive for photosynthesis. Indeed, in most species guard cells are the only epidermal cells that contain chloroplasts, and guard cell chlorophyll is implicated as a photoreceptor in the light responses of stomata. However, the greater quantum efficiency of blue light over red light in stimulating stomatal opening (for review and refs., see Assmann, 1993; Zeiger and Zhu, 1998) suggests that guard cells also possess a specific blue-light photoreceptor. A species of orchid (*Paphiopedilum harrisianum*) that lacks guard cell chlorophyll and therefore has guard cell sensitivity only to blue light, shows significant increases in fresh weight when grown under blue-enriched lighting, possibly illustrating the impact of the specific stomatal response to blue light on plant growth (Zeiger et al., 1985). In less exceptional species, the stomatal response to blue light may be particularly important under the blue-enriched illumination that prevails in early morning light and in sunflecks. As discussed below, fundamental questions still remain concerning the identity of the blue-light photoreceptor in guard cells, the cellular signals linking light perception to downstream regulators of guard cell solute content, and the nature of these downstream regulators.

THE BLUE-LIGHT PHOTORECEPTOR

Based on their absorption properties, carotenoids, flavins, and pterins have been proposed as blue-light photoreceptors (cryptochromes) in higher plants (Horwitz and Berrocal, 1997). In recent years much progress has been made in identifying the photoreceptors responsible for other blue-light responses in plants. Blue-light-specific in-

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Abbreviations: CDPK, Ca^{2+}-dependent protein kinase with a calmodulin-like domain; IP_3, inositol 1,4,5-trisphosphate; MLCK, myosin light chain kinase.

hibition of hypocotyl growth is now known to be mediated by the flavin- and pterin-binding photolyase homologs CRY1 and CRY2 (Ahmad and Cashmore, 1996; Ahmad et al., 1998). Phototropism appears to be mediated by a protein kinase, NPH1 (nonphototropic1), which has homology to other gene products known to be flavoproteins (Huala et al., 1997). *cry1,cry2* double mutants are also altered in some aspects of phototropism (Ahmad et al., 1998), suggesting that multiple photoreceptors may interact in this blue-light response.

To our knowledge, CRY1, CRY2, and NPH1 have not been implicated in the stomatal response to blue light. Instead, the carotenoid zeaxanthin has received much attention as a possible guard cell photoreceptor (Zeiger and Zhu, 1998). Zeaxanthin is a component of the photoprotective xanthophyll cycle of chloroplasts, in which excess irradiation energy is dissipated in the conversion of violaxanthin first to antheraxanthin and then to zeaxanthin. The absorption spectrum of zeaxanthin correlates reasonably well with the action spectrum for the guard cell blue-light response (e.g. Quiñones et al., 1996). In addition, good correlation exists between the levels of guard cell zeaxanthin and the extent of blue-light-stimulated stomatal opening. As summarized in Zeiger and Zhu (1998), DTT, a general reducing agent that prevents violaxanthin conversion, also inhibits blue-light-stimulated stomatal opening. Conversely, high-intensity background (red) light, which enhances the magnitude of the blue-light response, promotes zeaxanthin production. In cotton plants, in which the magnitude of the stomatal response to blue light is greater in adaxial than in abaxial stomata, the zeaxanthin content is also higher in the adaxial guard cells. Finally, the facultative CAM plant *Mesembryanthemum crystallinum* has stomata with a normal blue-light response and a functioning xanthophyll cycle when in C_3 mode, but loses both blue-light responsiveness and light-stimulated zeaxanthin accumulation upon conversion to CAM mode. Initial studies with the *npq1* (*nonphotochemical quenching 1*) mutant of Arabidopsis, which cannot convert violaxanthin to zeaxanthin (Nyogi et al., 1998), suggest that these mutant plants have little or no stomatal blue-light response. It will be of great interest to extend and confirm these Arabidopsis studies.

Despite these strong correlations, two observations serve as cautionary notes against the zeaxanthin hypothesis. First, the same types of correlations were observed between zeaxanthin content and phototropism of maize coleoptiles (Quiñones et al., 1996), yet none of the known mutations conferring phototropic insensitivity appear related to pathways of zeaxanthin production or turnover (see also Palmer et al., 1996). Second, treatment of plants with the herbicide SAN 9789 (norflurazon) virtually eliminates carotenoids (and chlorophyll), yet such plants still show transpiration increases in response to blue light (Karlsson et al., 1983). Although low levels of residual carotenoids may remain following norflurazon treatment, these data imply that the magnitude of the blue-light response does not necessarily correlate with the concentration of any given carotenoid. Thus, at present no definitive conclusion can be drawn regarding the nature of the blue-light photoreceptor of guard cells.

