Annals of Botany **92**: 477–485, 2003
doi:10.1093/aob/mcg173, available online at www.aob.oupjournals.org

INVITED REVIEW

Encoding Specificity in Plant Calcium Signalling: Hot-spotting the Ups and Downs and Waves

CARL K.-Y. NG[1] and MARTIN R. MCAINSH[2,*]

[1]*Department of Botany, University College Dublin, Belfield, Dublin 4, Republic of Ireland and* [2]*Department of Biological Sciences, Institute of Environmental and Natural Sciences, Lancaster University, Bailrigg, Lancaster LA1 4YQ, UK*

Received: 8 May 2003 Returned for revision: 2 June 2003 Accepted: 15 June 2003 Published electronically: 21 August 2003

Calcium ions function as intracellular second messengers in regulating a plethora of cellular processes from acclimative stress responses to survival and programmed cell death. The generation of specificity in Ca^{2+} signals is dependent on influx and efflux from the extracellular milieu, cytosol and intracellular organelles. One aspect of plant Ca^{2+} signalling that is currently attracting a great deal of interest is how 'Ca^{2+}-signatures', specific spatio-temporal changes in cytosolic-free Ca^{2+}, encode the necessary information to bring about this range of physiological responses. Here, current information is reviewed on how Ca^{2+}-signatures are generated in plant cells and how stimulus-specific information can be encoded in the form of Ca^{2+}-signatures.

Key words: Calcium, waves, oscillations, hot-spots, cytosolic free calcium ($[Ca^{2+}]_{cyt}$), guard cells, ion channels

INTRODUCTION

The Ca^{2+} ion is now firmly established as a ubiquitous signalling molecule in plants. Numerous plant signal transduction pathways have been shown to use Ca^{2+} as an integral signalling component (Sanders *et al*., 1999, 2002). The universality of the Ca^{2+} ion in signalling highlights the importance of understanding how specificity can be encoded in elevations in the cytosolic concentration of this ion (Sanders *et al*., 1999, 2002; Berridge *et al*., 2000). Perhaps the best example of this in plants is the response of stomata to the plant hormones abscisic acid (ABA) and auxin, which bring about the diametrically opposite effects of stomatal closure and opening, respectively, via changes in guard cell turgor mediated in both cases through increases in the concentration of cytosolic-free Ca^{2+} ($[Ca^{2+}]_{cyt}$) (McAinsh *et al*., 1990; Irving *et al*., 1992). One plausible explanation is that each stimulus generates a unique increase in $[Ca^{2+}]_{cyt}$. The spatial and temporal components of this increase in $[Ca^{2+}]_{cyt}$, or 'Ca^{2+}-signature' as it is sometimes called, then dictate the outcome of the final response (McAinsh *et al*., 1997; McAinsh and Hetherington, 1998; Ng *et al*., 2001*b*). It has been suggested that the key to generating stimulus-specific Ca^{2+}-signatures lies in the ability to access differentially the cellular machinery controlling Ca^{2+} influx and release from intracellular stores (McAinsh *et al*., 1997; Blatt, 2000; Evans *et al*., 2001; Ng *et al*., 2001*b*; Schroeder *et al*., 2001).

Ca^{2+}-mobilizing signalling intermediates that have been implicated in mediating elevations in $[Ca^{2+}]_{cyt}$ in plants include, inositol-1,4,5-trisphosphate ($InsP_3$), inositol hexakisphosphate ($InsP_6$), nicotinic acid adenine dinucleotide phosphate (NAADP), phosphatidylinositol 3- and 4-phosphate (PI3P and PI4P), cyclic adenosine 5′-diphosphoribose (cADPR), hydrogen peroxide (H_2O_2) and sphingosine-1-phosphate (S1P) (Gilroy *et al*., 1990; McAinsh *et al*., 1996; Leckie *et al*., 1998; Staxén *et al*., 1999; Lemtiri-Chlieh *et al*., 2000; Navazio *et al*., 2000; Pei *et al*., 2000; Ng *et al*., 2001*a*; Jung *et al*., 2002). At the cellular level, it is possible that this complexity may contribute to the spatio-temporal variations in the Ca^{2+}-signatures, needed for specifying stimulus-specific responses. Furthermore, the locale within the cell of such elevations in $[Ca^{2+}]_{cyt}$, whether the appropriate response elements are present in a given region of the cell to decode the information encrypted in the Ca^{2+} signal, may also be important (Trewavas and Malhó, 1997; McAinsh and Hetherington, 1998; Malhó *et al*., 1998; Evans *et al*., 2001; Ng *et al*., 2001*b*). It is not the intention here to provide an exhaustive review of Ca^{2+} signalling in plants; for this information, the reader is referred to Sanders *et al*. (1999, 2002). Instead, the purpose of this review is to draw attention to the different types of Ca^{2+} elevations that occur in plants, which can take the form of hot-spots, puffs, sparks, oscillations and waves, and the importance of stimulus-specific Ca^{2+}-signatures for encoding information necessary for eliciting the appropriate physiological responses in plant cells. The various forms of Ca^{2+} elevations are discussed below, with examples given in the figures: hot-spots (Fig. 2), puffs and sparks (Figs 1 and 3), oscillations (Fig. 5) and waves (Fig. 1).

HOT-SPOTTING: ELEMENTAL EVENTS

Studies using animal cells have shown that elevations in $[Ca^{2+}]_{cyt}$ induced by agonists, e.g. $InsP_3$ and cADPR,

