Ca²⁺ signalling and control of guard-cell volume in stomatal movements
Michael R Blatt

Stomatal guard cells are unique as a plant cell model and, because of the depth of knowledge now to hand on ion transport and its regulation, serve as an excellent model for the analysis of stimulus-response coupling in higher plants. Parallel controls – mediated by Ca²⁺, H⁺ protein kinases and phosphatases – regulate the gating of the K⁺ and Cl⁻ channels that facilitate solute flux for stomatal movements. A growing body of evidence now indicates that oscillations in the cytosolic free concentration of Ca²⁺ contribute to a ‘signalling cassette’, which is integrated within these events through an unusual coupling with membrane voltage. Additional developments during the past two years point to related events in membrane traffic that play complementary roles in stomatal control. Research in these areas, especially, is now adding entirely new dimensions to our understanding of guard cell signalling.

Introduction
Stomata are pores that form between pairs of specialised cells, called guard cells, and that are found on the epidermis of all aeral parts of most higher plants. Guard cells open and close the stomata to regulate gas exchange between the intercellular spaces within the plant tissue and the surrounding environment, thereby influencing two of the processes that are most important to the vegetative plant, photosynthesis and transpiration. Thus, the control of stomatal aperture has long been recognised as directly influencing two of the processes that underpin these events [2–4]. Nonetheless, major gaps remain in our understanding of these signalling pathways. Even the function and processing of the [Ca²⁺]i signal, itself, is proving surprisingly complex. Furthermore, key developments during the past two years point to related events in membrane traffic that play equally important roles in stomatal control. Research in these areas, especially, is adding entirely new dimensions to our understanding of guard-cell signalling and of plant cell biology in general.

Stomatal movements are achieved through changes in guard-cell volume, which, in turn, are driven by the accumulation (during opening) and loss (during closing) of osmotically active solutes (i.e. KCl and/or other K⁺ salts). Flux of these inorganic solutes must take place across the plasma membrane because mature guard cells lack functional plasmodesmata [1,2]. K⁺ transport is dominated by two classes of K⁺ channels. The first of these permits K⁺ movement into, but not out of, the cell and thus gives rise to an inward-rectifying current (I[K⁺]in); the second facilitates K⁺ flux out of, but not into, the cell and therefore appears as an outward-rectifying current (I[K⁺]out). These two currents contribute to K⁺ flux during stomatal opening and closing, respectively, and can be clearly distinguished by their whole-cell, single-channel and pharmacological properties [2–4]. The movement of Cl⁻ through so-called ‘slow’ or ‘non-inactivating’ Cl⁻ channels (I(Cl)) accounts for much of the charge balancing of the fluxes of K⁺, especially during stomatal closure when it is essential that the voltage across the guard-cell plasma membrane is brought positive of the K⁺ equilibrium voltage (E[K⁺]) for the loss of K⁺ through I[K⁺]out [4].

Clearly, effective control of stomatal aperture, and hence of guard-cell volume, requires the coordinated regulation of both K⁺ and Cl⁻ fluxes, and implies a high degree of integration between the signalling pathways that determine the activities of I[K⁺]in, I[K⁺]out and I(Cl). We know now (see Figure 1) that ABA first, evokes an inward-directed current, mediated at least in part by I[K⁺]in; second, inactivates I[K⁺]in which normally mediates K⁺ uptake [7]; and third, activates the current through I[K⁺]out which together with I(Cl) facilitates a net loss of KCl. At least two parallel signalling pathways that engender changes in cytosolic-free calcium concentration ([Ca²⁺]i) and pH (pH(i)) underpin these events [2–4]. Nonetheless, major gaps remain in our understanding of these signalling pathways.

