The Plant Cell, Vol. 11, 691–706, April 1999, www.plantcell.org

Communicating with Calcium

Dale Sanders,[a,1] Colin Brownlee,[b] and Jeffrey F. Harper[c]

[a] Plant Laboratory, Biology Department, University of York, York YO1 5YW, United Kingdom
[b] The Laboratory, Marine Biological Association, Citadel Hill, Plymouth PL1 2PB, United Kingdom
[c] Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

INTRODUCTION

Calcium as a Ubiquitous Signal in Plants

All living cells use a network of signal transduction pathways to conduct developmental programs, obtain nutrients, control their metabolism, and cope with their environment. A major challenge for cell biologists is to understand the "language" of these signaling systems. For simplicity, signaling pathways usually have been studied in isolation, with experimenters attempting to define a single pathway through which a given stimulus evokes a response. However, cells are not simple, and for any given stimulus (input), the final response (output) is likely to be the result of complex interaction, or cross-talk, between multiple pathways (Trewavas and Malhó, 1997; Jenkins, 1998). Presumably, this cross-talk evolved as a mechanism to enable a relatively small number of messengers to help cells process a much larger array of potential stimuli in an appropriate fashion. In plant cells, the list of messengers used by signaling pathways includes Ca^{2+}, lipids, pH, and cyclic GMP (cGMP). However, no single messenger has been demonstrated to respond to more stimuli than has cytosolic free Ca^{2+} ($[Ca^{2+}]_c$). A partial list of stimuli that evoke changes in $[Ca^{2+}]_c$ is shown in Table 1.

Our knowledge of $[Ca^{2+}]_c$ in plant cell signaling has been markedly enhanced by the development of reliable techniques to measure free Ca^{2+} intracellularly (Read et al., 1993). Early studies (Brownlee and Wood, 1986; Miller and Sanders, 1987; Felle, 1989) with Ca^{2+}-selective microelectrodes yielded consistent estimates of $[Ca^{2+}]_c$. However, electrodes are rather unwieldy and are unable to report detailed spatial information, so they have been largely supplanted by fluorescent dyes and the luminescent protein aequorin (Read et al., 1993). Ca^{2+}-indicating fluorescent dyes usually are microinjected into the cell or sometimes loaded as the free acid at low pH. Their fluorescence yields are sufficient to enable ready imaging of heterogeneities in $[Ca^{2+}]_c$,; for dyes in which an isosbestic point is present in the excitation or emission spectrum, ratiometric images allow quantification of the signal independent of dye concentration. Alternatively, changes in $[Ca^{2+}]_c$ can be visualized with the Ca^{2+}-sensitive luminescent protein aequorin. The use of transgenic plants stably expressing apoaequorin—from which aequorin can be spontaneously reconstituted on addition of the luminophore coelenterazine—circumvents the requirement for microinjection, although low-intensity emission of aequorin so far has limited spatial resolution of $[Ca^{2+}]_c$ signals to the multicellular level (Knight et al., 1998).

Although calcium signals have been implicated in many stimulus–response phenomena, rigorous proof that a $[Ca^{2+}]_c$ transient is an essential intermediate has been difficult to obtain. A strong argument for a critical involvement of Ca^{2+} requires the demonstration not only that a stimulus evokes a change in $[Ca^{2+}]_c$ but also that two additional experimental criteria are met. First, a block of the $[Ca^{2+}]_c$ transient also should block the downstream response, and second, appropriate Ca^{2+}-sensitive response elements should be present. Meeting these criteria represents a formidable technical challenge, but strong evidence exists to support a central role of $[Ca^{2+}]_c$ in a range of events, including phytochrome action (Shacklock et al., 1992; Bowler et al., 1994; Neuhaus et al., 1997), stomatal closure in response to a number of stimuli (MacRobbie, 1997), and tip growth of root hairs, pollen tubes, and algal rhizoids (e.g., Brownlee and Wood, 1986; Wymer et al., 1997; Holdaway-Clarke et al., 1998; Malhó et al., 1998).