SOLUTE REGULATION AND BLUE LIGHT

A fundamental component of the blue-light response in guard cells is H^+ extrusion into the apoplast. Loss of protons from the guard cells contributes to a hyperpolarization of the plasma membrane, i.e. the cell becomes more negative inside. Hyperpolarization creates an electrical gradient that provides the driving force for the uptake of positively charged K^+, and activates K^+-selective ion channels in the guard cell membrane through which this uptake occurs. H^+ extrusion is also presumed to be crucial for Cl^- uptake via a process such as H^+/Cl^- cotransport. H^+ extrusion can be measured as a blue-light-stimulated acidification of the solution surrounding isolated guard cell protoplasts (Shimazaki et al., 1986).

Two transport mechanisms have been hypothesized to mediate guard cell H^+ extrusion. One is a H^+-ATPase and the other is a redox chain residing at the guard cell membrane. Electrophysiological measurements using the patch-clamp technique have shown that blue light stimulates a current across the guard cell membrane that, energetically, could only result from an active-transport process such as H^+ extrusion by a H^+-ATPase (Assmann et al., 1985). On the other hand, experiments measuring blue-light-stimulated medium acidification initially did not show inhibition of guard cell H^+ extrusion by vanadate (Shimazaki et al., 1986). Because vanadate is a standard inhibitor of plasma membrane H^+-ATPases, this negative result lent indirect support to the redox hypothesis. It was later discovered, however, that the presence of Cl^- in the medium could interfere with vanadate uptake by guard cells (Schwartz et al., 1991). Experiments subsequently performed in the absence of external Cl^- clearly demonstrated sensitivity of blue-light-stimulated guard cell swelling to vanadate, providing strong support for the involvement of a H^+-ATPase (Amodeo et al., 1992). Several H^+-ATPase genes are expressed in guard cells (Hentzen et al., 1996), and it will be interesting to determine whether one of these is specifically coupled to the signal transduction chain for blue light. Because of the convincing evidence for H^+-ATPase mediation of H^+ extrusion, the blue-light-stimulated reduction of exogenously provided electron acceptors detected at the guard cell plasma membrane (Vani and Raghavendra, 1989; Gautier et al., 1992) may indicate that a plasma membrane redox chain modulates the activity of the H^+-ATPase following a blue-light stimulus.

In addition to K^+ accumulation driven by membrane hyperpolarization, blue light also stimulates an increase in intracellular concentrations of the organic acid malate (Ogawa et al., 1978). It is thought that blue light increases the activity of the enzyme PEP carboxylase, which produces the precursor of malate—oxaloacetic acid—by carboxylation of PEP. PEP carboxylase does not appear to be directly light regulated; increased cytosolic K^+ concentrations and alkalinization of the guard cell cytosol upon H^+ extrusion are hypothesized mechanisms for activation of this enzyme (for review, see Assmann, 1993). Under certain

conditions blue light also stimulates Suc accumulation in guard cells (for review, see Talbott and Zeiger, 1998). This process is insensitive to DCMU, suggesting that it does not proceed via photosynthetic carbon fixation (Poffenroth et al., 1992). Suc may accumulate under these conditions from starch breakdown that occurs under blue light or from Suc uptake (Reddy and Das, 1986; Lu et al., 1997).

BLUE-LIGHT SIGNAL TRANSDUCTION CHAIN

Exogenous application of Ca^{2+} strongly inhibits stomatal opening, including opening driven by blue light (Parvathi and Raghavendra, 1997), and both hydrolytic activity and H^+-pumping activity of the H^+-ATPase are inhibited by micromolar levels of Ca^{2+} (Kinoshita et al., 1995), the levels typically found when a stimulus elevates cytosolic Ca^{2+} in plant cells. It was therefore a surprise to discover that some of the most effective pharmacological inhibitors of blue-light-stimulated H^+ extrusion and stomatal opening are those that inhibit kinases dependent on Ca^{2+} for their activity (Shimazaki et al., 1992, 1993; Table I). These inhibitors belong to a group of compounds that inhibit MLCK, which phosphorylates myosin. Kinases of this type are dependent on the Ca^{2+}-binding protein calmodulin for their activity, and calmodulin inhibitors are also effective against the blue-light response (Table I). The inhibitors are not simply poisoning the cells or acting nonspecifically, because in the presence of these inhibitors guard cells are still able to pump protons and drive stomatal opening in response to another opening signal, the fungal toxin fusicoccin (Shimazaki et al., 1993).