* For correspondence. Fax +44 1524 843854, e-mail m.mcainsh@lancaster.ac.uk

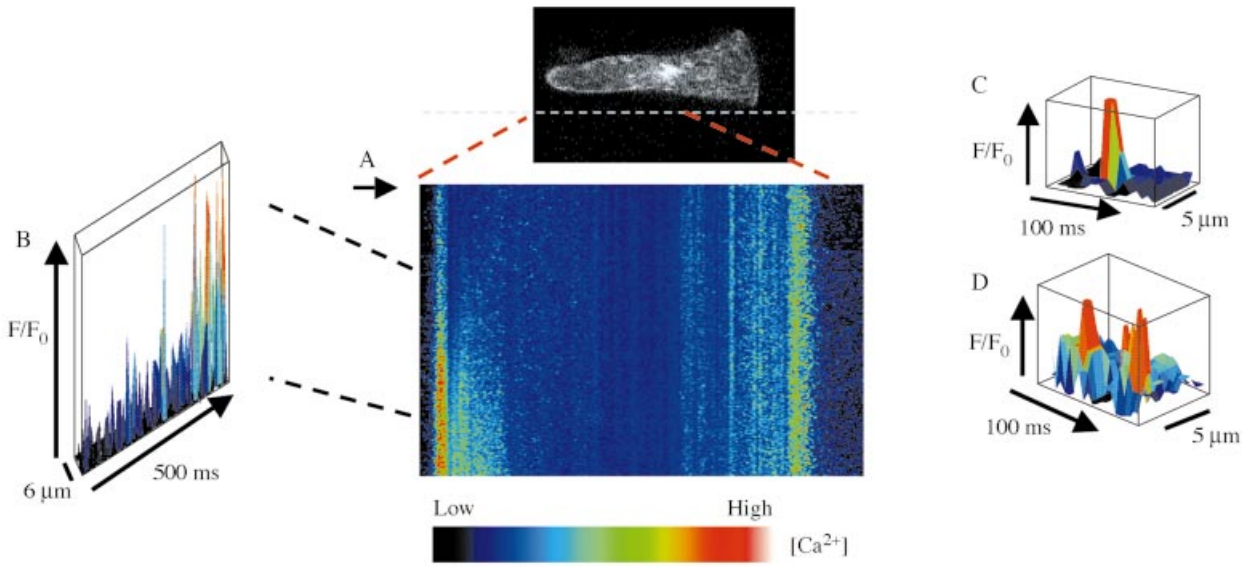


FIG. 1. Elemental Ca^{2+} elevations during Ca^{2+} wave propagation in a *Fucus* rhizoid cell. (A), Single-line confocal scans of Ca^{2+} Green fluorescence along the longitudinal axis of the cell during the initation of Ca^{2+} waves are displayed sequentially to show the relative change in fluorescence following hypo-osmotic treatment (to 50 % sea water). (B), Three-dimensional plot shows a non-uniform increase in Ca^{2+} during the onset of the Ca^{2+} wave in the rhizoid apex. (C), Elemental Ca^{2+} elevations in the perinuclear region. (D), Elemental Ca^{2+} events appear to arise repetitively at the same location in perinuclear region. Reproduced, with persmission, from Goddard *et al.* (2000).

through their respective Ca^{2+} release-channels, $InsP_3R$ and ryanodine receptors (RyR), respectively, involve a hierarchically distinguishable series of sub-threshold events (Berridge *et al*., 2000; Bootman *et al*., 2001). Low levels of stimulation result in single channel events, leading to Ca^{2+} elevations known as 'quarks' and 'blips'. The Ca^{2+} release associated with higher levels of stimulations is termed 'puffs' and 'sparks'. Quarks, blips, puffs and sparks constitute what is collectively termed 'elemental events' and form the fundamental building blocks for global Ca^{2+} signalling, e.g. Ca^{2+} waves. In plants, the best examples of elemental events in Ca^{2+} signalling were obtained using *Fucus serratus* embryos by Brownlee and co-workers (Goddard *et al*., 2000; Fig. 1). They showed that hypo-osmotic shock induced unitary Ca^{2+} elevations in discrete domains within the cytosol of *Fucus* embryos. These discrete elevations in $[Ca^{2+}]_{cyt}$ are in the range 200–300 nM and last between 15 and 30 ms (Fig. 1C). Interestingly, these unitary increases in $[Ca^{2+}]_{cyt}$ occur repetitively and are spatially separated by regions with fewer events (Fig. 1D). Unitary elevations in Ca^{2+} of similar magnitudes, amplitudes and spatial dimensions were also induced by UV-photolysis of caged-$InsP_3$, and suggest a role for $InsP_3$ in the generation of osmotically induced Ca^{2+} signals, although direct evidence for this is lacking (Goddard *et al*., 2000). The observation of these unitary increases in $[Ca^{2+}]_{cyt}$ in *Fucus* provides the first evidence in support of elemental events as fundamental building blocks in plant Ca^{2+} signalling.

In stomatal guard cells, stimulus-induced elevations in $[Ca^{2+}]_{cyt}$ also show marked spatial heterogeneities (Fig. 2). McAinsh *et al*. (1992) observed that ABA-induced elevations in $[Ca^{2+}]_{cyt}$ were unevenly distributed and appeared as

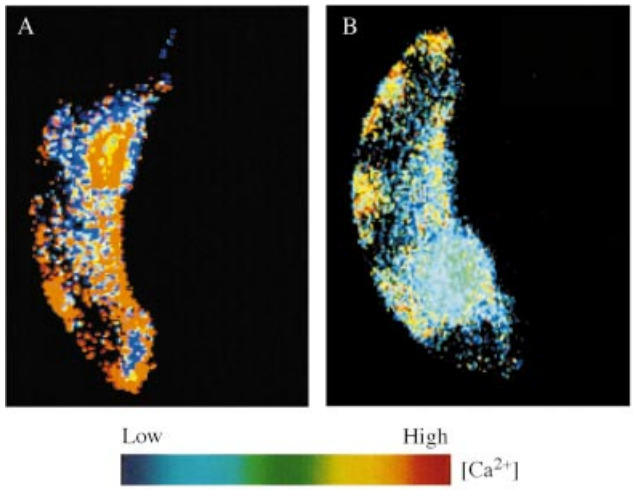


FIG. 2. Spatial heterogeneities in guard cell $[Ca^{2+}]_{cyt}$ in response to 100 nM ABA (A) and 1 mM $[Ca^{2+}]_{ext}$ (B). $[Ca^{2+}]_{cyt}$ levels are colour-coded; blue indicates low $[Ca^{2+}]_{cyt}$, red indicates high $[Ca^{2+}]_{cyt}$. These data suggest that plant cells have the capacity to encode specificity in the Ca^{2+} signal in the form of localized increases in $[Ca^{2+}]_{cyt}$. Reproduced, with permission, from McAinsh *et al.* (1992, 1995).

