Evidence for an Extracellular Reception Site for Abscisic Acid in Commelina Guard Cells

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The phytohormone abscisic acid (ABA) triggers stomatal closing as a physiological response to drought stress. Several basic questions limit our understanding of the mechanism of ABA reception in guard cells. Whether primary ABA receptors are located on the extracellular side of the plasma membrane, within the intracellular space of guard cells, or both remains unknown. Furthermore, it is not clear whether ABA must be transported into guard cells to exert control over stomatal movements. In the present study, a combination of microinjection into guard cells and physiological assays of stomatal movements have been performed to determine primary sites of ABA reception in guard cells. Microinjection of ABA into guard cells of Commelina communis L. resulted in injected cytosolic concentrations of 50 to 200 μM ABA and in additional experiments in lower concentrations of approximately 1 μM ABA. Stomata with ABA-loaded guard cells (n > 180) showed opening similar to stomata with uninjected guard cells. The viability of guard cells following ABA injection was demonstrated by neutral red staining as well as monitoring of stomatal opening. Extracellular application of 10 μM ABA inhibited stomatal opening by 98% at pH 6.15 and by 57% at pH 8.0. The pH dependence of extracellular ABA action may suggest a contribution of an intracellular ABA receptor to stomatal regulation. The findings presented here show that intracellular ABA alone does not suffice to inhibit stomatal opening under the imposed conditions. Furthermore, these data provide evidence that a reception site for ABA-mediated inhibition of stomatal opening is on the extracellular side of the plasma membrane of guard cells.

ABA is a vital plant hormone, which, among other functions, is known to induce dormancy in seeds and buds, inhibit root elongation, and reduce the effects of drought stress on plants (Walton, 1980). Studies of Arabidopsis show that two genetic loci each affect the control of processes such as seed germination, seedling growth, gas exchange regulation, and protein synthesis by ABA, indicating common initial signaling mechanisms (Koornneef et al., 1984; Finkelstein and Somerville, 1990). During drought conditions, ABA acts on stomatal pores in the leaf epidermis to cause closure or to inhibit the opening of stomata. Stomatal pores, which regulate transpirational H₂O loss and CO₂ uptake from the atmosphere, are bounded by pairs of guard cells. Stomatal aperture is controlled by guard cell turgor, which is mediated by ionic fluxes across guard cell plasma membranes.

Although the physiology of ABA action has been studied in detail, the molecular mechanisms of ABA reception and initial events in ABA-induced signal transduction remain unknown. In guard cells, several key ion channels responsible for ABA-induced stomatal closure have been characterized (Schroeder et al., 1987; Schroeder and Hagiwara, 1989; for reviews, see Schroeder and Hedrich, 1989; Keams and Assmann, 1993). Whether ABA or other plant hormones exert their primary actions via activation of extracellular receptors or by binding to intracellular receptors or both are fundamental unanswered questions in plant biology.

Evidence for the plant hormone auxin has shown that extracellular binding sites exist that may contribute to auxin action (Venis et al., 1990; Babier-Brygoo et al., 1991; Marten et al., 1991; Jones and Herman, 1993). Results concerning the primary site of ABA reception, however, have so far been equivocal. As yet unconfirmed data from experiments using immunohistochemical staining or photoaffinity labeling have suggested that ABA binds to proteins in the plasma membrane of guard cells (Hornberg and Weiler, 1984; Curvetto and Delmastro, 1986). However, evidence exists that ABA uptake may be required for stomatal regulation. Cellular ABA accumulation is known to depend on extracellular pH (Kaiser and Hartung, 1981). Studies in which the extracellular pH was varied showed that ABA was less effective at alkaline pH (Ogunkami et al., 1973), indicating that ABA uptake may be necessary for the regulation of stomatal aperture. A different study showed a lower threshold concentration for ABA at an acidic pH of 5.5 but a more rapid ABA response at a neutral pH of 6.8 (Paterson et al., 1988). In addition, other studies show that alkaline pH does not reduce ABA effects (Hartung, 1983).

Extracellular application of ABA to guard cells produces early and rapid increases in cytosolic Ca²⁺ (McAinsh et al., 1990; Schroeder and Hagiwara, 1990), together with rapid activation of nonselective ion channels, which provide a mechanism for Ca²⁺ influx and, even in the absence of extracellular Ca²⁺, for depolarization (Schroeder and Hagiwara, 1990). Concomitantly, ABA triggers a rapid transient of K⁺ efflux (MacRobbie, 1990), which requires depolarization. In spite of the rapid nature of these early responses, which occur within seconds, these data have not excluded the possibility that ABA uptake into guard cells may be required for their activation.