Abbreviations
ABA abscisic acid
ARF ADP-ribosylation factor G-protein
Ca²⁺ concentration of cytosolic free calcium
ADP ribose
E[K⁺] K⁺ equilibrium voltage
GEF guanine-nucleotide exchange factor
I(Cl) non-inactivating Cl⁻ current
I[K⁺]in inward-rectifying K⁺ current
I[K⁺]out outward-rectifying K⁺ current
IP₃ inositol-1,4,5-trisphosphate
Nt-Syr1 Nicotiana syntaxin-related protein 1
PLD phospholipase D
SNARE soluble N-ethylmaleimide-sensitive factor attachment protein receptor
[Ca\(^{2+}\)]\(_{i}\) increases and Ca\(^{2+}\) channels

Early debates centred around the origins of the [Ca\(^{2+}\)]\(_{i}\) signal, whether it is internal or external to the guard cell. Considerable evidence now indicates that increases in [Ca\(^{2+}\)]\(_{i}\) arise from both Ca\(^{2+}\) entry across the plasma membrane and release from intracellular stores [8–10]. Furthermore, the vacuole is certainly not the only source of Ca\(^{2+}\) within the cell [8]. Some uncertainty remains about the relative contributions of various pathways, which may reflect an inherent redundancy (and, hence, plasticity) within the Ca\(^{2+}\) cascade. Nonetheless, we can expect that the specialisation, and localisation to selected membranes within the cell, of [Ca\(^{2+}\)]\(_{i}\)-signalling elements is also important for signal encoding [11,12].

Within the guard cell, Ca\(^{2+}\) release and Ca\(^{2+}\)-mediated suppression of I\(_{K_{in}}\) are mediated by inositol-1,4,5-trisphosphate (IP\(_{3}\)) [13,14], which is produced rapidly in response to ABA [15,16]. This lipid metabolite probably interacts with IP\(_{3}\)-sensitive Ca\(^{2+}\) channels within the cell to potentiate a rise in [Ca\(^{2+}\)]\(_{i}\) [17]. Several studies have, however, described a second class of endomembrane Ca\(^{2+}\)-release channels in higher plants [17,18]. These channels are sensitive to the alkaloid ryanodine and are activated by binding cyclic ADP-ribose (cADPR), a metabolite of nicotinamide adenine dinucleotide.

A statistical analysis of cADPR-injected mesophyll had implicated a role for this agonist in r29A and kin2 gene expression evoked by ABA [19]. More recently, Leckie et al. [20••] have provided direct evidence of cADPR-sensitive, Ca\(^{2+}\)-permeable channels in guard-cell vacuoles and suggested that they function as a Ca\(^{2+}\)-release pathway during ABA stimulation. In support of this hypothesis, they observed increases in [Ca\(^{2+}\)]\(_{i}\) following injection of cADPR into guard cells, and a slowing of stomatal closure in response to ABA when guard cells were preloaded with the cADPR antagonist 8NH\(_{2}\)-cADPR. Grabov and Blatt [21•] offer parallel evidence that voltage-evoked increases in [Ca\(^{2+}\)]\(_{i}\) are suppressed by ryanodine in intact guard cells. The latter study is of particular interest because it offers the first quantitative analysis of the [Ca\(^{2+}\)]\(_{i}\) dependence of I\(_{K_{in}}\). Grabov and Blatt [22••] had previously found evidence of a hyperpolarisation-activated Ca\(^{2+}\) channel at the plasma membrane, implicated by the sensitivity of [Ca\(^{2+}\)]\(_{i}\) increases to the membrane voltage. In the more recent study [21•], they made use of changes in clamp voltage to elicit changes in [Ca\(^{2+}\)]\(_{i}\), monitoring I\(_{K_{in}}\) under voltage clamp. They found a high (‘switch-like’) sensitivity of the current to [Ca\(^{2+}\)]\(_{i}\), with an apparent K\(_{1/2}\) (~330 µM) only marginally above the mean resting [Ca\(^{2+}\)]\(_{i}\). Whether ABA affects the [Ca\(^{2+}\)]\(_{i}\) dependence of I\(_{K_{in}}\) is not known; however, ABA does strongly influence the voltage sensitivity of
mediated activation of Cl⁻ channels \[28,29\], has been shown to give rise to 'Ca²⁺ signatures' that encode important roles in defining the \([Ca²⁺]i\) release pathways within the guard cell, can be expected to depend on animal ryanodine-receptor Ca²⁺ channels \[23\], and contribute to Ca²⁺ release \[24\]. Nonetheless, Ca²⁺ channels have also been identified in the endoplasmic reticulum (ER) of mechanosensitive organs in *Bryonia* \[25\] and probably the ER of *Lepidium* roots \[26\]. In *Brassica* florets, Ca²⁺ release appears to be triggered by IP₃ in vesicular compartments that are distinct from the vacuole \[27\]. Furthermore, in the giant alga *Chara*, the action potential (which is initiated by Ca²⁺-mediated activation of Cl⁻ channels \[28,29,30\]) draws on IP₃-dependent Ca²⁺ release from a non-vacuolar compartment close to the plasma membrane. In a particularly elegant study, Plieth *et al.* \[31\] preloaded *Chara* with Mn²⁺, targeting either all intracellular stores (by incubation in Mn²⁺ medium) or only the central vacuole (by injection with Mn²⁺). They found that Mn²⁺ quenches the fluorescence of the Ca²⁺-sensitive dye Fura2 and, because it also permeates many Ca²⁺ channels, it provides an assay for divergent release. When they evoked action potentials, Plieth *et al.* \[31\] observed a quenching of Fura2 fluorescence in cells that were uniformly loaded with Mn²⁺, but not in cells in which Mn²⁺ was loaded only into the vacuole. Whether spatial differences in Ca²⁺ release underpins [Ca²⁺]i signalling in guard cells has yet to be determined, but there is certainly evidence of local differences in [Ca²⁺]i dynamics between the peripheral cytosol and regions of the cell close to the nucleus \[22\].