'hot-spots' and Ca^{2+}-quiescent regions. Spatial heterogeneity in guard cell $[Ca^{2+}]_{cyt}$ has also been reported by Gilroy et al. (1991) and McAinsh *et al*. (1995). It is possible that the spatial heterogeneities in $[Ca^{2+}]_{cyt}$ elevations could result from (*a*) differential accessibility of the primary stimulus to only a subset of the signalling machinery, or (*b*) the non-uniform distribution of the intracellular signalling machinery. These observations suggest the potential for

encoding specificity in the form of localized increases in $[Ca^{2+}]_{cyt}$. It is tempting to suggest that these localized Ca^{2+} 'hot-spots' observed in guard cells represent elemental events (puffs and sparks). However, due to the spatial and temporal resolution used in these studies, it is likely that these localized elevations in $[Ca^{2+}]_{cyt}$ represent longer transients in $[Ca^{2+}]_{cyt}$, as opposed to the unitary Ca^{2+} elevations observed in *Fucus* in response to osmotic stress and UV-photolysis of caged-$InsP_3$.

TRIGGERING WAVES

Ca^{2+} waves represent a form of global increase in $[Ca^{2+}]_{cyt}$ that are triggered by the clustering of unitary Ca^{2+} elevations leading to Ca^{2+}-induced Ca^{2+}-release (CICR). Ca^{2+} waves have been extensively studied in animal cells (Berridge *et al*., 2000; Bootman *et al*., 2001). It is now well established that Ca^{2+} signalling in plants can also take the form of a propagating Ca^{2+} wave from studies using *Fucus* (Taylor *et al*., 1996). Goddard *et al*. (2000) showed an increase in the number of unitary Ca^{2+} elevations following exposure to hypo-osmotic stress, leading subsequently to clustering and more prolonged elevations (Fig. 3A and B). Interestingly, they observed that clustering and prolonged elevations preceded the formation of Ca^{2+} waves. This suggests that the clustering of unitary Ca^{2+} elevations acts as the trigger for the generation of a Ca^{2+} wave (Fig. 3C). In this respect, the unitary Ca^{2+} elevations in *Fucus* are reminiscent of Ca^{2+} quarks/blips reported in animal cells and suggest that the clustering of such elemental events may be representative of Ca^{2+} puffs and sparks.

Importantly, the authors showed that the spatio-temporal changes in $[Ca^{2+}]_{cyt}$ can vary with the strength of the hypo-osmotic shock treatment, suggesting that variations in the signature of the Ca^{2+} wave can determine downstream physiological responses (Goddard *et al*., 2000). In rhizoid cells that had undergone nuclear division but not partition wall formation, Ca^{2+} waves were observed to propagate from two nuclear regions, with most cells showing an initial transient Ca^{2+} elevation in the rhizoid apex, followed by Ca^{2+} elevations in the apical nuclear region (n1) that subsequently spread to the sub-apical nucleus (n2) (Fig. 3C). However, in 20 % of the cells, Ca^{2+} elevations were observed to occur in the region of the sub-apical nucleus (n2) either before or simultaneously with Ca^{2+} elevations in the rhizoid apex followed by Ca^{2+} elevations in the apical nucleus (n1) (Fig. 3D). Additionally, the authors showed that these Ca^{2+} elevations were highly correlated with the distribution of endoplasmic reticulum (ER) and that these specific patterns of Ca^{2+} wave generation may encode the necessary information for differential regulation of cell volume changes and the rate of cell division (Goddard *et al*., 2000).

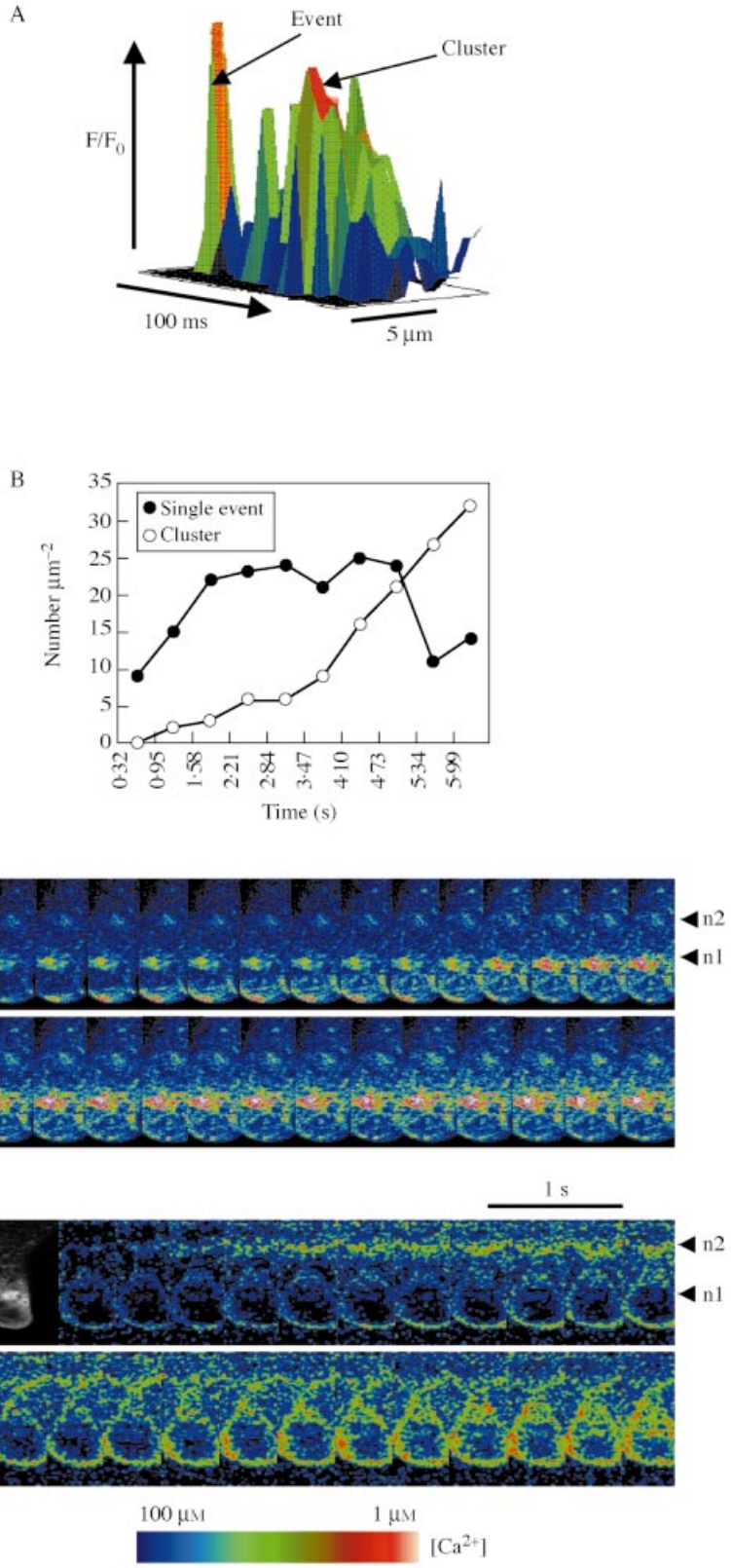
Recently, Coelho *et al*. (2002) showed that the generation of reactive oxygen species (ROS) are tightly linked to the generation of Ca^{2+} waves in *Fucus* and suggested the following sequence of events may occur during osmotic stress (Fig. 4). (*a*) Sensing of osmotic changes by an unidentified osmosensor results in extracellular production of ROS. (*b*) This initial ROS burst then activates the influx of Ca^{2+} through Ca^{2+}-permeable channels on the plasma membrane. Interestingly, H_2O_2 has also been reported to activate plasma membrane Ca^{2+}-permeable channels in guard cells (Pei *et al*., 2000). (*c*) Ca^{2+} influx then leads to elevations in $[Ca^{2+}]_{cyt}$, triggering CICR from the ER via $InsP_3$-sensitive release mechanisms. (*d*) This $InsP_3$-dependent release of Ca^{2+} from the ER results in the formation of a Ca^{2+} wave followed by mitochondrial uptake of Ca^{2+} and subsequent mitochondrial production of ROS. Although the significance of the subsequent production of ROS by the mitochondria remains to be established, this study nevertheless highlights the importance of peripheral ROS production and Ca^{2+} influx through plasma membrane Ca^{2+}-permeable channels in the generation of Ca^{2+} waves during acclimative responses to osmotic stress in *Fucus*.