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Stomatal pores provide an opportune system to study ABA reception and its initial signal transduction mechanisms for several reasons: (a) the response of single guard cells can be readily monitored by measuring stomatal aperture, (b) the delivery of various physiological stimuli can be made to guard cells within epidermal strips, and (c) several molecular mechanisms that control ABA-induced stomatal movements have been identified. Furthermore, microinjections of dyes and second messengers into guard cells have recently shown that the cytosol of guard cells can be accessed using microelectrodes (Blatt et al., 1990; Gilroy et al., 1990, 1991; Mcainsh et al., 1990, 1992). In the present study we have used iontophoretic microinjection to load ABA into the cytosol of guard cells. In conjunction with monitoring the aperture of stomatal pores in leaf epidermal strips, a potent cell biological system is provided to gain initial insight into the question of whether extracellular ABA reception is required for control of stomatal movements.

MATERIALS AND METHODS

Plant Material

Commelina communis L. plants were grown in a controlled environment growth chamber (Conviron E15) at 25°C with a 16-h light/8-h dark day/night cycle, under fluorescent and incandescent illumination at a photon fluence rate of 100 μE m⁻² s⁻¹. Plants were grown in potting soil (Uni-Gro; L&L Nursery Supply Inc., Chino, CA) and watered with deionized water. Epidermal strips were isolated from the third fully expanded leaf of 4- to 6-week-old plants.

Epidermal Strip Preparation and Incubation

Variability in the extent of stomatal responses to physiological stimuli has been reported (Gorton et al., 1989). To minimize experimental variation, pairs of epidermal strips from the same leaf were used for control experiments and treatments. Furthermore, injected and uninjected guard cells (approximately 10 injected and >20 uninjected guard cells per epidermal strip) were mapped to allow repeated aperture measurements of individual stomatal pores over time (Gorton et al., 1989). Each leaf was halved vertically, and two identical strips of abaxial epidermis were gently isolated under water to minimize epidermal cell damage and desiccation. The epidermal strips were anchored on two sides with high-vacuum grease (Dow Corning, Midland, MI) in perfusion chambers prior to microinjection. The epidermal strips were incubated in 50 or 100 mM KCl with either 10 mM Mes-Tris (pH 6.15) or 10 mM Hepes-KOH (pH 8.0) containing either 0 or 10 mM ABA (± cis-trans isomer, Sigma). In all experiments stomata were initially closed. To promote stomatal opening, the epidermal strips were incubated under white light with a photon fluence rate of 500 μE m⁻² s⁻¹. In all experiments epidermal strips from the same leaves were used for control treatments. Data from experiments in which stomata of control treatments did not open in response to light were omitted from further analysis. During the incubation period (up to 135 min), the chambers were perfused with CO₂-free air (21% O₂, 79% N₂; Parsons Medical, San Diego, CA), and the temperature was controlled at 27°C. Measurements of stomatal aperture were made using an ocular micrometer and high-resolution, differential interference microscopy at the times indicated in the figure legends.

Microinjection

Microelectrodes were pulled from filamented glass (Sutter Instruments Co., San Rafael, CA, catalog No. QF 100-70-10) to a tip diameter of approximately 250 nm. The electrode tip was filled with 3 μL of a filtered solution of 10 mM ABA (free acid), 20 mM KOH (from a stock of 100 mM ABA dissolved in 200 mM KOH), and 10 mM Mes. The final pH of the microinjected solution was adjusted to 7.2 by addition of approximately 6 mM HCl. The osmolality was adjusted to 525 mmol kg⁻¹ by addition of d-mannitol. For experiments in which the final injected cytosolic ABA concentration was approximately 1 μM, the electrode tip was filled with a solution containing 100 μM ABA, 100 mM KCl, 10 mM Mes (pH 7.5) (suggested by A. Schwartz and S.M. Assmann). The electrode was backfilled with 1 M KCl. Electrode maneuvering and guard cell impalement were accomplished with a micromanipulator (Narishige, Tokyo, Japan) and an inverted microscope (Diaphot; Nikon, Tokyo, Japan) under ×400 magnification using high-resolution, differential interference-contrast optics. Impalement of guard cells became apparent both microscopically and by the simultaneous appearance of hyperpolarized membrane potentials. One or two guard cells per closed stoma were iontophotically injected (approximately 10 stomata injected per epidermal strip). Iontophoretic injection was achieved using alternating 5-s pulses of −200 and 0 pA of current applied through the electrode for 2 min (Gilroy et al., 1991) using an Axopatch-1D patch-clamp amplifier in current clamp mode (Axon Instruments, Inc., Foster City, CA). To avoid external effects of any ABA possibly leaking from the injection microelectrode during the microinjection procedure, the epidermal strip was continuously perfused with incubation medium using a peristaltic pump (Rainin, Woburn, MA). Epidermal strips were incubated in neutral red to verify the viability of injected guard cells at the end of all experiments (Gilroy et al., 1991).