To summarise, both the pharmacological and kinetic characteristics, as well as the spatial distribution of Ca²⁺ release pathways within the guard cell, can be expected to play important roles in defining the nature of the [Ca²⁺]i response to ABA.

**[Ca²⁺]i oscillations**

Ca²⁺ mediates a diversity of responses, even within a single cell. The issue of how a single second messenger could give rise to such varied responses was resolved only when it was recognised that the frequency and location of a sequence of repetitive increases in [Ca²⁺]i within the cell might determine the nature of the response(s) \[12,32,33\]. Frequency modulation of [Ca²⁺]i increases or 'spikes' has been shown to give rise to 'Ca²⁺ signatures' that encode specific responses, including gene expression \[34,35\] and calmodulin-dependent protein kinase activity \[36\]. In animals, [Ca²⁺]i oscillations initiate Ca²⁺ entry across the plasma membrane; local [Ca²⁺]i elevation then triggers further Ca²⁺ release from intracellular stores (i.e., Ca²⁺-induced Ca²⁺ release [CICR]) before the Ca²⁺ is eliminated from the cell or sequestered within organelles. The result is repetitive [Ca²⁺]i spikes, each with a duration of a few seconds or less.

Interest in [Ca²⁺]i signalling in plants has been heightened by observations that [Ca²⁺]i may oscillate in response to external stimuli \[37–40\]. In guard cells, however, these oscillations often take place over periods of more than 10 min, with discrete [Ca²⁺]i maxima lasting for 2–4 min or more when evoked by a rise in the extracellular Ca²⁺ concentration or CO₂ \[40,41\]. A similar oscillation pattern has now been observed in response to ABA. Staxen *et al.* \[42\] reported [Ca²⁺]i increases from approximately 200 nM at rest to 400–600 nM within 15–30 min of exposure to 10 nM ABA. Significantly, treatments with higher concentrations of ABA that resulted in a pronounced decrease in stomatal aperture also produced a reduced amplitude in the [Ca²⁺]i oscillations and a longer duration of the [Ca²⁺]i elevation. A role for IP₃-mediated Ca²⁺ release was suggested because the ABA-evoked oscillations were partially suppressed by treatment with the phospholipase C antagonist U73122.