OSCILLATIONS: SIMPLY UP AND DOWN?

Since the initial discovery of external Ca^{2+} ($[Ca^{2+}]_{ext}$)-induced oscillations in $[Ca^{2+}]_{cyt}$ in guard cells (McAinsh *et al*., 1995), stimulus-induced oscillations in $[Ca^{2+}]_{cyt}$ have also been observed in other cell types, including pollen tubes (Holdaway-Clarke *et al*., 1997), roots (Kiegle *et al*., 2000) and root hairs (Ehrhardt *et al*., 1996). Oscillations in $[Ca^{2+}]_{cyt}$ result from the dynamic balance of fluxes of Ca^{2+} into and out of the cytosol and include release and uptake from intracellular stores (vacuole and ER) and nucleus as well as influx and efflux across the plasma membrane. These fluxes are mediated through the regulated activities of Ca^{2+} channels and Ca^{2+}-pumps located on the plasma and endomembranes (Bunney *et al*., 1999; Sanders *et al*., 1999, 2002; Pauly *et al*., 2000; Harper, 2001; Miedema *et al*., 2001). Oscillations in $[Ca^{2+}]_{cyt}$ allow for information to be encoded in both the amplitude and frequency (Fig. 5) (Berridge *et al*., 1988; Fewtrell, 1993; McAinsh *et al*., 1997; McAinsh and Hetherington, 1998; Evans *et al*., 2001; Ng *et al*., 2001*b*). Of the various cell types that have been used to study stimulus-induced oscillations in $[Ca^{2+}]_{cyt}$, the guard cell has emerged as the most intensively used system.

Studies in the Hetherington and McAinsh laboratories using manganese quenching and imaging have shown that $[Ca^{2+}]_{ext}$-induced oscillations in $[Ca^{2+}]_{cyt}$ in guard cells of *Commelina communis* result from both Ca^{2+} influx through the plasma membrane and release from intracellular stores (McAinsh *et al*., 1995). Later studies from the Blatt and Schroeder laboratories using *Vicia faba* and *Arabidopsis thaliana* guard cells demonstrated that Ca^{2+} influx through the plasma membrane is gated by hyperpolarization-dependent Ca^{2+} channels (Grabov *et al*., 1998, 1999; Hamilton *et al*., 2000; Pei *et al*., 2000). Interestingly, Blatt and co-workers showed that the influx of Ca^{2+} through the plasma membrane Ca^{2+} channels are coupled to oscillations in plasma membrane potentials, and that ABA can regulate this influx of Ca^{2+} by increasing the probability of channel opening and by shifting the voltage sensitivity of these channels to more depolarizing potentials. Together these results suggest that ABA-induced oscillations in $[Ca^{2+}]_{cyt}$ is the result of influx of Ca^{2+} through the plasma membrane due in part to channel gating by oscillations in membrane potentials and greater probabilities of Ca^{2+} channel opening

(Grabov *et al*., 1998, 1999; Hamilton *et al*., 2000). More recently, the Schroeder laboratory showed that ABA induced H_2O_2 production, and subsequent H_2O_2-activation of Ca^{2+} influx through hyperpolarization-activated guard cell plasma membrane Ca^{2+}-permeable channels can contribute to increases in $[Ca^{2+}]_{cyt}$ (Pei *et al*., 2000).



In addition to the role of plasma membrane Ca^{2+} influx, a variety of second messenger systems exist for the release of Ca^{2+} from intracellular stores, for the generation of increases in $[Ca^{2+}]_{cyt}$. One of the earliest indications of an intracellular route for Ca^{2+} release in guard cells was reported by the Trewavas laboratory (Gilroy *et al*., 1990). They showed that UV-photolysis of caged $InsP_3$ microinjected into the cytosol of guard cells elevated $[Ca^{2+}]_{cyt}$ and stimulated stomatal closure. This artificial elevation of cytosolic $InsP_3$ could also reversibly inactivate inward-rectifying K^+ channels ($I_{K,in}$), whilst at the same time activating an inward current that depolarizes the plasma membrane (Blatt *et al*., 1990). Many elements of an $InsP_3$-mediated signalling pathway have been identified in plants, and Lee *et al*. (1996) have shown that ABA induced the rapid turnover of phosphoinositides in guard cell protoplasts. Staxén *et al*. (1999) have also demonstrated the involvement of a phosphoinositide-specific phospholipase C (PI-PLC)-$InsP_3$ Ca^{2+}-mobilizing pathway in the regulation of guard cell turgor by ABA but not $[Ca^{2+}]_{ext}$. Their data suggest that ABA-induced oscillations in $[Ca^{2+}]_{cyt}$ have the potential to encode information on the strength of the initial ABA stimulus and for maintaining steady-state stomatal apertures (Staxén *et al*., 1999). Hunt *et al*. (2003) have subsequently confirmed the role of PI-PLC in the control of stomatal aperture by ABA. Another inositol phosphate that has been implicated in ABA-mediated changes in guard cells is $InsP_6$ (Lemtiri-Chlieh *et al*., 2000). ABA has been shown to induce a rapid production of $InsP_6$ and that loading of $InsP_6$ into the cytosol of guard cell protoplasts inhibits $I_{K,in}$ in a Ca^{2+}-dependent manner. It will be of interest to determine the exact nature of the Ca^{2+}-dependence of $InsP_6$-mediated inhibition of $I_{K,in}$ and the modulation of $[Ca^{2+}]_{cyt}$ by $InsP_6$.

