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Plasma Membrane Ion Channel Regulation during Abscisic Acid-Induced Closing of Stomata

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Plasma membrane ion channel regulation during abscisic acid-induced closing of stomata

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SUMMARY

The plant growth regulator abscisic acid triggers closing of stomata in the leaf epidermis in response to water stress. Recent tracer flux studies, patch-clamp studies, fluorometric Ca^{2+} measurements and microelectrode experiments have provided insight into primary transduction mechanisms by which abscisic acid causes stomatal closing. Data show that abscisic acid activates non-selective Ca^{2+}-permeable ion channels in the plasma membrane of guard cells. The resulting elevation in the free Ca^{2+} concentration in the cytosol of guard cells, and the resulting membrane depolarization as well as other unidentified Ca^{2+}-independent mechanisms are suggested to contribute to activation of voltage- and second messenger-dependent anion channels and outward rectifying K^+ channels. Recent data suggest the involvement of two types of anion channels in the regulation of stomatal movements, which provide highly distinct mechanisms for anion efflux and depolarization. A novely characterized 'S-type' anion channel is likely to provide a key mechanism for long-term depolarization and sustained anion efflux during closing of stomata. Patch-clamp studies have revealed the presence of a network of K^+, anion and non-selective Ca^{2+}-permeable channels in the plasma membrane of a higher plant cell. The integrated control of these guard cell ion channels by abscisic acid can provide control over K^+ and anion efflux required for stomatal closing.

1. INTRODUCTION

(*a*) *Higher plant Ca^{2+} channels*

Transmembrane fluxes of Ca^{2+} play a central role in the initiation of signal transduction pathways controlling numerous processes in higher plant cells, including growth, gas exchange regulation, enzyme activity, secretion and movements (Hepler & Wayne 1985; Leonard & Hepler 1990). Activation of Ca^{2+} channels results in elevation of the cytosolic Ca^{2+} concentration from basal levels of approximately 100 nM to elevated levels greater than 200 to 300 nM. This rise in cytosolic Ca^{2+} can trigger cell biological processes mainly by modulation of protein kinases, ion channels and other cellular control enzymes (Leonard & Hepler 1990). Early evidence for Ca^{2+}-channel action during hormonal signal transduction in plants were obtained in studies of cytokinin-induced budding in *Funaria*, using Ca^{2+} ionophores and pharmacological blockers of voltage-dependent mammalian Ca^{2+} channels (Saunders & Hepler 1982, 1983). Until recently however, direct evidence for plant Ca^{2+} channels was restricted to data showing voltage-dependent Ca^{2+}-channel activation in algae (Findlay 1962; Williamson & Ashley 1982; for a review see Tazawa *et al.* 1987).

Recent advances suggest that several distinct classes of Ca^{2+} channels exist in higher plant cells (for review, see Schroeder & Thuleau 1991). These Ca^{2+} channels can be categorized into two general classes of ion channels: plasma membrane Ca^{2+} channels, which allow Ca^{2+} influx from the cell wall space into the cytosol (e.g. Schroeder & Hagiwara, 1990*a*; Cosgrove & Hedrich 1991; Thuleau *et al.* 1992); and Ca^{2+}-release channels located in the membrane of intracellular organelles which allow release of Ca^{2+} from cellular stores (Alexandre *et al.* 1990; Brosnan & Sanders 1990).

Recent research in several plant systems has shown that Ca^{2+}-independent mechanisms, in addition to Ca^{2+}-dependent processes, can control cellular responses. How Ca^{2+}-independent and Ca^{2+}-dependent processes interact to produce a cellular response remains unknown in higher plant cells. Control of stomatal movements provides a system in which substantial insights to higher plant signaling mechanisms have been gained. In this article advances will be discussed in the understanding of Ca^{2+}-dependent and Ca^{2+}-independent processes by which the plant growth regulator abscisic acid may trigger closing of stomata. Ca^{2+}-permeable ion channels and other second messenger and voltage-dependent ion channels are suggested to provide key mechanisms for promoting stomatal closing.