Carboxyfluorescein Experiments

To estimate the approximate cytosolic concentration range of ABA achieved by iontophoretic injection, microelectrodes were filled with 10 μM to 20 μM carboxyfluorescein adjusted to pH 7.2 with KOH and with the osmolality adjusted to 525 mmol kg⁻¹ by the addition of mannitol. Guard cells were iontophotically injected (as above). Microelectrode and cell fluorescence were recorded by passing light from a 100-W mercury lamp through a fluorescein isothiocyanate filter set (Omega Optical, excitation 480 nm, emission 535 nm). Fluorescence measurements were made using a pinhole diaphragm and a photo multiplier (Thorn EMI Electron Tubes, Inc., Rockaway, NJ) and recorded and analyzed using an Axolab interface (Axon Instruments, Inc.) and a 25-MHz 386-AT microcomputer (Schroeder and Hagiwara, 1990). Fluorescence intensities were measured from 9.6 μm² of guard cell cytoplasmic regions exposed through a pinhole diaphragm (the less-fluorescent areas directly over the vacuole and the...
Extracellular Guard Cell ABA Receptor

RESULTS

Iontophoretic Microinjection of Commelina Guard Cells

To study the primary reception site of ABA in guard cells, it was necessary to apply known concentrations of ABA either extracellularly or to the guard cell cytosol. Single-cell microinjection was used to access the guard cell cytosol. The efficiency of the iontophoretic microinjection technique was analyzed using the fluorescent dye carboxyfluorescein. Upon impalement of guard cells with the microelectrode and injection of negative current, rapid appearance of the dye in the cytoplasm was observed (Fig. 1). Localization of dye in the cell periphery and in chloroplasts and the nucleus indicated cytoplasmic rather than vacuolar injection. The observed results of more than 40 injections of carboxyfluorescein into stomatal guard cells indicated that the iontophoretic injection technique used in this study allowed reproducible delivery into the cytosol.

Quantitation of carboxyfluorescein microinjection by single-cell fluorescence photometry was used to estimate the efficiency of iontophoretic microinjection of ABA. From these experiments it was estimated that iontophoretic injection of ABA resulted in a concentration of 50 to 200 μM ABA in the guard cell cytosol. The efficiency of ABA microinjection determined here correlates closely with microinjection efficiencies reported for guard cells in recent studies (Gilroy et al., 1990, 1991; McAlinsh et al., 1990; Lee et al., 1993). These estimates give the cytosolic concentration of microinjected ABA and do not include endogenous ABA in guard cells (Harris et al., 1988). The injected concentration range of ABA is severalfold higher than the concentration of ABA found in Vicia faba guard cells following water stress, which was within the range of 7 to 13 μM (Harris et al., 1988).

To test whether guard cells microinjected with ABA would remain viable for the duration of these experiments, epidermal strips were stained with the viability stain neutral red at the completion of all experiments. Up to 2.5 h after microinjection of ABA, 80 to 90% of the cells remained viable as determined by the ability to accumulate neutral red in the vacuole and sustain visible cytoplasmic streaming. In addition, stomata with microinjected guard cells were competent to open, which requires guard cell viability, as described below. Approximately 10% of the injected guard cells showed immediate cessation of cytoplasmic streaming during microinjection and a visible deflation in turgor, indicating a vacuolar location of the electrode tip. Data from these cells were not included in this study.

Microinjection of ABA

The question of whether ABA, applied directly to the guard cell cytoplasm, could inhibit stomatal opening was addressed using the iontophoretic microinjection technique. Aperture measurements of noninjected stomata and of stomata within epidermal strips from the same leaf externally treated with ABA were made in parallel and served as controls for all experimental trials. Guard cells were microinjected with ABA (50-200 μM) and exposed to light

Figure 1. Carboxyfluorescein was iontophoretically microinjected into the cytosol of one stomatal guard cell in an epidermal strip from the abaxial side of a Commelina leaf.