Why Calcium?

Metabolism in all cells requires the presence of orthophosphate (P_i) and phosphorylated organic compounds, particularly for cytosolic reactions associated with transduction of free energy. The low solubility product of Ca^{2+} with P_i would have required the early evolution of mechanisms for maintenance of $[Ca^{2+}]_c$ at a level that would certainly be well below

[1] To whom correspondence should be addressed. E-mail ds10@york.ac.uk; fax 44-1904-434317.

Table 1. Some Physiological Stimuli That Elevate $[Ca^{2+}]_c$ in Plant Cells

Stimulus	Example of Response	Reference
Red light	Photomorphogenesis	Shacklock et al. (1992)
Abscissic acid	Stomatal closure	McAinsh et al. (1990)
Gibberellin	α-Amylase secretion	Bush and Jones (1988)
Salinity/drought	Proline synthesis	Knight et al. (1997)
Hypoosmotic stress	Osmoadaptation	Taylor et al. (1996)
Touch	Growth retardation	Knight et al. (1991)
Fungal elicitors	Phytoalexin synthesis	Knight et al. (1991)
Cold	*KIN1* gene expression	Knight et al. (1996)
Heat shock	Thermotolerance	Gong et al. (1998)
Oxidative stress	Free radical scavenger induction	Price et al. (1994)
NOD factors	Root hair curling	Ehrhardt et al. (1996)

the millimolar concentrations that prevail in seawater. Thus, transport systems that export Ca^{2+} from the cytosol are present in all cells to sustain steady state values of $[Ca^{2+}]_c$ in the submicromolar range. This homeostatic mechanism would have been ideal for subsequent evolution of Ca^{2+}-based signaling pathways. Specifically, the elevation of $[Ca^{2+}]_c$ by a factor of 10 or 20 can occur more rapidly than would be possible for ions or solutes that are maintained at millimolar levels.

The chemistry of Ca^{2+} provides further favorable features for signal transduction. The ability of the ion to coordinate a considerable number (commonly six to eight) of uncharged oxygen atoms enables the evolution of protein conformations in which remote domains can participate in calcium binding (McPhalen et al., 1991). Calcium-induced conformational changes can in fact be exploited to elicit downstream events in signaling pathways.

Cytosolic Free Calcium, Buffering, and the Energization of Ca^{2+} Transport

The cytosolic free calcium concentration typically is maintained at ~200 nM (Bush, 1995). However, the Ca^{2+} content of the cytosol is far higher than this because of the high affinity for Ca^{2+} to a range of binding proteins. Prominent among these proteins is calmodulin, which, by binding in turn to other proteins, serves as an adaptor of Ca^{2+} signals. Much of the calcium that enters the cytosol during signaling is rapidly bound. Estimates from neuronal cells suggest that only 0.1 to 1% of an imposed Ca^{2+} load remains free (Brinley et al., 1977; Gorman and Thomas, 1980), with the rest being absorbed. The tendency for the majority of Ca^{2+} ions to associate with cytosolic proteins has the effect of markedly reducing the effective diffusion coefficient of Ca^{2+} in the cytosol (Thomas, 1982). Restricted rates of diffusion in the cytosol underlie the occurrence of localized and spatially distinct patterns in Ca^{2+} signaling.

The presence of intracellular organelles that are able to accumulate Ca^{2+} effectively contributes to the buffering of a Ca^{2+} load, tending to dampen Ca^{2+} signals. Whereas the endoplasmic reticulum (ER), mitochondria, and even chloroplasts are all known to have the capacity to sequester Ca^{2+} (Bush, 1995), it is vacuoles that, by virtue of their size and capacity for Ca^{2+} accumulation, represent the most prominent sink for Ca^{2+} in most cells. The relative contributions of biochemical and organellar buffering have not been estimated in plant cells. However, in the mycelial fungus *Neurospora crassa*, buffering of Ca^{2+} is extremely effective, with vacuolar sequestration probably playing the major role (Miller et al., 1990).