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(*b*) *Stomatal regulation*

Stomatal pores permit diffusion of CO_2 into leaves while modulating the transpiration of water vapour to the atmosphere. This exchange of gases is regulated by movements of guard cell pairs that surround stomatal pores. Physiological signals including light stimuli trigger opening of stomata (Raschke 1979). Under drought conditions, the plant growth regulator abscisic acid (ABA) can act as a trigger for stomatal closing (Raschke 1979; MacRobbie 1981). Stomatal movements are controlled by changes in the ion content of guard cells, which are mediated by large ion fluxes across guard cell membranes (Raschke 1979; MacRobbie 1988; Schroeder & Hedrich 1989).

Mechanisms that regulate stomatal opening and stomatal closing are tightly coupled within the plant (Raschke 1979). Stomatal opening and closing are regulated by metabolic events and by changes in membrane potential, which are produced by modulation of specific ion transport mechanisms (Schroeder & Hedrich 1989). To provide an accurate understanding of individual molecular mechanisms which contribute to stomatal regulation it is useful to separately study membrane-associated mechanisms which promote stomatal opening and stomatal closing by patch-clamp recordings. In addition to promoting ion efflux, abscisic acid-induced stomatal closing would require simultaneous inhibition of ion channels which allow ion uptake during stomatal opening as briefly summarized in the following.

2. K^+ UPTAKE CHANNELS AND STOMATAL OPENING

Stomatal opening requires K^+ uptake (Raschke 1979; MacRobbie 1988). In most eukaryotic and prokaryotic cell systems K^+ uptake has been shown to be mediated by several active transport mechanisms which include K^+-ATPases, and coupled cotransport or antiport systems (Kyte 1981; Hesse *et al.* 1984; Sachs 1987; Rodriguez-Navarro *et al.* 1986). Such active K^+-uptake mechanisms are likely to function as high-affinity transporters in higher plant cells (Rodriguez-Navarro *et al.* 1986). Patch-clamp studies have shown that higher plant cells make use of an additional, perhaps unique mechanism for net K^+ uptake, by passive long-term transport through inward rectifying K^+ channels (Schroeder *et al.* 1984, 1987; Ketchum *et al.* 1989; Moran & Satter 1989; for review, see Hedrich & Schroeder 1989). The plasma membrane proton pump provides the driving force for K^+ channel-mediated K^+ uptake in guard cells (Raschke 1979; Assmann *et al.* 1985; Shimazaki *et al.* 1986; Schroeder 1988). Initial data indicating that proton pump driven net K^+ uptake can be carried by K^+ channels (Schroeder *et al.* 1984, 1987; Assmann *et al.* 1985) led to an early counter hypothesis regarding this novel type of cellular K^+ uptake mechanism (Blatt 1987). This counter hypothesis stated: 'It is argued, that passive (diffusional) mechanisms are unlikely to contribute to K^+ uptake during stomatal opening, despite membrane potentials which, under certain, well-defined conditions, lie negative of the potassium equilibrium potential likely prevailing.' (Blatt 1987, p. 272). In guard cells, biophysical, pharmacological and cell biological studies have however strongly supported the model (Schroeder *et al.* 1984, 1987) that inward rectifying Ca^{2+}-regulated K^+ channels represent a major pathway for K^+ uptake into guard cells during stomatal opening (Schroeder *et al.* 1984, 1987; Schroeder 1988; Schroeder & Hagiwara 1989; Fairley-Grenot & Assmann 1991; Schroeder & Fang 1991; Thiel *et al.* 1992). These inward-rectifying K^+ channels have been found by several laboratories in other types of higher plant cells (for a review, see Hedrich & Schroeder 1989) and have been suggested to provide a general pathway for K^+ uptake (Schroeder *et al.* 1987). A recent study has shown that these K^+ channels can function as low affinity K^+-uptake transporters. This study further suggested that inward rectifying K^+ channels may also provide a contribution to K^+ uptake at external K^+ concentrations lower than 300 μM where active high-affinity transporters provide the major mechanism for K^+ uptake (Schroeder & Fang 1991; Hedrich & Schroeder 1989).