More recently, Jung *et al*. (2002) used a pharmacological approach to demonstrate that PI3P and PI4P are components of the ABA signal transduction pathway mediating stomatal closure, and that inhibitors of PI3-kinase and PI4-kinase (wortmanin and LY294002) inhibited ABA-induced oscillations in $[Ca^{2+}]_{cyt}$. Together, these data suggest that inositol phosphates are important regulatory components in guard cell ABA signalling. Other Ca^{2+}-mobilizing second messengers known to function in regulation of guard cell turgor include cADPR and the recently identified sphingolipid metabolite, S1P. Leckie *et al*. (1998) observed that most cells microinjected with cADPR showed a sustained

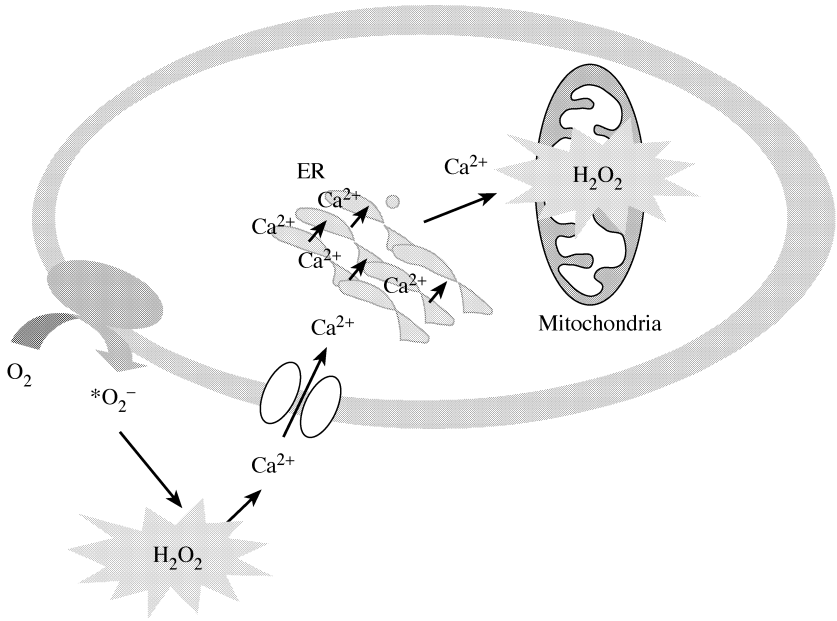


FIG. 4. Proposed signalling pathway during hyperosmotic stress in *Fucus* embryos. Osmotic change is sensed by an unidentified sensor leading to the production of extracellular ROS production (which may be important for strengthening the cell wall). The H_2O_2 formed also diffuses into the cell, leading to localized peripheral intracellular increase. The extracellular H_2O_2 also increases Ca^{2+}-channel activity. This leads to Ca^{2+}-induced Ca^{2+} release from intracellular stores (ER) resulting in Ca^{2+} wave propagation and mitochondrial ROS production. Reproduced, with permission, from Coelho *et al.* (2002).

FIG. 3. (Opposite) Elemental Ca^{2+} elevations during Ca^{2+} wave propagation in a *Fucus* rhizoid cell and patterns of Ca^{2+} elevations following hypo-osmotic treatment in dividing cells. (A), Elemental Ca^{2+} events in the perinuclear region either occur individually or appeared to cluster into more prolonged elevations. (B), Number of discrete elevations at the rhizoid apex increased initially during the first 1·5 s of wave propagation and then declined with the appearance of more prolonged elevations. (C), Ca^{2+} Green to Teax Red ratio images during the onset of a hypo-osmotically induced Ca^{2+} waves (100 % sea water to 50 % sea water) shows an initial elevation of Ca^{2+} in the rhizoid apex which declines before the onset of Ca^{2+} elevation arising in the apical nucleus region. (D), A minority of cells shows a variation of the pattern shown in part C where the Ca^{2+} elevations were observed to arise in the subapical nucleus simultaneous with the apical Ca^{2+} elevation. Reproduced, with permission, from Goddard *et al.* (2000).