A MLCK has not yet been detected in plants, and no role has been identified to date for the involvement of a plant myosin in stomatal regulation. Furthermore, none of these kinase inhibitors is completely specific. Therefore, it is currently unclear whether these inhibitors are targeting a plant MLCK or another plant kinase that is susceptible to these inhibitors. One candidate for the latter category are the CDPKs, which have an intrinsic calmodulin-like domain and are therefore inhibited by compounds that target calmodulin (for review, see Roberts and Harmon, 1992). Guard cells do express CDPK (Li et al., 1998), but the sensitivity of guard cell CDPK to MLCK inhibitors remains to be tested. It is also not currently known whether the relevant kinase phosphorylates the H^+-ATPase molecule itself or a signaling intermediary.

Given the effectiveness of exogenously supplied Ca^{2+} as an inhibitor of stomatal opening, it seems implausible that a general elevation of cytosolic Ca^{2+} is a component of the blue-light response. Spatially distinct elevations of Ca^{2+}, perhaps within the localized vicinity of the relevant kinase, could play a role. One might think that it would be easy to test such hypotheses by imaging of cytosolic Ca^{2+} concentrations using fluorescent indicator dyes, but unfortunately the ratiometric Ca^{2+}-indicator dyes typically require excitation by UV/blue light as part of the measurement process, meaning that one cannot readily obtain a value for resting Ca^{2+} levels before blue-light stimulation. It may prove feasible to use longer-wavelength indicators such as Ca^{2+} crimson, which is not ratiometric but could give a qualitative indication of changes in cytosolic Ca^{2+} concentrations.

STOMATAL CLOSURE INDUCED BY ABA

When a plant endures water stress in drying soil, ABA is synthesized in the roots and translocated to the leaf through the transpiration stream. ABA is redistributed by a pH change in the apoplast of the leaf, and may also be synthesized by the guard cells themselves. These processes result in an increase of ABA levels around or inside guard cells. The increased concentration of ABA stimulates stomatal closure and reduces transpirational water loss from the leaf. Stomatal closure occurs when the accumulated K^+, Cl^-, and organic solutes are released from the guard cells into the external space. Because most of the K^+ salts accumulate in the vacuoles of guard cells in open stomata, these

Table I. *Effectiveness of MLCK and calmodulin inhibitors on inhibition of blue-light-stimulated H^+ pumping by guard cell protoplasts of fava bean*

All data are from Shimazaki et al. (1992, 1993).

Inhibitor	Major Target	Concentration	H^+ Pumping
		μM	*% of control*
K-252a	Broad spectrum of kinases	1	26
ML-7	MLCK	50	24
ML-9	MLCK	50	45
KT5926	MLCK	1	20
H-8	cAMP/cGMP-dependent protein kinases	50	101
H-7	Protein kinase C	50	92
Calphostin C	Protein kinase C	1	80
KN-62	Ca^{2+}/calmodulin kinase II	50	98
Trifluoperazine	Calmodulin	50	36
Prenylamine	Calmodulin	50	62
HT-74	Calmodulin	25	17
W-7	Calmodulin	50	88
		200	11
W-5	"Inactive" W-7 analog	50	94
		200	78

ions must pass through both the vacuolar and plasma membranes during stomatal closure (Ward et al., 1995; Fig. 1).

PERCEPTION OF ABA

There has been no conclusive identification of an ABA receptor(s) in plant cells. ABA microinjected into guard cells induces stomatal closure, suggesting that an ABA reception site is localized internally (Allan et al., 1994; Schwartz et al., 1994). The higher efficiency of ABA action with low pH in the external medium, as has been shown in the inhibition of stomatal opening, K^+ uptake channels, and blue-light-dependent proton pumping (Schwartz et al., 1994; Goh et al., 1996), suggests that the active form for inhibition of stomatal responses is the protonated form of ABA. These results suggest that because the protonated form of ABA easily permeates the plasma membrane, ABA can act inside the guard cell. An external site of action has also been proposed (e.g. Anderson et al., 1994), and these two possibilities are not mutually exclusive.