Recent molecular genetic studies have resulted in a breakthrough showing that K^+ uptake-deficient yeast mutants can be complemented by *Arabidopsis* genes which show homology to outward rectifying K^+ channel genes from animal systems (Anderson *et al.* 1992; Sentenac *et al.* 1992). If these *Arabidopsis* outward rectifier homologues do indeed encode outward-rectifying K^+ channels, these findings would not correlate directly to earlier results from patch clamp studies suggesting the importance of inward-rectifying K^+ channels for K^+ uptake. Whether these genes encode outward rectifying K^+ channels in plants remains unknown. Identification of the molecular function of these ion channel homologues should provide an additional test of the hypothesis that inward rectifying K^+ channels function as a general mechanism for K^+ uptake in higher plants (Schroeder *et al.* 1987).

Abscisic acid inhibits stomatal opening and K^+ uptake (Raschke 1979; MacRobbie 1981). Data have shown that inositol phosphates and guanine nucleotides inhibit inward rectifying K^+ channels by elevation in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) (Schroeder & Hagiwara 1989; Blatt *et al.* 1990; Fairley-Grenot & Assmann 1991). This Ca^{2+}-dependent inhibition of inward rectifying K^+ channels has been suggested to provide a molecular basis by which Ca^{2+} inhibits stomatal opening and K^+ uptake (Schroeder & Hagiwara 1989). In addition, data suggest that abscisic acid also inhibits the plasma membrane proton pump (Shimazaki, *et al.* 1986), which provides the driving force for K^+ channel-mediated K^+ uptake.

3. ABSCISIC ACID-DEPENDENT CLOSING OF STOMATA

In addition to inhibition of stomatal opening, abscisic acid induces stomatal closing. Stomatal closing is produced by release of K^+ and anions from guard

cells (Raschke 1979; MacRobbie 1981). In brief, the following model has been suggested by which abscisic acid can control plasma membrane ion channels which regulate ion efflux during stomatal closing (Schroeder & Hedrich 1989): abscisic acid causes a rise in $[Ca^{2+}]_{cyt}$ which in turn inhibits inward rectifying K^+ channels and activates guard cell anion channels (Schroeder & Hagiwara 1989; Hedrich *et al.* 1990). The resulting anion efflux through anion channels causes depolarization, thereby driving K^+ efflux through outward rectifying K^+ channels (Schroeder *et al.* 1984, 1987). Simultaneous opening of anion and K^+ channels results in long-term ion release and turgor and volume reduction leading to stomatal closing. This model (Schroeder & Hedrich 1989) has been confirmed by a number of studies (for reviews, see Schroeder & Hedrich 1989; Mansfield *et al.* 1990). In the following, this report will focus on the extension and refinement of previously postulated models for stomatal closing (MacRobbie 1988; Schroeder & Hedrich 1989) based on new findings.

4. ABA-INDUCED CA^{2+} ELEVATIONS

Data have shown that ABA-mediated closing of stomata can proceed in a Ca^{2+}-dependent manner (De Silva *et al.* 1985; Schwartz *et al.* 1988; for reviews, see MacRobbie 1988; Schroeder & Hedrich 1989; Mansfield *et al.* 1990). Increases in cytosolic Ca^{2+} have been suggested to play a key role in inducing stomatal closing by ABA (De Silva *et al.* 1985; Schwartz *et al.* 1988; Schroeder & Hagiwara 1989; McAinsh *et al.* 1990; Gilroy *et al.* 1990). Fluorometric recordings using Ca^{2+} indicator dyes have shown that abscisic acid can trigger an elevation in $[Ca^{2+}]_{cyt}$ of guard cells (McAinsh *et al.* 1990; Schroeder & Hagiwara 1990*a*; Gilroy *et al.* 1991) (figure 1). Furthermore, elevation in the cytosolic Ca^{2+} concentration suffices to produce stomatal closing (Gilroy *et al.* 1990). Simultaneous application of patch clamp techniques and optical Ca^{2+} measurements have revealed that ABA-induced $[Ca^{2+}]$ elevations are variable in *Vicia faba* guard cells (Schroeder & Hagiwara 1990*a*). Such variable ABA-induced elevations in Ca^{2+} have been independently reported in studies on stomata from *Commulina communis* and have led to the suggestion that Ca^{2+}-dependent as well as Ca^{2+}-independent transduction events contribute to ABA-induced stomatal closing (Gilroy *et al.* 1991).