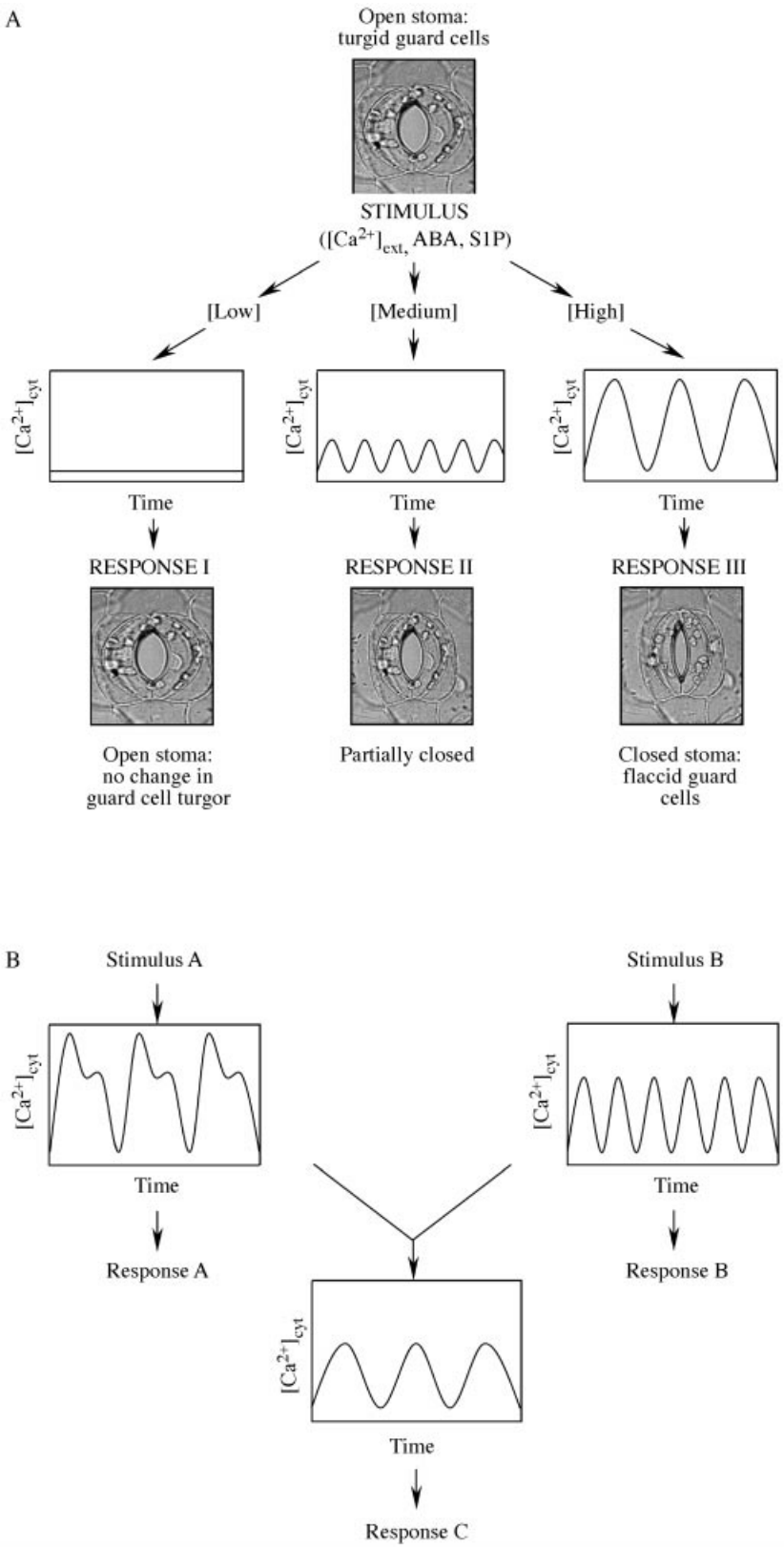


FIG. 5. Encoding signalling information in plant Ca^{2+} signatures. (A), In guard cells the strength of the stimulus has been correlated directly with the pattern of $[Ca^{2+}]_{cyt}$ oscillations (i.e. the period, frequency and amplitude) which, in turn, dictates the resultant steady-state stomatal aperture. (B), Guard cells are able to integrate signalling information from a number of stimuli that induce oscillations in $[Ca^{2+}]_{cyt}$ applied simultaneously to generate a novel Ca^{2+} signature when formulating the final stomatal aperture. Reproduced, with permission, from Evans *et al.* (2001).

increase in $[Ca^{2+}]_{cyt}$ lasting in excess of 10 min while oscillations were observed in some cells. The increase in $[Ca^{2+}]_{cyt}$ elicited by cADPR ranged from 0·05 to 0·4 μM. In the case of S1P, the increase in $[Ca^{2+}]_{cyt}$ take the form of oscillations that have a period and amplitude that is characteristic of the concentration of S1P used. The differential pattern of $[Ca^{2+}]_{cyt}$ induced by different concentrations of S1P can be correlated with the rate of stomatal closure and is further evidence in support of oscillations in the control of guard cell turgor (Ng and Hetherington, 2001; Ng *et al*., 2001*a*).

The removal of Ca^{2+} from the cytosol is also an important factor in the generation of oscillations in $[Ca^{2+}]_{cyt}$ causing the downward phase of the oscillation. The importance of cytosolic Ca^{2+} removal is highlighted by Allen *et al*. (2000) who showed that the V-type ATPase activity is required for returning $[Ca^{2+}]_{cyt}$ to resting levels. The authors showed in the *det3* (*de-etiolated 3*) mutant of arabidopsis (which exhibits decreased endomembrane energization due to a 60 % decline in the expression of the C-subunit of the V-type ATPase) that a correlation exists between stomatal closure and $[Ca^{2+}]_{ext}$-induced $[Ca^{2+}]_{cyt}$ oscillations. Guard cells of *det3* mutants do not close in response to $[Ca^{2+}]_{ext}$ and do not exhibit oscillations in $[Ca^{2+}]_{cyt}$ compared with wild-type guard cells. Interestingly, $[Ca^{2+}]_{cyt}$ oscillations induced by cold treatment and ABA were not affected in guard cells of the *det3* mutants and suggest a scenario where multiple pathways of cytosolic Ca^{2+} removal exist and that interact differentially to bring about the complexities observed in stimulus-induced oscillations in $[Ca^{2+}]_{cyt}$.

The mechanism by which stimulus-specific information may be encoded in $[Ca^{2+}]_{cyt}$ oscillations has been studied in the Schroeder laboratory. In an elegant study, Allen *et al*. (2001) showed that the information encrypted in elevations in $[Ca^{2+}]_{cyt}$ can be separated into two components: (1) 'Ca^{2+}-reactive' and (2) 'Ca^{2+}-programmed'. These two distinct components encode information relating to short-term and long-term acclimative responses, respectively. The 'Ca^{2+}-reactive' component of the elevation in $[Ca^{2+}]_{cyt}$ occurs rapidly and encodes information for the initiation of stomatal closure. This rapid reactive component usually takes the form of the initial transient elevation in $[Ca^{2+}]_{cyt}$. On the other hand, the 'Ca^{2+}-programmed' component encodes information related to the maintenance of steady-state stomatal apertures and is usually represented by a series of oscillations. The number of transients (a complete cycle consisting of an upward and downward phase) determines the final steady-state aperture: the greater the number of transients, the smaller the steady-state stomatal aperture (Allen *et al*., 2001). Taken together, these data make a compelling case for the encryption of important physiological information in the form of $[Ca^{2+}]_{cyt}$ oscillations.