Ultimately, homeostasis of $[Ca^{2+}]_c$ must be achieved by export across the plasma membrane because both biochemical buffering and intracellular sequestration have finite capacities. Hence, a considerable electrochemical potential difference for $Ca^{2+}(\Delta\overline{\mu}_{Ca})$ exists across all membranes in the cell, with the energized transport of Ca^{2+} invariably in a direction out of the cytosol. At the plasma membrane, the Ca^{2+} concentration ratio (inside/outside) is typically of the order of 10^{-4}, and the presence of a cytosolic negative electrical potential difference of approximately −150 mV yields a $\Delta\overline{\mu}_{Ca}$ of approximately −50 kJ mol^{-1}. Although the potential difference across the membranes of organelles, such as the vacuole and ER, is likely to be less negative than that across the plasma membrane, large driving forces nevertheless prevail and require energized removal of Ca^{2+} from the cytosol. Efflux of Ca^{2+} from the cytosol is mediated by pumps powered by either ATP hydrolysis or a proton motive force. By contrast, the passive entry of Ca^{2+} into the cytosol is mediated by ion channels.

ENCODING CALCIUM SIGNALS: AN ISSUE OF TRANSPORT

Cytosolic Ca^{2+} signals can be thought of as the result of two opposing functions: influx into and efflux out of the cytosol.

In theory, the characteristics of the transport systems catalyzing influx and efflux are the primary determinants of the dynamic form of a Ca^{2+} signal. However, Ca^{2+} binding proteins, by virtue of their buffer capacity, will in practice act as significant modifiers of $[Ca^{2+}]_c$ dynamics.

Research has emphasized the role of transporters in regulating $[Ca^{2+}]_c$ at the vacuolar, ER, and plasma membranes. Figure 1 gives an overview of the Ca^{2+} transport events at these membranes, which are the subject of more detailed discussion below. Additionally, Ca^{2+} fluxes across endomembranes, such as the chloroplast envelope, mitochondrial inner membrane, and nuclear membrane, might be equally important in determining the pattern or form of Ca^{2+} signals (Kreimer et al., 1985; Johnson et al., 1995; Santella and Carafoli, 1997).

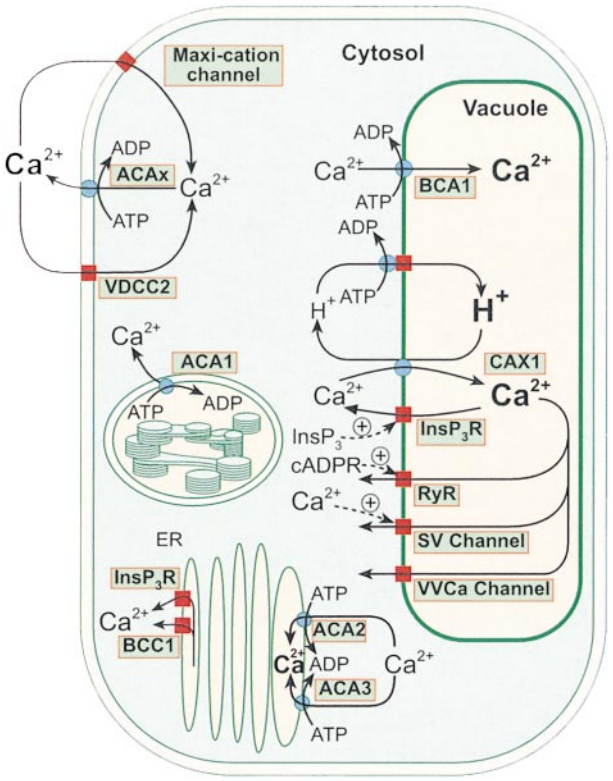

Figure 1. Schematic Representation of Major Identified Ca^{2+} Transport Pathways in Plant Cell Membranes.