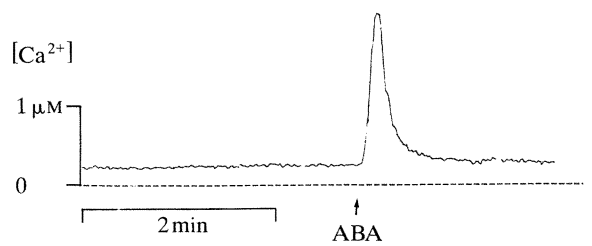

Figure 1. Rapid extracellular perfusion of *Vicia faba* guard cells with 1 μM abscisic acid triggers a rise in the cytosolic Ca^{2+} concentration. Reproduced with permission from Schroeder & Hagiwara (1990*a*).

Studies on *Vicia faba* guard cells have revealed that ABA-induced Ca^{2+} influx via Ca^{2+}-permeable channels in the plasma membrane contributes to rises in $[Ca^{2+}]_{cyt}$ (Schroeder & Hagiwara 1990*a*). Interestingly these ABA-activated ion channels were shown to be non-selective, apparently allowing transmembrane flux of K^+ along with Ca^{2+} influx. The non-selective ion permeability of ABA-activated channels was determined by accurate reversal potential measurements. In addition, wash-out of previously characterized outward rectifying K^+ channels (Schroeder *et al.* 1987; Schroeder 1988) and guard cell anion currents (Schroeder & Hagiwara 1989) was ensured (Schroeder *et al.* 1987; Schroeder & Hagiwara 1989, 1990*a*). With respect to ion selectivity, ABA-activated ion channels resemble several types of chemically modulated Ca^{2+}-permeable ion channels in animal systems which are non-selective Ca^{2+}-permeable ion channels, such as the NMDA receptor ion channel (Madison *et al.* 1991), and cyclic nucleotide-regulated ion channels (Fesenko *et al.* 1985). Chemical activation of ABA-activated currents provides a potent approach for determining relative ion permeabilities of ion channels as single cells can be rapidly and reversibly perfused with ABA (Schroeder & Hagiwara 1990*a*). This method of permeability determination, which has led to accurate identifications of relative ion permeabilities of many chemically modulated ion channels in neurobiological systems (Hille 1984), has recently been questioned (Blatt 1991). The suggestion that currents rapidly-activated by ABA were due to activation of K^+, Cl^- and other non-Ca^{2+}-permeable currents (Blatt 1991) was excluded by abolishment of other known currents, by direct determination of ion permeabilities by reversal potential measurements and by additional simulataneous controls using cytosolic Ca^{2+} measurements (Schroeder & Hagiwara 1990*a*). Biphasic activation of two ion channel types with opposed ion selectivities was further excluded in these experiments with a frequency resolution of 2 KHz. The hypothesis that non-selective Ca^{2+}-permeable channels can be attributed to activation of 'K^+, Cl^- and other non-Ca^{2+} currents' (Blatt 1991, p. 102) is therefore unlikely.

An additional property of ABA-activated Ca^{2+}-permeable channels is that these channels repeatedly activate and inactivate in concert within a single guard cell during continuous ABA application, giving rise to transient elevations followed by plateau elevations in $[Ca^{2+}]_{cyt}$ (Schroeder & Hagiwara 1990*a*; Gilroy *et al.* 1991). This repetitive activation pattern suggests that rapid intermediate coupling mechanisms may be required between ABA exposure to guard cells and activation of plasma membrane Ca^{2+} channels (Schroeder & Hagiwara 1990*a*). Preliminary patch clamp studies have shown that *Commelina communis* guard cells produce similar ABA responses (J. I. Schroeder, unpublished data) to those described in *Vicia faba* (Schroeder & Hagiwara 1990*a*).

Tracer flux measurements have recently revealed that abscisic acid triggers two phases of K^+ efflux from guard cells (MacRobbie 1990). This study showed that the initial phase of K^+ efflux was rapid and

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independent of Ca^{2+} influx. Potassium efflux produced by ABA-activated depolarization (Schroeder & Hagiwara 1990*a*) may provide the mechanism for this initial rapid phase of ABA-stimulated K^+ efflux. In addition MacRobbie (1990) has shown that extracellular removal of abscisic acid after incubation periods of 2 min or longer does not halt progression of ABA-induced stomatal closure. This finding, as well as the variable ABA-activated Ca^{2+} response in guard cells (Schroeder & Hagiwara 1990*a*; Gilroy *et al.* 1991), support the hypothesis that a chain of transduction events is involved in ABA-mediated stomatal closure and that Ca^{2+}-independent coupling processes precede Ca^{2+} increases in the cytoplasm (MacRobbie 1990). In addition these results indicate that transient increases in cytosolic Ca^{2+} may suffice to prime Ca^{2+}-dependent mechanisms participating in stomatal closure (MacRobbie 1990; Schroeder & Hagiwara 1990*a*).