CONCLUSIONS AND FUTURE PROSPECTS

The potential for encoding specificity in the Ca^{2+} signal has been demonstrated by the ability of guard cells to generate stimulus-specific temporal changes in $[Ca^{2+}]_{cyt}$ in the form of oscillations. Additional information can also be encoded in the form of complex patterns of $[Ca^{2+}]_{cyt}$ oscillations generated in response to two or more stimuli (Hetherington *et al*., 1998; McAinsh *et al*., 2000; Ng *et al*., 2001*b*). The ability to modulate the pattern of the Ca^{2+}-signature suggests the presence of a complex cellular machinery capable of integrating stimuli perception into a meaningful physiological response. While $[Ca^{2+}]_{cyt}$ oscillations play an important role in turgor regulation in stomatal guard cells, stimulus-specific patterns of Ca^{2+} wave formation and propagation appear to be important in specifying the information necessary for differential regulation of cell volume changes and the rate of cell division. The ability for encryption of stimulus-specific information immediately raises the question of how the cell is able to decode the information into a meaningful physiological response. It has been suggested that a variety of Ca^{2+}-binding proteins, Ca^{2+}-dependent protein kinases and protein phosphatases may provide the diversity needed for decoding the encrypted Ca^{2+} signal (Luan *et al*., 1993; Berkowitz *et al*., 2000; Guo *et al*, 2002). Webb and Hetherington (1997) have suggested that signalling components may be organized in the form of a 'cassette' and function as focal points for signal convergence and integration. Trewavas and Malhó (1997) also suggested a similar concept of a 'transducon', large protein complexes where signalling components may be spatially organized within the cellular milieu. The hypotheses that signalling components are organized into spatially distinct cassettes/transducons was lent credence by the recent observation of Guo *et al*. (2002) who showed the physical interaction of ScaBP5, a Ca^{2+}-binding protein with PKS3, its interacting protein kinase. PKS3 in turn interacts with ABI2 and ABI1. This suggests that ScaBP5, PKS3 and ABI2 and/or ABI1 are spatially organized, perhaps in a cassette/transducon, for efficient transduction of the ABA signal. Insights into how stimulus-specific signals are encrypted and the underlying mechanism(s) for decoding the Ca^{2+} signal into meaningful physiological responses are likely to be gained through the systematic identification of genes, their products and the interactions between the various signalling components.

ACKNOWLEDGEMENTS

We are grateful to the Biotechnology and Biological Sciences Research Council, UK (M.R.M.), The Royal Society, UK (M.R.M.) and University College Dublin (C.K.-Y.N.) for research funding.

LITERATURE CITED

Allen GJ, Chu SP, Harrington CL, Schumacher K, Hoffmann T, Tang YY, Grill E, Schroeder JI. 2001. A defined range of guard cell calcium oscillation parameters encodes stomatal movements. *Nature* **411**: 1053–1057.

Allen GJ, Chu SP, Schumacher K, Shimazaki CT, Vafeados D, Kemper A, Hawke SD, Tallman G, Tsien RY, Harper JF *et al.* 2000. Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in *Arabidopsis det3* mutants. *Science* **289**: 2338–2342.

Berkowitz G, Zhang X, Mercier R, Leng Q, Lawton M. 2000. Co-expression of calcium-dependent protein kinase with the inward

rectified guard cell K+ channel KAT1 alters current parameters in *Xenopus laevis* oocytes. *Plant and Cell Physiology* **41**: 785–790.

Berridge MJ, Cobbold PH, Cuthbertson KSR. 1988. Spatial and temporal aspects of cell signalling. *Philosophical Transactions of the Royal Society of London Series B* **320**: 325–343.

Berridge MJ, Lipp P, Bootman MD. 2000. The versatility and universality of calcium signalling. *Nature Reviews – Molecular Cell Biology* **1**: 11–21.

Blatt MR. 2000. Cellular signalling and volume control in stomatal movements in plants. *Annual Review of Cell and Developmental Biology* **16**: 221–241.

Blatt MR, Thiel G, Trentham DR. 1990. Reversible inactivation of K^+ channels in *Vicia* stomatal guard cells following the photolysis of caged inositol 1,4,5-triphosphate. *Nature* **346**: 766–769.

Bootman MD, Lipp P, Berridge MJ. 2001. The organisation and functions of local Ca^{2+} signals. *Journal of Cell Science* **114**: 2213–2222.

Bunney TD, Shaw PJ, Watkins PAC, Taylor JP, Beven AF, Wells B, Calder GM, Drøbak BK. 1999. ATP-dependent regulation of nuclear Ca^{2+} levels in plant cells. *FEBS Letters* **476**: 145–149.

Coelho SM, Taylor AR, Ryan KP, Sousa-Pinto I, Brown MT, Brownlee C. 2002. Spatiotemporal patterning of reactive oxygen production and Ca^{2+} wave propagation in *Fucus* rhizoid cells. *Plant Cell* **14**: 2369–2381.

Ehrhardt DW, Wais R, Long SR. 1996. Calcium spiking in plant root hairs responding to rhizobium nodulation signals. *Cell* **85**: 673–681.

Evans NH, McAinsh MR, Hetherington AM. 2001. Calcium oscillations in higher plants. *Current Opinion in Plant Biology* **4**: 415–420.

Fewtrell C. 1993. Ca^{2+} oscillations in nonexcitable cells. *Annual Review of Physiology* **55**: 427–454.

Gilroy S, Read ND, Trewavas AJ. 1990. Elevation in cytoplasmic calcium by caged calcium or caged inositol trisphosphate initiates stomatal closure. *Nature* **346**: 769–771.

Gilroy S, Fricker MD, Read ND, Trewovas AJ. 1991. Role of calcium in signal transduction of *Commelina* guard cells. *Plant Cell* **3**: 333–344.

Goddard H, Manison NFH, Tomos D, Brownlee C. 2000. Elemental propagation of calcium signals in response-specific patterns determined by environmental stimulus strength. *Proceedings of the National Academy of Sciences of the USA* **97**: 1932–1937.

Grabov A, Blatt MR. 1998. Membrane voltage initiates Ca^{2+} waves and potentiates Ca^{2+} increases with abscisic acid in stomatal guard cells. *Proceedings of the National Academy of Sciences of the USA* **95**: 4778–4783.

Grabov A, Blatt MR. 1999. A steep dependence of inward-rectifying potassium channels on cytosolic free calcium concentration increase evoked by hyperpolarisation in guard cells. *Plant Physiology* **119**: 277–287.

Guo Y, Xiong L, Song CP, Gong D, Halfter U, Zhu JK. 2002. A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signalling in *Arabidopsis*. *Developmental Cell* **3**: 233–244.

Hamilton DWA, Hills A, Köhler B, Blatt MR. 2000. Ca^{2+} channels at the plasma membrane of stomatal guard cells are activated by hyerpolarisation and abscisic acid. *Proceedings of the National Academy of Sciences of the USA* **97**: 4967–4972.