Figure 2. Iontophoretic microinjection of ABA does not inhibit stomatal opening. One guard cell (indicated by arrow) was microinjected using a microelectrode containing ABA. A, Stomata following microinjection at time 0 min. B, The same stomata after 135 min of incubation under light. The abaxial epidermal strip was incubated in 50 mM KCl, 10 mM Hepes-HCl (pH 8.0). This experiment is representative of more than 100 guard cells microinjected with ABA under these conditions. Bars indicate 20 μm.
in the absence of external ABA (n > 180 ABA-injected guard cells in this study). Figure 2A shows two initially closed stomata following ABA microinjection and prior to exposure to light. In Figure 2B, the same two stomata are shown following incubation under white light for 135 min. In all experimental trials, the stomata with injected guard cells (n > 100) opened similarly to the un.injected control stomata. Stomata in which both surrounding guard cells were microinjected with ABA (n = 10) also opened similarly to un injected control stomata (data not shown). These results indicated that intracellular ABA alone could not inhibit stomatal opening. In additional control experiments, stomata microinjected with 50 to 200 μM ABA and incubated in the presence of 10 μM extracellular ABA at pH 6.15 remained closed (n = 20).

To quantify the effect of internal ABA on stomatal opening, apertures were measured for stomata with guard cells injected with 50 to 200 μM internal ABA throughout a 135-min incubation. As shown in Figure 3, stomata with one guard cell microinjected with ABA consistently had rates and maximum stomatal opening that were similar to un injected controls. When guard cells were injected with lower cytosolic ABA concentrations of approximately 1 μM (n = 24 guard cells), stomatal pores also opened similarly to controls.

It is possible that microinjected ABA requires the presence of additional CaCl₂ in the bath solution for inhibition of stomatal opening (Schwartz et al., 1988). Stomata of guard cells injected with 50 to 200 μM ABA, with 0.5 mM CaCl₂ added to the bath solution, also opened similarly to controls (n = 10). These data further support the observation (Fig. 2) that internal ABA alone was not sufficient to inhibit stomatal opening.

**Extracellular Application of ABA**

The ability of low concentrations of externally applied ABA to inhibit stomatal opening was tested. To exclude the possibility that externally applied ABA could be taken up by guard cells, the experiments were conducted at an extracellular pH of 8.0. ABA predominates in the deprotonated form at pH 8.0 and uptake is eliminated (Kaiser and Hartung, 1981; Baier and Hartung, 1991). Epidermal strips with initially closed stomata (Fig. 4A) were incubated under light in the presence of 10 μM external ABA. As shown in Figure 4B, after 135 min the stomata exposed to 10 μM external ABA remained closed or did not open as wide as nontreated controls (n > 400 stomata in 20 experiments). Parallel control experiments in the absence of external ABA showed wider stomatal opening than ABA-treated stomata. This confirmed that ABA in the external solution contributes to inhibition of stomatal opening.

Taken together, the above experiments (Figs. 2–4) demonstrate a requirement for external ABA in the inhibition of stomatal opening under the imposed conditions. To further analyze whether the uptake of externally applied ABA may also contribute to the inhibition of stomatal opening, the effect of ABA on stomatal aperture was quantitatively and directly compared at pH 6.15 and 8.0 using epidermal strips from the same leaves. Guard cells were exposed to 0 or 10 μM external ABA at pH 6.15 or 8.0, placed under light, and perfused with CO₂-free air for 2 h. Externally ABA at both pH 6.15 and 8.0 inhibited stomatal opening (Fig. 5), and inhibition was more effective at pH 6.15 in all experiments. Extracellular ABA inhibited stomatal opening without addition of exogenous Ca²⁺ to the bath solution in all experiments. These data suggest that basal free Ca²⁺ levels in the cell wall space of guard cells and in the unbuffered bath solutions were sufficient for ABA-induced inhibition of stomatal opening under the imposed conditions and/or that Ca²⁺-independent events contribute to inhibition of stomatal opening (MacRobbie, 1990). Stomatal opening was inhibited an average of 98% by 10 μM external ABA at pH 6.15 and 57% at pH 8.0. These results may suggest that, in addition to an extracellular ABA requirement, the pH-dependent uptake of ABA contributes to the inhibition of stomatal opening and/or that other processes involved in stomatal regulation are pH dependent.