Although activation of Ca^{2+}-permeable channels has been shown to contribute to ABA-induced increases in $[Ca^{2+}]_{cyt}$, release of Ca^{2+} from intracellular organelles may also contribute to ABA-mediated rises in Ca^{2+} as this possibility has not been excluded in previous studies (McAinsh *et al.* 1990; Schroeder & Hagiwara, 1990*a*; Gilroy *et al.* 1991). In guard cells, microinjection of caged inositol 1,4,5 trisphosphate ($InsP_3$) and subsequent flash photolysis triggered an elevation in $[Ca^{2+}]_{cyt}$ and stimulated stomatal closing (Gilroy *et al.* 1990). Microinjection of caged $InsP_3$ into guard cells was shown to inhibit inward rectifying K^+ channels (Blatt *et al.* 1990), which correlates to findings that cytosolic Ca^{2+} elevation inhibits these inward rectifying K^+ channels (Schroeder & Hagiwara 1989). Microinjection experiments have shown that $InsP_3$ can exert control over stomatal movements (Gilroy *et al.* 1990; Blatt *et al.* 1990). Whether abscisic acid or one of a number of other stimuli of stomatal closing trigger $InsP_3$-dependent Ca^{2+} release in guard cells remains to be determined.

5. CA^{2+}- AND VOLTAGE-DEPENDENT ANION CHANNELS AND VOLTAGE-DEPENDENT K^+ CHANNELS

Increases in $[Ca^{2+}]_{cyt}$ have been shown to inhibit inward rectifying K^+ channels and to activate voltage-dependent anion channels in guard cells (Schroeder & Hagiwara 1989). Ca^{2+}-activation of anion channels results in efflux of anions through anion-selective channels (Keller *et al.* 1989) which, in turn, depolarizes guard cells sufficiently to activate outward rectifying K^+ channels (Schroeder *et al.* 1987; for review, see Schroeder & Hedrich 1989). In addition to this depolarization-induced activation of K^+ channels, a direct ABA-dependent enhancement of K^+-channel currents has been shown (Blatt 1990). This ABA-induced enhancement of K^+ currents was found to be relatively slow (Blatt 1990) and therefore appears to be significantly different from rapid ABA-activation of non-selective channels (Schroeder & Hagiwara 1990*a*) and ABA-induced initial K^+ efflux transients (MacRobbie 1990). Although ABA may directly modulate outward rectifying K^+ channels, the observed enhancement of K^+ currents (Blatt 1990) may be controlled by ABA-mediated second messenger-dependent processes. Simultaneous activation of anion channels and K^+ channels would permit ion efflux across the plasma membrane, which in turn leads to stomatal closing (Schroeder & Hagiwara 1989; Keller *et al.* 1989; for reviews, see MacRobbie 1988; Schroeder & Hedrich 1989). Recent studies have provided further support for the hypothesis (Schroeder *et al.* 1984, 1987) that outward rectifying K^+ channels provide the pathway for K^+ release during stomatal closing (for a review, see Blatt 1991).

Results have suggested that other second messengers, in addition to $[Ca^{2+}]_{cyt}$, may be important for regulating voltage-dependent anion channels (Schroeder & Hagiwara 1989; Hedrich *et al.* 1990; Schroeder & Hagiwara 1990*b*). Additional second-messenger transduction processes may be triggered by ABA, as the ABA activation of Ca^{2+}-permeable channels appears to occur by means of preceding coupling mechanisms (Schroeder & Hagiwara 1990*a*). Such preceding coupling mechanisms may be involved in simultaneous control of Ca^{2+}-permeable channels (Schroeder & Hagiwara 1990*a*), anion channels (Hedrich *et al.* 1990; Schroeder & Hagiwara 1989) and K^+ channels (Blatt 1990). This suggestion however requires further analysis. Whole-cell patch-clamp experiments executed in conjunction with $[Ca^{2+}]_{cyt}$ measurements may allow further insight into such rapid initial signal transduction events of ABA-mediated stomatal closure.