Harper JF. 2001. Dissecting calcium oscillators in plant cells. *Trends in Plant Science* **6**: 395–397.

Hetherington AM, Gray JE, Leckie CP, McAinsh MR, Ng C, Pical C, Priestley AJ, Staxén I, Webb AAR. 1998. The control of specificity in guard cell signal transduction. *Philosophical Transactions of the Royal Society of London Series B* **353**: 1489–1494.

Holdaway-Clarke TL, Feijo JA, Hackett GR, Kunkel JG, Hepler PK. 1997. Pollen tube growth and the intracellular cytosolic calcium gradient oscillate in phase while extracellular calcium influx is delayed. *Plant Cell* **9**: 1999–2010.

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

Irving HR, Gehring CA, Parish RW. 1992. Changes in cytostolic pH and calcium guard cells precede stomatal movements. *Proceedings of the National Academy of Sciences of the USA* **89**: 1790–1794.

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

Kiegle E, Moore CA, Haseloff J, Tester MA, Knight MR. 2000. Cell-type-specific calcium responses to drought, salt and cold in the *Arabidopsis* root. *Plant Journal* **23**: 267–278.

Leckie CP, McAinsh MR, Allen GJ, Sanders D, Hetherington AM. 1998. Abscisic acid-induced stomatal closure mediated by cyclic ADP-ribose. *Proceedings of the National Academy of Sciences of the USA* **95**: 15837–15842.

Lee YS, Choi YB, Suh S, Lee J, Assmann SM, Joe CO, Kelleher JF, Crain RC. 1996. Abscisic acid-induced phosphoinositide turnover in guard cell protoplasts of *Vicia faba*. *Plant Physiology* **110**: 987–996.

Lemtiri-Chlieh F, MacRobbie EAC, Brearley CA. 2000. Inositol hexakisphosphate is a physiological signal regulating K^+-inward rectifying conductances in guard cells. *Proceedings of the National Academy of Sciences of the USA* **97**: 8687–8692.

Luan S, Li W, Rusnak F, Assmann SM, Schreiber SL. 1993. Immunosuppresants implicate protein phosphatase regulation of K+ channels in guard cells. *Proceedings of the National Academy of Sciences of the USA* **90**: 2202–2206.

Malhó R, Moutinho A, van der Luit A, Trewavas AJ. 1998. Spatial characteristics of calcium signalling: the calcium wave as a basic unit of plant cell calcium signalling. *Philosophical Transactions of the Royal Society of London Series B* **353**: 1463–1473.

McAinsh MR, Hetherington AM. 1998. Encoding specificity in Ca^{2+} signalling systems. *Trends in Plant Science* **3**: 32–36.

McAinsh MR, Brownlee C, Hetherington AM. 1990. Abscisic acid-induced elevation of guard cell cytosolic Ca^{2+} precedes stomatal closure. *Nature* **343**: 186–188.

McAinsh MR, Brownlee C, Hetherington AM. 1992. Visualising changes in cytosolic-free Ca^{2+} during the response of stomatal guard cells to abscisic acid. *Plant Cell* **4**: 1113–1122.

McAinsh MR, Brownlee C, Hetherington AM. 1997. Calcium ions as second messengers in guard cell signal transduction. *Physiologia Plantarum* **100**: 16–29.

McAinsh MR, Clayton H, Mansfield, TA, Hetherington AM. 1996. Changes in stomatal behaviour and guard cell cytosolic free calcium in response to oxidative stress. *Plant Physiology* **111**: 1031–1042.

McAinsh MR, Gray JE, Hetherington AM, Leckie CP, Ng C. 2000. Calcium signalling in stomatal guard cells. *Biochemical Society Transactions* **28**: 476–481.

McAinsh MR, Webb AAR, Taylor JE, Hetherington AM. 1995. Stimulus-induced oscillations in guard cell cytosolic-free calcium. *Plant Cell* **7**: 1207–1219.

Miedema H, Bothwell JHF, Brownlee C, Davies JM. 2001. Calcium uptake by plant cells – channels and pumps acting in concert. *Trends in Plant Science* **6**: 514–519.

Navazio L, Bewell MA, Siddiqua A, Dickson GD, Galione A, Sanders D. 2000. Calcium release from the endoplasmic reticulum of higher plants elicited by the NADP metabolite nicotinic acid adenine dinucleotide phosphate. *Proceedings of the National Academy of Sciences of the USA* **97**: 8693–8698.

Ng CKY, Hetherington AM. 2001. Sphingolipid-mediated signalling in plants. *Annals of Botany* **88**: 957–965.

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

Ng CKY, McAinsh MR, Gray JE, Hunt L, Leckie CP, Mills L, Hetherington AM. 2001*b*. Calcium-based signalling systems in guard cells. *New Phytologist* **151**: 109–120.

Pauly N, Knight MR, Thuleau P, van der Luit A, Moreau M, Trewavas AJ, Ranjeva R, Mazars C. 2000. Cell signalling: control of free calcium in plant cells. *Nature* **405**: 754–755.

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

Sanders D, Brownlee C, Harper JF. 1999. Communicating with calcium. *Plant Cell* **11**: 691–706.

Sanders D, Pelloux J, Brownlee C, Harper JF. 2002. Calcium at the crossroads of signalling. *Plant Cell* (Supp 1): S401–S417.

Schroeder JI, Allen GJ, Hugovieux V, Kwak JM, Waner D. 2001.

Guard cell signal transduction. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**: 627–658.

Staxén I, Pical C, Montgomery LT, Gray JE, Hetherington AM. 1999. Abscisic acid induces oscillations in guard-cell cytosolic free calcium that involve phosphoinositide-specific phospholipase C. *Proceedings of the National Academy of Sciences of the USA* **96**: 1779–1784.

Taylor AR, Manison NFH, Fernandez C, Wood JW, Brownlee C. 1996. Spatial organization of calcium signalling involved in cell volume control in the *Fucus* rhizoid. *Plant Cell* **8**: 1935–1949.

Trewavas AJ, Malhó R. 1997. Signal perception and transduction: the origin of the phenotype. *Plant Cell* **9**: 1181–1195.

Webb AAR, Hetherington AM. 1997. Convergence of the ABA, CO_2 and extracellular calcium signal transduction pathways in stomatal guard cells. *Plant Physiology* **114**: 1557–1560.