Further experiments were conducted to determine the ef-
Effects of microinjected ABA on the time course of stomatal opening at an extracellular pH of 8.0. Guard cells were injected with ABA and incubated for 135 min under white light at an external pH of 8.0. Stomata with guard cells that were microinjected with ABA opened at a rate and to an extent that was similar to uninjected control stomata (Fig. 6). These data may indicate that the decreased effectiveness of external ABA in the inhibition of stomatal opening at pH 8.0 (Fig. 5) was not solely due to a decrease in ABA uptake and a lack of intracellular ABA receptor activation. Although a possibility of functional intracellular receptors for ABA was not excluded, the presented data demonstrate a requirement for extracellular ABA in the inhibition of stomatal opening.

**DISCUSSION**

Phytohormones such as auxin, GAs, and ABA have important functions in the control of plant growth, development, and responses to the environment. Functional reception sites at the extracellular side of the plasma membrane have been implicated for the hormones auxin (Venis et al., 1990; Barbier-Brygoo et al., 1991; Marten et al., 1991; Jones and Herman, 1993) and GAs (Hooley et al., 1991). However, the cellular location of ABA receptors has remained equivocal. Stomatal guard cells provide a well-suited system to study ABA receptor location.

**ABA Reception by Guard Cells**

Under drought stress conditions, ABA accumulates rapidly in leaves (for review, see Walton, 1980) and can decrease transpirational water loss by triggering stomatal closure. The physiological response of stomatal guard cells to exogenously applied ABA is well characterized. Studies have shown that an extracellular concentration of 1 \(\mu M\) ABA is sufficient to induce maximum stomatal closure in *Commelina* (McAinsh et al., 1990). Although externally applied ABA triggers stomatal closure in excised epidermal strips, it has remained unclear if binding to an extracellular receptor contributes to this response. High-affinity ABA-binding sites have been indicated in the plasma membrane of *V. faba* guard cells (Hornberg and Weiler, 1984; Curvetto and Delmastro, 1986). However, it is not clear if these binding sites represent ABA uptake transporters or ABA receptors. Several distinct mechanisms for ABA reception are therefore possible, including: (a) intracellular ABA receptors requiring ABA uptake into guard cells and cytosolic ABA accumulation, (b) ABA receptors at the extracellular side of the plasma membrane that trigger intracellular signaling events, and (c) extracellular ABA receptors acting in parallel with intracellular receptors, resulting in a requirement for both extracellular and cytosolic ABA. To gain insight into which of these ABA reception mechanisms can be excluded and which mechanisms function in the inhibition of stomatal opening, we utilized the technique of microinjection of ABA into the cytosol of guard cells and the physiological assay of stomatal aperture measurement.

**Extracellular Location of Guard Cell ABA Receptors**

As reported here, stomata with one or both guard cells microinjected cytosolically with ABA consistently opened at a similar rate and to a similar maximum aperture as uninjected stomata (Figs. 2, 3, and 6). These data indicate that intracellular ABA alone was not sufficient to inhibit stomatal opening. Externally applied ABA (10 \(\mu M\)) inhibited stomatal opening (Figs. 4 and 5). Taken together, these data demonstrate that extracellular ABA was required to inhibit stomatal opening under the conditions imposed here. However, the possibility that, following external application, ABA uptake had an additional role in the inhibition of opening could not be ruled out by these observations (Fig. 5). The experimental approach presented here focuses on whether extracellular ABA is required. Data using co-injection of ABA and carboxyfluorescein have indicated a contribution of intracellular ABA to stomatal regulation using a complementary approach.

Figure 5. External ABA at pH 6.15 and 8.0 inhibits stomatal opening. Epidermal strips were incubated at pH 6.15 or 8.0 in the presence and absence of 10 \(\mu M\) external ABA. Apertures of mapped stomata \(n = 40\) for each bar were measured before the addition of ABA and after 2 h of exposure to extracellular ABA and incubation under white light. Data are from one representative experiment of five. Error bars denote SE.

Figure 6. Microinjected ABA does not inhibit stomatal opening at an extracellular pH of 8.0. Guard cells were microinjected with ABA and abaxial epidermal strips were incubated as described in the legend to Figure 2. Uninjected guard cells \(n = 20\); ■, stomata with one guard cell injected with ABA (50–200 \(\mu M\); \(n = 10\)). Data are from one representative experiment of seven. Error bars denote SE.
that focuses on a requirement for intracellular ABA (Schwartz et al., 1993).