6. TWO TYPES OF GUARD CELL ANION CHANNELS

A recent study has shown that two types of anion channels prevail in the plasma membrane of guard cells (Schroeder & Keller 1992). Both anion channel types are regulated by Ca^{2+} (Schroeder & Hagiwara 1989; Hedrich *et al.* 1990). These two anion channel types have highly divergent voltage-dependent properties and are therefore likely to control different aspects of stomatal movements (Schroeder & Keller 1992).

One anion channel type (Keller *et al.* 1989) has been shown to be activated by Ca^{2+} and intracellular nucleotides (Hedrich *et al.* 1990). These anion channels show rapid activation and deactivation kinetics (R-type anion channels) (Hedrich *et al.* 1990; Schroeder & Keller 1992). In addition, these rapidly activating anion channels inactivate during prolonged stimulation (Hedrich *et al.* 1990; Schroeder & Keller 1992). The inactivation of these R-type anion channels suggests that this class of ion channels may allow initial short term anion efflux and transient depolarization in response to physiological signals, while long term anion efflux may be limited due to decay in anion channel currents (Hedrich *et al.* 1990; Schroeder & Keller 1992).

Recent data have led to the interesting discovery for auxin regulation that these R-type anion channels are

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activated by the plant growth regulator indole-3-acetic acid (auxin) (Marten *et al.* 1991), which induces short-term depolarizations in higher plant cells (Bates & Goldsmith 1983). Martin *et al.* (1991) report that R-type ion channels are not directly modulated by ABA. It is interesting to note that auxin stimulates stomatal opening and not stomatal closing, giving rise to an apparent paradox with respect to the hypothesis that these R-type anion channels may control closing of stomata (Keller *et al.* 1989; Hedrich *et al.* 1990). Whether R-type anion channels play a role in abscisic acid-mediated stomatal closure remains to be determined. Findings of auxin activation of these anion channels (Marten *et al.* 1991), as well as the reduced magnitude of these anion channels after inactivation (Keller *et al.* 1989; Hedrich *et al.* 1990; Schroeder & Keller 1992), suggest that R-type anion channels may provide an initial and transient contribution to anion efflux. It is therefore unlikely that R-type anion channels can account solely for the large and long-term anion efflux observed during stomatal closing (MacRobbie 1981).

A possible solution to the above mentioned apparent paradox and newly arising question of, how physiologically required long-term anion efflux and depolarization are accomplished during closing of stomata has resulted from a recent study. Recent findings have led to the unequivocal characterization of a second type of voltage-dependent anion channel behaviour in the plasma membrane of *Vicia faba* guard cells (Schroeder & Hagiwara 1989; Schroeder & Keller 1992). This second type of anion current shows depolarization induced activation (Schroeder & Keller 1992). Initial characterization of these ion channels had shown that this type of anion channel can be regulated by elevation in the cytosolic Ca^{2+} concentration which induces long-term depolarizations of guard cells (Schroeder & Hagiwara 1989). Evidence that these Ca^{2+}-activated anion currents have highly distinct properties from the above mentioned R-type anion channels was gained by analysis of the voltage-dependent properties of these ion channels (Schroeder & Keller 1992). This type of anion channel shows slow voltage-dependent activation and deactivation kinetics (S-type anion channels). In addition these anion channels can be activated in a sustained manner for periods of several minutes without showing inactivation (Schroeder & Keller 1992). This sustained activation of anion channels suggests that S-type anion channels trigger long-term depolarizations of guard cells as have been reported in response to ABA (Kusamo 1981; Ishikawa *et al.* 1983). S-type anion channels may therefore provide the central driver of long-term stomatal closing by regulating long-term anion efflux, depolarization and the resulting K^+ efflux through depolarization-activated K^+ channels (Schroeder & Hagiwara 1989; Schroeder & Keller 1992).