Energized transport systems are represented as blue circles. ACA1, ACA2, and ACA3 are Arabidopsis Ca^{2+} ATPases that have been localized to the membranes shown. The direction of Ca^{2+} pumping for ACA1 is hypothetical. ACAx refers to a plasma membrane ATPase that has been identified biochemically but not at a molecular level. BCA1 is a *Brassica oleracea* Ca^{2+} ATPase that localizes to the vacuolar membrane. CAX1 is a Ca^{2+}/H^+ antiporter from Arabidopsis, probably located at the vacuolar membrane. Red squares represent Ca^{2+}-permeable ion channels, identified through electrophysiological approaches. None has been identified yet at a molecular level. BCC1, *Brionica* Ca^{2+} channel; $InsP_3R$, putative $InsP_3$ receptor; RyR, putative ryanodine receptor, activated by cADPR; SV channel, slowly activating vacuolar channel; VDCC2, voltage-dependent Ca^{2+} channel 2; VVCa channel, vacuolar voltage-gated Ca^{2+} channel. See text for further discussion and references.

Calcium Efflux through H^+/Ca^{2+} Antiporters and Calcium ATPases

The transport systems that energize efflux from the cytosol are proposed to provide three critical housekeeping functions. First, they restore $[Ca^{2+}]_c$ to resting levels, thereby terminating Ca^{2+} signals. Second, they load Ca^{2+} into compartments such as the ER and vacuole to be used as sources for regulated Ca^{2+} release. Third, they supply Ca^{2+} to various organelles to support specific biochemical functions. For example, high levels of Ca^{2+} in the ER are required for proper protein processing through the secretory pathway (Rudolph et al., 1989; Gill et al., 1996).

A fundamental question is whether, in addition to their housekeeping functions, any of these efflux pathways help shape the dynamic form of a calcium signal and thereby help define the information encoded in calcium release. If efflux is subject to regulation, then elucidating the signals that control these efflux systems will be equally important in identifying the signals that open various calcium channels. Pioneering work in two nonplant systems (*Xenopus* oocytes and *Dictyostelium*) has demonstrated that increasing the abundance or activity of a Ca^{2+} pump can indeed alter signal transduction (Camacho and Lechleiter, 1993; Cubitt et al., 1998; Lechleiter et al., 1998). Thus, the potential signaling importance of efflux systems in plants must be seriously considered.

Carriers

H^+/Ca^{2+} antiporters can in principle drive "uphill" transport of Ca^{2+} in which a proton motive force is maintained, although in plants this usually requires a H^+/Ca^{2+} stoichiometry of at least three (Blackford et al., 1990). The first plant H^+/Ca^{2+} antiporter to be cloned and functionally expressed was CAX1p (Calcium exchanger 1; Hirschi et al., 1996). The gene was identified by its ability to restore growth on high-Ca^{2+} media to a yeast mutant defective in vacuolar Ca^{2+} transport. CAX1p appears to transport Ca^{2+} at low concentrations (K_m is ~13 μM), consistent with kinetic studies on H^+/Ca^{2+}-antiport activity conducted with oat root vacuoles (e.g., Schumaker and Sze, 1986). Although it is reasonable to speculate that CAX1p is localized to the plant vacuole, there is evidence for H^+/Ca^{2+} antiporters in other membranes, such as the plasma membrane (Kasai and Muto, 1990). Thus, the localization of various antiporters may require careful evaluation at the whole-cell level.

Pumps

Calcium pumps belong to the superfamily of P-type ATPases that directly use ATP to drive ion translocation. Two distinct Ca^{2+} pump families have been proposed, based on protein sequence identities (Axelsen and Palmgren, 1998). Members of the type IIA and IIB families, respectively, include the ER-type and plasma membrane–type Ca^{2+} pumps first identified in animal cells. Previously, ER- and plasma membrane–type pumps were distinguished by three criteria: (1) localization to either the ER or plasma membrane, respectively; (2) differential sensitivity to inhibitors (e.g., ER-type inhibition by cyclopiazonic acid and thapsigargin); and (3) direct activation of plasma membrane–type pumps by calmodulin (Evans and Williams, 1998).