In the present study inhibition of stomatal opening by ABA was analyzed rather than ABA-mediated stomatal closing to ensure viability of guard cells after microinjection. In calibration experiments, high levels of microinjected carboxyfluorescein inhibited stomatal opening and reduced cell viability as judged by neutral red staining (n > 40, see "Materials and Methods"). Further experiments would therefore be required to determine if the presented results also apply to ABA-induced stomatal closing.

Dependence of ABA Effectiveness on Extracellular pH

At an extracellular pH of 8.0, a condition that eliminates ABA uptake (Kaiser and Hartung, 1981; Baier and Hartung, 1991), externally applied ABA consistently inhibited stomatal opening (Fig. 5), although to a lesser extent than at pH 6.15. Furthermore, stomata with guard cells microinjected with ABA and incubated at pH 8.0 opened at a rate similar to uninjected control stomata (Fig. 6). These data further support a requirement for an ABA receptor at the extracellular side of the plasma membrane that functions in the regulation of stomatal aperture.

However, the data reported here do not completely exclude the presence of an additional intracellular ABA receptor. Alternate explanations for the decreased inhibition of stomatal opening at pH 8.0 are pH effects on plasma membrane ion channels and pumps that control stomatal movements or a direct pH dependence of ABA binding to receptors at the plasma membrane. Previous studies have suggested that a decreased effectiveness of extracellular ABA to induce stomatal closure at alkaline pH is correlated with a decreased rate of ABA binding to high-affinity sites at the plasma membrane of guard cells (Hornberg and Weiler, 1984).

Studies of the Arabidopsis thaliana ABA-insensitive mutants abi1 and abi2 have shown that early ABA-induced signaling events are shared in different physiological responses of different cell types (Pinkelstein and Somerville, 1990). Recent studies using different techniques to those used here, specifically of α-amylase secretion in barley aleurone cells (Gilroy and Jones, 1994) and ABA-induced expression of the wheat Em gene in a transient assay (R. Quatrano, personal communication), also suggest an extracellular reception site for ABA.

Stability of Microinjected ABA

The possibilities that microinjected ABA was either rapidly degraded in guard cells, leaked out of guard cells, or was microinjected at concentrations too high to be effective in this study need to be considered. Stomatal opening was observed in response to ABA injection, which requires guard cell viability. Additional experiments, in which ABA was injected at approximately 100-fold lower concentrations (1 µM), also did not show an inhibition of stomatal opening. Furthermore, there is no evidence for reduced ABA effectiveness on stomatal regulation or other responses at high ABA concentrations (Walton, 1980). For example, induction of β-glucuronidase activity from ABA-inducible promoter-β-glucuronidase constructs in transformed rice protoplasts was proportional to ABA concentrations from 1 to 100 µM (Marcotte et al., 1988). The stability of ABA in guard cells is demonstrated by its accumulation for up to 8 h in V. faba guard cells to concentrations of 7 to 13 µM following dehydration stress of leaves (Harris et al., 1988; Harris and Outlaw, 1991). Therefore, it is unlikely that ABA was microinjected at concentrations that were too high to inhibit stomatal opening.

ABA is metabolized slowly to phaseic acid and 4'-dihydrophaseic acid with a half-time of 6 to 8 h in leaf discs of V. faba (Mertens et al., 1982). Upon the removal of dehydration stress, the ABA concentration of V. faba guard cells returned to prestress levels with a half-time of 2 h (Harris and Outlaw, 1991). However, responses of guard cells to ABA have been reported to occur more rapidly. Recent data show that a brief (2 min) exposure to extracellular ABA is sufficient to trigger the signaling cascade leading to long-term K+ efflux from guard cells and a resulting decrease in stomatal aperture (MacRobbie, 1990). Furthermore, the initial rates of stomatal opening were not measurably affected by ABA microinjection (Figs. 3 and 6). Therefore, the possibility that rapid degradation of ABA resulted in the complete lack of effectiveness of microinjected ABA in this study is unlikely. Since guard cells remained viable throughout the duration of the experiments reported here, significant leakage of ABA from guard cells, within several minutes, was unlikely. In addition, any leakage of injected ABA may contribute to an inhibition of stomatal opening that was never observed in this study.

In conclusion, this report provides strong evidence for the requirement of an ABA receptor at the extracellular side of the plasma membrane of guard cells which functions in the regulation of stomatal aperture. Furthermore, data show that intracellular ABA alone does not suffice to inhibit stomatal opening under the imposed conditions. The identification of ABA receptors and components of ABA-induced signaling pathways using cell biological, molecular biological, and genetic approaches should provide further insight into the mechanism of ABA reception.

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