Both R-type and S-type anion channels are activated by cellular messengers and depolarization. The activation potentials of both anion channels differ (Schroeder & Keller 1992) and depend on recording conditions (Marten *et al.* 1991). Whether these two anion channel types are differentially regulated by second messengers remains unknown. ABA-activated non-selective Ca^{2+}-permeable channels may provide a mechanism for a suggested 'predepolarization' of guard cells for activation of voltage-dependent anion channels and the induction of stomatal closing (Schroeder & Hagiwara 1990*b*). Recent flux studies by MacRobbie (1990) correlate to this suggestion as discussed in § 4. However, detailed properties such as activation mechanisms and the voltage dependence of non-selective Ca^{2+}-permeable ion channels and anion channels need to be characterized to assess this possibility.

7. EVIDENCE FOR Ca^{2+}-INDEPENDENT REGULATION OF STOMATAL CLOSING

As referred to throughout this text, data are accumulating which suggest Ca^{2+}-independent regulation of stomatal, closing. In brief, these recent studies have shown:

1. Ca^{2+}-activated S-type anion currents depend on additional unknown cytosolic factors (Schroeder & Hagiwara 1989).
2. R-type anion currents are enhanced by ATP and nucleotides (Hedrich *et al.* 1990).
3. Ca^{2+}-independent activation of outward rectifying K^+ channels (Blatt 1990; Blatt *et al.* 1990).
4. Two phases of ABA-induced ion efflux in guard cells: one rapid Ca^{2+}-independent phase followed by a Ca^{2+}-dependent phase (MacRobbie 1990).
5. ABA-induced repetitive activation of Ca^{2+}-permeable currents, suggesting second-messenger-dependent coupling between ABA reception and channel opening (Schroeder & Hagiwara 1990*a*).
6. Variable effects of ABA on cytosolic Ca^{2+} (Schroeder & Hagiwara 1990*a*; Gilroy *et al.* 1991).
7. The most striking evidence that Ca^{2+} is not the sole signal for control of stomatal closing has arisen from fluorometric studies which show ABA-induced stomatal closure, without a measurable increase in cytosolic Ca^{2+} (Gilroy *et al.* 1991) and studies showing elevation in cytosolic Ca^{2+} during stomatal opening and ABA-induced alkalization of cytosolic pH in orchids (Irving *et al.* 1992).

Ca^{2+}-independent transduction steps may precede, follow and parallel Ca^{2+}-dependent signaling events. For example, recent research has suggested that the reduction of inward rectifying K^+ channel currents by inositol 1,4,5-trisphosphate and by guanosine-triphosphate (Blatt *et al.* 1990; Fairley-Grenot & Assmann 1991) precedes Ca^{2+} inhibition of these channels (Schroeder & Hagiwara 1989). It is likely that further research on the regulation of guard cell ion channels, by stimuli such as cytosolic alkalization (Irving *et al.* 1992), will provide an understanding of the yet unknown Ca^{2+}-independent mechanisms involved in control of stomatal closing.

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8. CONCLUSIONS

Recent studies in several laboratories have provided first insights to the molecular mechanisms of abscisic acid-induced signal transduction in guard cells. We have identified ion channels which are likely to play important roles in the chain of events by which abscisic acid may trigger stomatal closing, including ABA-activated non-selective Ca^{2+}-permeable channels, Ca^{2+}- and voltage-dependent S-type anion channels and inward and outward rectifying K^+ channels. A number of studies on guard cells in several laboratories have provided insights to the activation, regulation and function of these higher plant ion channels. Patch-clamp studies in conjunction with tracer flux studies, microelectrode recordings and Ca^{2+} measurements suggest specific roles for several ion channel types in the control of signal transduction in higher plants (for a review, see MacRobbie 1988; Schroeder & Hedrich 1989). Recent data have led to support and refinement of a suggested model for stomatal regulation (Schroeder & Hedrich 1989) as described in this article. It should be noted that many additional types of ion channels are present in the guard cell plasma membrane (e.g. Cosgrove & Hedrich 1991) which are likely to provide further control over stomatal movements. Future studies are likely to provide insight to such additional types of important ion channels and answers to the many arising questions, some of which have been delineated here. Several studies have suggested that yet unidentified Ca^{2+}-independent regulatory links between guard cell ion channels are likely to be of importance in the control of stomatal closing. Further studies on guard cells should provide an understanding of how Ca^{2+}-dependent and Ca^{2+}-independent mechanisms interact during the control of signal transduction in a higher plant cell.

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