This pattern of slow and irregular oscillations in [Ca²⁺]i raises questions about the origins and role of this signal in the guard cell. One explanation relates [Ca²⁺]i oscillations to the voltage status of the cell. Guard cells, like many higher-plant cells, have two states of membrane voltage: one state close to Eₖ, and the other largely K⁺-insensitive and typified by voltages well negative of Eₖ \[43,44\]. Transitions between these states take place in response to stimuli, including ABA, that can give rise to oscillations in voltage with periods between 10 s and many minutes \[43,45\]. Because membrane hyperpolarisation also evokes a Ca²⁺ influx and a consequent rise in [Ca²⁺]i, it is most likely that the [Ca²⁺]i oscillations mirror an underlying oscillation in plasma membrane voltage \[22\]. These characteristics — and the effect of ABA in altering the voltage threshold for the [Ca²⁺]i rise \[22\] — suggest a role for [Ca²⁺]i elevation in a feedback mechanism that 'tunes' the [Ca²⁺]i-dependent currents Iₖ, in and Iₐ, in to the prevailing transport status of the guard cell. Thus, [Ca²⁺]i feedback creates a response 'cassette' for osmotic balance (Figure 2), switching the membrane between states in which net uptake and net loss of K⁺ and Cl⁻ take place \[8,44\].

**Membrane traffic and cell volume**

The activation of Ca²⁺ channels may also play a role in events one step removed from the regulation of K⁺ and Cl⁻ fluxes. Between the open and closed state of the stomatal pore, the volumes of the guard cells often change by a factor of two or more and extensive reorganisation of the vacuolar membrane is known to occur \[2,46\]. Such volume changes cannot be accommodated by lateral expansion and compression of the bilayer \[47,48\] and must, therefore, be accompanied by substantial changes in total membrane matter. A coordinate regulation of vesicular traffic to and from both the plasma membrane and the tonoplast is therefore implied. MacRobbie \[49\] began to explore some...
of these issues in *Chara* more than two decades ago. Yet, despite their implied function in stomatal movements, the dynamics of membrane trafficking within the guard cell were largely ignored until recently. Two recent developments have no placed membrane trafficking in the limelight of stomatal physiology and ABA signalling.

In the first of these, Homann [50•] and Kubitscheck *et al.* [51••] used *in vivo* capacitance recording and membrane-surface labelling with styryl dyes to quantify membrane exocytosis and endocytosis in guard-cell protoplasts during osmotically driven volume changes. Capacitance recording makes use of sine-wave retardation to determine membrane capacitance [52–54], which is directly proportional to the membrane surface area. An increase in capacitance represents the sum of all exocytotic events less the sum of all endocytotic events. Endocytotic events can subsequently be isolated by simultaneously monitoring internalisation of fluorescent styryl dyes, such as FM1-43 [55]. Homann [50•] found that the membrane surface area of *Vicia* guard-cell protoplasts increased under hypo-osmotic conditions and decreased after hypertonic treatment, and that the rate of change in each case was graded in response to the magnitude of the osmotic potential difference. Kubitscheck *et al.* [51••] subsequently combined these techniques with FM1-43 dye measurements. Their results demonstrate a rapid internalisation of membrane during hyperosmotic stimulation that is quantitatively consistent with the decrease in plasma membrane surface area (and also capacitance). Intriguingly, the dye also accumulated gradually within the cell in a ring 1–2 µm below the plasma membrane, even in the absence of an osmotic step. This steady internalisation suggests that constitutive endocytosis continues under constant osmotic pressure, resulting in the accumulation of a pool of sub-plasma membrane vesicles. Whether these vesicles are available for re-incorporation into the plasma membrane remains to be determined. The role of ABA and of Ca²⁺ in these events can also be expected to draw attention in the near future. So far, all of the evidence indicates that Ca²⁺ does not have a direct impact on the membrane trafficking that is evoked by osmotic gradients [50•]. It may be that osmotic potential- and ABA-sensitive trafficking controls overlap only in part. Whether or not this is the case, experimental results point to a highly dynamic process of membrane cycling that is responsive to membrane tension.
The second development has come from the identification of the *Nicotiana* syntaxin homologue Nt-Syr1. Syntaxins belong to a group of integral membrane proteins that form the core of the molecular machinery for vesicle trafficking and membrane fusion (see below). Surprisingly, Leyman et al. [56••] isolated the Nt-Syr1 gene by expression cloning in a heterologous screen designed to identify an ABA receptor. Their studies demonstrate not only that ABA enhances Nt-Syr1 transcript and Nt-Syr1 protein levels in the plant but also that disrupting Nt-Syr1 function, with either the Clostridial neurotoxin BotN/C (an endopeptidase that specifically cleaves the syntaxin) or by loading guard cells with the soluble (carboxy-truncated) portion of Nt-Syr1, prevents both K⁺ and Cl⁻ channel responses to ABA in vivo. These results, and observations of a high molecular weight band recognised by Nt-Syr1 antibodies, suggest that Nt-Syr1 functions in a complex that is competitively blocked through substitution with the carboxy-truncated protein. They also indicate that Nt-Syr1 functions within or very close to the early steps of the ABA signal cascade, either as a scaffolding protein, a second messenger or a modulator of the activity/availability of one or more signalling elements.