INVOLVEMENT OF Ca^{2+} IN STOMATAL CLOSURE BY ABA

ABA and Ca^{2+} have a synergistic effect on the inhibition of stomatal opening (De Silva et al., 1985), suggesting that ABA-induced stomatal movements might involve Ca^{2+}. A link between ABA and Ca^{2+} has been directly demonstrated by an increase of cytosolic-free Ca^{2+} in guard cells of *C. communis* in response to ABA, detected using fluorescent Ca^{2+} indicators (McAinsh et al., 1990). The ABA-triggered increase in cytosolic Ca^{2+} precedes stomatal closure. The increase in cytosolic Ca^{2+} stimulated by ABA is probably due to both Ca^{2+} uptake from the extracellular space and Ca^{2+} release from intracellular stores. Simultaneous measurement of cytosolic Ca^{2+} and ionic currents across the plasma membrane of guard cell protoplasts from fava bean indicate that Ca^{2+} influx across the membrane participates in the increase in cytosolic Ca^{2+} in response to ABA (Schroeder and Hagiwara, 1990). Influx of Ca^{2+} across the plasma membrane from the apoplast occurs through ABA-activated, nonselective, Ca^{2+}-permeable channels. Stretch-activated Ca^{2+}-selective channels (Cosgrove and Hedrich, 1991) could also participate in the ABA response, but this has not been tested directly.

Ca^{2+} stored in intracellular organelles such as vacuoles is another candidate for a Ca^{2+} source. Release of Ca^{2+} from the vacuole into the cytosol seems to have several pathways in guard cells. Gilroy et al. (1990) have shown that guard cells are competent to release Ca^{2+} in response to microinjected IP_3; and Lee et al. (1996) have suggested that ABA may stimulate inositol phospholipid turnover in guard cell protoplasts of fava bean, resulting in IP_3 production. Ca^{2+} can also be released by microinjection of cyclic ADP-Rib into the cytosol of guard cells (McAinsh et al., 1997). In addition to presumed Ca^{2+} channels gated by ligands such as IP_3 and cyclic ADP-Rib, slow-vacuolar channels, which are permeable to both Ca^{2+} and K^+, and voltage-gated Ca^{2+} channels have also been identified in the vacuolar membrane of guard cells; these two distinct Ca^{2+}-permeable channels may also be pathways for Ca^{2+} release in response to ABA (Ward et al., 1995). Ward and Schroeder (1994) have suggested that the slow-vacuolar channels may release Ca^{2+} in response to elevations in cytosolic Ca^{2+}, a phenomenon known as Ca^{2+}-induced Ca^{2+} release. According to this theory, activation of vacuolar K^+ channels after an increase in cytosolic Ca^{2+} could depolarize the tonoplast, thereby activating the slow-vacuolar channels.

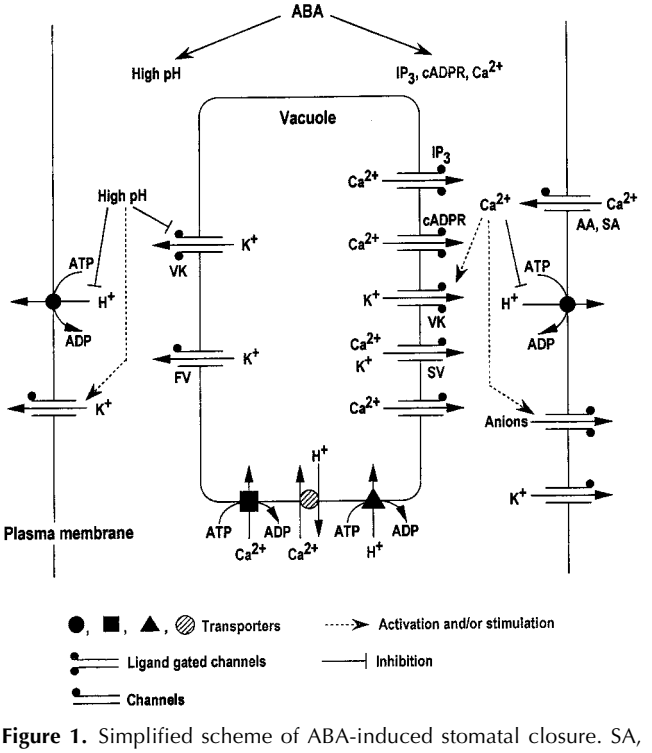

Figure 1. Simplified scheme of ABA-induced stomatal closure. SA, Stretch-activated; AA, ABA-activated.