Several genes encoding type IIA (ER-type) pumps have been cloned from plants, including LCA1 from tomato (Wimmers et al., 1992), OCA1 from rice (for OsCa–ATPase; Chen et al., 1997), and ECA1/ACA3 from Arabidopsis (for ER-type Ca^{2+}–ATPase/Arabidopsis Ca^{2+}–ATPase isoform 3; Liang et al., 1997). There is evidence that ECA1p/ACA3p is located in the ER. However, in contrast to the animal prototypes, non-ER locations have been suggested for other isoforms. For example, immunodetection of LCA1-related pumps in membrane fractionation experiments identified ER-type pumps in both tonoplast and plasma membrane locations (Ferrol and Bennett, 1996).

Three plant genes encoding type IIB (plasma membrane–type) pumps have been reported: *ACA1* and *ACA2* from Arabidopsis (Huang et al., 1993; Harper et al., 1998) and *BCA1* from *Brassica oleracea* (Malmstrom et al., 1997). The corresponding plant proteins are distinguished from animal plasma membrane–type pumps by (1) localization at membranes other than the plasma membrane, and (2) a unique structural arrangement with putative autoinhibitory domains at the N terminus instead of the C terminus. Evidence suggests that ACA1p is in a plastid inner envelope membrane, based on membrane fractionation and immunodetection with an anti-ACA1 polyclonal antibody (Huang et al., 1993). BCA1p appears to be in the vacuolar membrane, based on correspondence with a peptide sequence obtained from a purified vacuolar ATPase (Malmstrom et al., 1997). ACA2p was shown to fractionate with the ER, as indicated by immunodetection of ACA2p in membrane fractions and confirmed by cytological visualization of an ACA2p tagged with a C-terminal green fluorescent protein (Harper et al., 1998; Hong et al., 1999).

Functional Overlap?

The vacuole and ER provide two examples in which multiple efflux pathways have been identified in the same organelle. In vacuoles, there is evidence for both a H^+/Ca^{2+} antiporter (like CAX1p) and a pump (like BCA1p). In the ER, there is evidence for two different types of calcium pumps, a type IIA (like ACA3p/ECA1p) and type IIB (like ACA2p). It is not clear what unique functional properties are provided by the different efflux pathways.

Regulation

It is expected that the Ca^{2+} efflux pathways are regulated in plants, by analogy to fungal and animal systems. The yeast Ca^{2+} exchanger (VCX1p) is inhibited by calcineurin, a calmodulin-activated phosphatase (Cunningham and Fink, 1996). In animals, both ER- and plasma membrane–type Ca^{2+} pumps appear to be regulated by multiple signal transduction pathways, including calmodulin and multiple kinases (e.g., CaMKII and protein kinase C; Carafoli, 1997; Stokes, 1997; Penniston and Enyedi, 1998). The ER-type pumps can be regulated in response to cytoplasmic signals as well as a feedback system that senses the Ca^{2+} load within the ER lumen (Bhogal and Colyer, 1998; Mogami et al., 1998). In plants, the clearest example of regulation is the direct activation of at least some type IIB pumps by Ca^{2+}/calmodulin (e.g., ACA2p and BCA1p).

Calcium Influx through Ca^{2+}-Permeable Ion Channels

Calcium-permeable channels have yet to be definitively identified at a molecular level in plants. Nevertheless, a wheat cDNA clone, *LCT1* (for low-affinity cation transporter 1), complements yeast mutants defective in Ca^{2+} influx (Schachtman et al., 1997; Clemens et al., 1998). Although the sequence of *LCT1* provides no clues to its likely relationship to previously identified ion channels, an exciting possibility is that LCT1p provides a physiologically significant pathway for Ca^{2+} uptake in plants.