Further evidence of membrane trafficking in stomatal control?

Two additional lines of evidence hint at roles for membrane trafficking in guard-cell volume control and ABA signalling. First, in screening for ABA-hypersensitive mutants of *Arabidopsis*, Cutler et al. [57] identified Era1, the gene that encodes a protein farnesyl-transferase. Farnesyl- and geranylgeranyl-transferases covalently add lipid moieties to small GTPases, anchoring the latter at the membrane surface [58] to target GTPase activity in a variety of cellular functions. Although the substrate(s) for Era1 is not known, Rac- and Rho-type GTPases, which are commonly farnesylated. In fact, a Rho-like GTPase, Rop1, that is localised to the apex of *Pisum* pollen tubes [60] has been implicated in microfilament-based intracellular transport of vesicles. Intriguingly, actin organisation has also been suggested to regulate guard-cell ion channels and stomatal movements, although the function of actin in this case has been interpreted in the context of control by mechanical stress rather than membrane trafficking [61–63].

The Era1 farnesyl-transferase also contributes to ABA signalling. Pei et al. [64•] have reported an enhanced
sensitivity of guard-cell Cl\(^{-}\) channels to ABA in the era1 mutant and in wild-type Arabidopsis plants after treatment with broad-range farnesyl-transferase antagonists. The era1 phenotype is dominant and persists throughout the life cycle of the plant. So, although these results do not distinguish between possible roles for Era1 as an ABA-signalling element per se and as a secondary element necessary to maintain the signalling machinery, they do implicate a role for small GTPases and potentially for membrane trafficking in guard-cell function.

Second, Jacob et al. \cite{65*} have recently reported a transient rise in the phospholipase D (PLD) activity of guard-cell protoplasts following stimulation with ABA. They also observed a reduction in the activity of I\(_{K,\text{in}}\) that complemented the action of the cADPR antagonist nicotinamide when the protoplasts were treated with the PLD by-product phosphatidic acid. It is worth noting that both PLD and phosphatidic acid have been implicated in secretion by the barley aleurone \cite{66}. Phosphatidic acid contributes to the regulation of vesicle trafficking at the Golgi apparatus \cite{67}, and PLD activity has been associated with the functioning of ARF- and Rho-GTPases in the secretory processes of mammals and yeast \cite{68,69}. Once again, the data suggest a link between membrane trafficking and ABA.

**Coordinating membrane trafficking and ion channel control**

How might membrane trafficking contribute to ion-channel control and ABA signalling? Membrane cycling and fusion between the membranes of eukaryotic cells are facilitated by a family of SNARE (i.e. soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins that are associated with each membrane \cite{70–72}. During synaptic transmission, which depends on vesicle fusion and release of a neurotransmitter, the target (t-SNARE) proteins syntaxin and SNAP-25 form a stable ternary complex with the vesicle (v-SNARE) protein synaptotobrevin that serves to bring the two membrane bilayers into close proximity for fusion \cite{73}. In yeast and plants, SNARE proteins are thought to be essential for the related functions of interorganelle transport, growth and cell division \cite{72,74}. Thus, SNAREs have been recognised as contributing to response mechanisms and not as components of signal cascades per se.