Ca^{2+} TARGETS IN THE PLASMA MEMBRANE

Accumulated K^+ and anions must be released from guard cells to the external space in a sustained fashion during stomatal closure. There are two major pathways for K^+ and anion release from guard cells: outward-rectifying K^+ channels (i.e. K^+ channels that are regulated such that they open only under conditions in which the electrochemical gradient favors K^+ efflux) and anion channels, respectively. During stomatal opening the membrane potential of guard cells can be more negative than −200 mV (Thiel et al., 1992), and both the outward K^+ and the anion channels are inactivated or are in a low active state. When the Ca^{2+} concentration increases to around 1 μM in the cytosol in response to ABA, Ca^{2+} inhibits the H^+ ATPase and activates anion channels in the plasma membrane (Kinoshita et al., 1995; Ward et al., 1995). Inhibition of the pump depolarizes the membrane, increasing the driving force for anion loss and thus causing further depolarization. Outward-rectifying K^+ channels activate in response to the depolarized membrane potential. Thus sustained release of

K^+ and anions is achieved. Ca^{2+} also inhibits inward-rectifying K^+ channels in the plasma membrane, and this could be a component of the pathway mediating ABA inhibition of stomatal opening.

Ca^{2+} TARGETS IN THE TONOPLAST

More than 90% of the K^+ and anions accumulated by guard cells are stored in the vacuole and must be released across the vacuolar membrane into the cytosol during stomatal closure. Ward et al. (1995) have identified a voltage-independent vacuolar K^+-selective channel and have suggested that it may be responsible for the release of K^+ from the vacuole. Elevated concentrations of cytosolic Ca^{2+} activate the vacuolar K^+-selective channels. Sustained efflux of K^+ from vacuoles requires either simultaneous efflux of anions or uptake of counterions for K^+ into the vacuole. Ward et al. (1995) have suggested that electrogenic H^+ pumps in the tonoplast provide the counterions for the sustained loss of K^+ from the vacuole, although these pumps have yet to be characterized at the molecular level in guard cells. Alkalinization of the guard cell cytosol by 0.2 to 0.3 pH units has been observed in response to ABA (Blatt and Armstrong, 1993), and this alkalinization would be consistent with the activation of an inward-directed electrogenic proton pump in the vacuolar membrane (Ward et al., 1995). Anions including Cl^- and malate must also be released from the vacuole during stomatal closure in response to ABA, but the underlying transport mechanisms are not yet elucidated.

Ca^{2+}-INDEPENDENT PATHWAYS

When ABA is applied to epidermal peels, a rapid increase in cytosolic Ca^{2+} often precedes stomatal closure, but there is no such Ca^{2+} increase in some guard cells even though all stomata close (McAinsh et al., 1990; Gilroy et al., 1991). This result suggests that there may be at least two transduction pathways in ABA signaling in guard cells: one that is Ca^{2+}-dependent and one that is Ca^{2+}-independent (Allan et al., 1994). Cytosolic alkalinization of guard cells (described above) may be a component of the Ca^{2+}-independent pathway. Blue-light-dependent H^+ pumping, which hyperpolarizes the membrane potential, is inhibited by ABA, and this may occur through the alkalinization of cytosolic pH (Goh et al., 1996). In guard cells in which the membrane potential resided well negative of the K^+ equilibrium potential, ABA shifted the potential to a more depolarized state (Blatt and Armstrong, 1993), probably due to ABA inhibition of the H^+ pump and/or activation of anion channels in the plasma membrane.

Alkalinization also appears to increase the availability of the outward K^+ channels that mediate K^+ loss from the guard cells in response to ABA; cytosolic Ca^{2+} elevation does not activate these channels (Blatt and Armstrong, 1993). Because most of the K^+ in turgid guard cells is accumulated in the vacuole, K^+ release from the vacuole in response to alkalinization might be expected. K^+-permeable fast-vacuolar channels in the tonoplast may play this role (Allen et al., 1998). Fast-vacuolar channels are active at resting Ca^{2+} concentrations, are partially inhibited at 1 μM Ca^{2+}, and remain active at high cytosolic pH (7.4). The Ca^{2+}-activated, vacuolar K^+-selective channels, an alternate pathway of K^+ release from the vacuole, are suppressed strongly at this pH level.