Even so, SNAREs are probably important for signal transduction, its plasticity and adaptation. SNAREs may contribute to ion-transport regulation simply by mediating the selective addition and removal of transport proteins from the membrane. These events can take place with remarkable rapidity. A recent study of the Arabidopsis GNM gene, which encodes a GTP–GDP exchange factor (GEF) for an ADP-ribosylation factor G-protein (ARF) GTPase, is a case in point. Steinmann et al. \cite{75*} report that treatments with the ARF–GEF antagonist brefeldin A result in dramatic alterations in protein localisation within 15–30 min, including a loss of the polar distribution of the putative auxin efflux carrier protein Pin1. Auxin efflux, itself, is altered within 10 min of brefeldin A treatments \cite{76}, suggesting that substantial changes to the make-up of the membrane transport proteins take place within the same time period. The data certainly highlight the dynamic flux of membrane and protein within the plant cell.

The role(s) carried out by SNAREs may prove to be much more subtle still. In neurons, SNAREs, including syntaxins, bind to and regulate Ca\(^{2+}\) channels \cite{77,78} as well as CFTR Cl\(^{-}\) channels \cite{79}. Syntaxins interact with proteins that may not be related to secretion processes directly, including the orphan G-protein-coupled receptor CIRL \cite{80} and tomosyn, a microfilament-associated protein \cite{81}. Furthermore, SNARE proteins have been associated with responses to membrane tension and osmotic stress \cite{82}. So, one possibility (Figure 3) is that SNARE proteins such as Nt-Syr1 \cite{56*} contribute to the scaffolding of an ABA-receptor complex and, therefore, serve dual roles by marrying the functions of a signalling element and of the machinery for membrane trafficking.

**Conclusions**

Research into the cellular and molecular mechanisms of stomatal control has begun to take on new dimensions in relating signalling pathways with response ‘cassettes’ that comprise several ion transporters and their control mechanisms. Understanding how these cassettes are assembled will present a major challenge in the coming years. Defining the interactions within these cassettes is already providing a clearer picture of the functions of Ca\(^{2+}\) as a secondary messenger. At the same time, attention to the related processes of membrane dynamics and trafficking promises to add to our understanding of both cellular transport and volume control, as well as to the guard-cell system as a higher-plant cell model.

**Update**

Following up the evidence of a hyperpolarisation-activated rise in [Ca\(^{2+}\)], Hamilton et al. \cite{84**} have now identified a small-conductance Ca\(^{2+}\) channel at the plasma membrane by patch-clamp of Vicia guard-cell protoplasts. The channel shows characteristics that are entirely consistent with those expected from the previous studies, including a dependence on negative voltage and block by Gd\(^{3+}\) and La\(^{3+}\) \cite{21*,22**}. Furthermore, ABA evokes a large increase in channel opening, even in isolated membrane patches. These results suggest that the ABA receptor is physically close to the Ca\(^{2+}\) channel and localised to the inner surface of the plasma membrane.

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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


17. Allen GJ, Muir SR, Sanders D: Release of Ca2+ from individual plant vacuoles by both InsP3(3) and cyclic ADP-ribose. Science 1995, 268:735-737.


This paper presents a highly significant milestone, demonstrating the action of cADPR in promoting Ca2+ flux across the tonoplast of guard cells and its possible link to ABA-mediated increases in [Ca2+].


The authors elegantly quantified the dependence of k, in on voltage-evoked [Ca2+], increases in vivo. Mn2+ loading and Fura2-fluorescence quench were used to demonstrate the role of intracellular Ca2+ release. The pharmacological data presented are consistent with a ryanodine-sensitive release pathway.


This is a seminal work that links plasma-membrane voltage to oscillations of [Ca2+] in guard cells. The data implicate the function of a plasma-membrane Ca2+-channel that is activated at negative voltages and demonstrate an action of ABA in altering the kinetics and voltage-dependence of these evoked [Ca2+] increases. A report of an ABA-sensitive, hyperpolarisation-activated Ca2+ channel at the guard-cell plasma membrane will follow shortly.


This is an excellent, quantitative analysis of ruthenium-red block of the ‘‘slow-vacuolar’’ channel of Beta vulgaris. The characteristics of this blockage are surprisingly similar to those of many ruthenium-red- and ryanodine-sensitive Ca2+-release channels in mammalian tissues. The data thus raise a question about targeting of ryanodine action and, possibly, cADPR at the tonoplast.


The authors use data from concurrent whole-cell voltage clamp and patch clamp recordings to demonstrate that Ca2+ channels are activated in discrete subpopulations, each with a fixed amplitude. The results suggest a localised, quantal action of a control factor and its release in close proximity to the plasma membrane.


An elegant demonstration showing that the rise in [Ca2+]i that creates the action potential across the Chara plasma membrane draws on Ca2+ release from non-vacuolar stores. The authors make use of Mn2+ fluorescence quench of cytosolic Fura2-dextran and Mn2+ microinjections to discriminate between vacuolar and non-vacuolar pools. The results also raise some intriguing questions about the coupling of membrane voltage to intracellular Ca2+ release. Compare these results with those of Grabov and Blatt [22••].


The authors present a remarkable synthesis of molecular, biochemical and biophysical techniques to demonstrate the modulation of protein kinase activity by an oscillating [Ca2+]i environment. The activity of immobilised calmodulin-dependent protein kinase II in phosphorylating a synthetic peptide substrate is shown to be strongly dependent on the frequency of repetitive elevations in [Ca2+]i, in vitro.
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38. Bauer CS, Pliegh C, Bethmann B, Popescu O, Hansen UP, Simonis W, Schonknecht G: Strontium-induced repetitive calcu-


40. McAnish MR, Webb AAR, Taylor JE, Hetherington AM: Stimulus-


This is an additional notation to the literature on [Ca\(^{2+}\)], and ABA action in guard cells. The authors demonstrate ABA-evoked oscillations in [Ca\(^{2+}\)], and link their occurrence to Ca\(^{2+}\) release from inositol-1,4,5-trisphosphate-sensitive stores.


The authors provide the first direct evidence of homeostatic endo/exocytotic vesicle traffic at the guard-cell plasma membrane in response to osmotic gradients. Intriguingly, the data do not support a role for [Ca\(^{2+}\)], in controlling these events.


This study represents a significant technical advance in the analysis of membrane trafficking in guard cells in vivo. The authors combined membrane capacitance and styryl-dye fluorescence recordings to examine the dynamics of exocytotic and endocytotic events at the plasma membrane. They found a rapid internalisation of membrane in response to hyperosmotic steps, which raises some interesting questions about membrane recycling during stomatal movements.


This paper describes the first application of expression-cloning in Xenopus oocytes to screen for a plant hormone receptor, with a secondary screen yielding the saxitin Nt-Syr1 from tobacco. The molecular, biochemical and electrophysiological evidence presented suggests that Nt-Syr1 possibly functions as part of a signalling protein complex coupling the ABA stimulus to control of guard-cell \(K^+\) and Ca channels. Structural homologies suggest a parallel function for Nt-Syr1 in vesicle trafficking, although this role remains to be established.


64. Pei ZM, Ghassemian M, Kwak CM, McCourt P, Schroeder JI: Role of \(\text{farnesyltransferase in ABA regulation of guard cell anion channels and water loss.}


The authors combined genetics, pharmacology and electrophysiology to gather evidence that \(\text{farnesyltransferase’s role in ABA-related signaling of guard cells. The substrate that is farnesylated has not yet been identified.}


The data presented in this paper suggest a role for phospholipase D and its metabolite phosphatic acid in the regulation of guard-cell \(K^+\) channels and stomatal movements by ABA.


This is a remarkable study of the function of the Arabidopsis GNOM gene product, a putative ARF–GTPase GTP–GDP exchange factor involved in Pin1 protein distribution at the plasma membrane. Disrupting GNOM function by mutation of the GNOM gene or by treatments with the ARF–GEF antagonist brefeldin A causes a rapid loss of Pin1 localisation to the basipetal region of the plasma membrane. The results highlight both the spatial localisation of a specific membrane protein (i.e., Pin1) within the cell and the dynamic nature of the processes that underpin the localisation.


84. Hamilton D, Hills A, Köhler B, Blatt M: Ca\textsuperscript{2+} channels at the plasma membrane of stomatal guard cells are activated by hyperpolarization and abscisic acid. Proc Natl Acad Sci USA 2000, in press. See 'Update'.