SIGNAL TRANSDUCTION AFTER ABA PERCEPTION

ABA disrupts cortical actin filaments in parallel with stomatal closure (Eun and Lee, 1997). If this event is not only correlative but also causative, it may reflect structural regulation of ion-channel activity or cytoskeletal regulation of signaling molecules. Among potential signaling molecules, protein kinases and phosphatases have been most strongly implicated in ABA signal transduction in guard cells. In fava bean, protein kinase inhibitors inhibit both anion channel activation and stomatal closure induced by ABA, and a protein phosphatase inhibitor, okadaic acid, activates anion channels (Schmidt et al., 1995). Activation of a Ser/Thr-type protein kinase (ABA-activated or ABA-related protein kinase) by ABA has been found in guard cells of fava bean using an in-gel assay technique (Li and Assmann, 1996; Mori and Muto, 1997). The kinase has a molecular mass of 48 kD, does not require Ca^{2+} for its activity, and appears to be specific to guard cells. It will be interesting to determine whether this kinase is involved in Ca^{2+}-independent ion-channel regulation in response to ABA. Because it has been shown that guard cell CDPK phosphorylates the inward K^+ channel protein KAT1 (Li et al., 1998), it is possible that phosphorylation by CDPK could be involved in Ca^{2+}-mediated ABA inhibition of stomatal opening. A Ca^{2+}-activated, calcineurin-like phosphatase has also been implicated in Ca^{2+} inhibition of the inward K^+ channels (Luan et al., 1993), and may be involved in the ABA response as well, perhaps targeting a different site on the channel molecule than CDPK phosphorylates or acting on a different component of the signal transduction pathway.

In guard cells of Arabidopsis, ABA-induced activation of anion channels and stomatal closure are suppressed by okadaic acid and enhanced by protein kinase inhibitors (Pei et al., 1997), suggesting the involvement of phosphatases. This is the opposite response to that found in *V. faba*, and the basis for the differences between species is not apparent. The ABA-insensitive mutants of Arabidopsis, *abi1* and *abi2*, show a wilty phenotype, indicating that stomatal regulation is impaired. Both *ABI1* and *ABI2* genes encode protein phosphatases of the 2C type (Leung and Giraudat, 1998). In guard cells from *abi1* and *abi2* mutant plants, ABA activation of one class of anion channels, the "slow" anion channels and ABA-induced stomatal closure are abolished (Pei et al., 1997). In contrast, in transgenic tobacco plants that express mutant abi1 protein, anion channel behavior is not altered, but the ABA responsiveness of both inward- and outward-rectifying K^+ channels is lost (Armstrong et al., 1995). Therefore, the protein phosphatases 2C seem to have an important role in ABA signaling, but may have different roles in different plant species.

CONCLUSIONS

It is not yet possible to draw firm conclusions regarding many aspects of guard cell responses to blue light and ABA. A few points are worth commenting upon, however. First, the H^+-ATPase is the only guard cell transporter to date known to be regulated by both blue light and ABA, and, as such, represents a site of convergence for the blue-light and ABA signaling pathways. As more detailed knowledge of these signal transduction pathways comes to light, it will be of interest to determine whether they converge on the H^+-ATPase itself or on an upstream regulatory molecule. Second, the multiplicity of ABA-sensitive transporters in guard cells may reflect the paramount importance of stomatal closure in plant survival: mesophytic plants can survive for some time on stored carbohydrate reserves, but succumb relatively quickly under conditions of water stress. This point is illustrated by the wilty *abi* mutants of Arabidopsis, which cannot close their stomates and must be grown under elevated levels of ambient humidity if they are to reach reproductive maturity. Finally, although this brief review has focused on blue light and ABA, we also emphasize that guard cells are multisensory cells. Other guard cell responses, e.g. stomatal opening in response to reduced intercellular CO_2 and stomatal closure in response to reduced ambient humidity, are also crucial components in the balancing act that guard cells play as they integrate the opposing demands of maximizing CO_2 uptake and minimizing transpirational water loss.

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