Despite the paucity of molecular detail, electrophysiological and biochemical studies have resulted in a considerable body of information on the permeability, gating, and pharmacological properties of Ca^{2+}-permeable channels, especially at the vacuolar and plasma membranes. In some cases, especially with respect to ligand-gated channels, insights are emerging into their roles in cell signaling.

Plasma Membrane

At least two major classes of Ca^{2+} channels reside in the plasma membrane (White, 1998). Those that are relatively nonselective with respect to cation and possess a high single-channel conductance are known as maxi-cation channels (White, 1993, 1994). The second class is relatively more selective for cation, exhibits a smaller single-channel conductance, and is known as voltage-dependent cation channel 2 (White, 1994; Pineros and Tester, 1995, 1997). Al-

though both classes of ion channel have been characterized most thoroughly in cereal roots, it is clear that Ca^{2+}-permeable channels exist in a variety of other cell types and tissues, including carrot and parsley suspension cultures (Thuleau et al., 1993; Zimmermann et al., 1997) and Arabidopsis mesophyll and root cells (Ping et al., 1992; Thion et al., 1996).

A common feature of most such channels is their capacity for activation by membrane depolarization. It is thought that such voltage gating might comprise the critical factor responsible for channel activation during signaling (Ward et al., 1995). Thus, a number of signals, including blue light (Spalding and Cosgrove, 1989), red light (Ermolayeva et al., 1996), nodulation (Nod) factors (Ehrhardt et al., 1992), and fungal elicitors (Kuchitsu et al., 1993), evoke rapid ($<$1 min) membrane depolarization, probably through initial activation of anion channels (Cho and Spalding, 1996; Ermolayeva et al., 1997). The resultant increase in Ca^{2+} channel activity could then facilitate an increase in $[Ca^{2+}]_c$. However, additional regulators of Ca^{2+} channel activity are likely to be required to preclude initiation of Ca^{2+} signals by the many factors (e.g., inorganic and organic nutrients) that have been shown to depolarize plant plasma membranes. The demonstration that the activity of depolarization-activated Ca^{2+} channels is apparently suppressed by direct or indirect interaction with microtubules (Thion et al., 1996) is in accord with the notion of voltage-independent control.

Evidence favors the presence of additional Ca^{2+} entry pathways into plant cells. In guard cells, for example, it is likely that abscisic acid (ABA) induces Ca^{2+} influx by activation of nonselective and voltage-independent cation channels (Schroeder and Hagiwara, 1990; Thiel et al., 1992). The inhibition of instantaneous current by okadaic acid (Thiel and Blatt, 1994) suggests that the activities of the channels that underlie the current might be regulated by protein phosphatase type 1 or 2A.

Endomembranes

At least four different Ca^{2+}-permeable channel types have been identified at vacuolar membranes (Allen and Sanders, 1997). Two of these channels are ligand gated, one by inositol 1,4,5-trisphosphate ($InsP_3$; Schumaker and Sze, 1987; Alexandre et al., 1990), the other by cyclic ADP-ribose (cADPR; Allen et al., 1995). The pharmacological properties of plant cADPR-gated channels resemble those of ryanodine receptors (Muir and Sanders, 1996), which, along with $InsP_3$ receptors, are responsible for mobilization of ER-based Ca^{2+} pools during signaling in animal cells. Microinjection of both $InsP_3$ and cADPR into guard cells has revealed that both compounds have the capacity to elevate $[Ca^{2+}]_c$, thereby demonstrating that $InsP_3$- and cADPR-gated channels are functional in Ca^{2+} release in plants (Gilroy et al., 1990; Leckie et al., 1998).

A property of both the $InsP_3$ receptors and the ryanodine receptors of animal cells is their capacity for activation by $[Ca^{2+}]_c$. This response is thought to underlie Ca^{2+}-induced Ca^{2+} release (CICR), which can be fundamental to the amplification of Ca^{2+} signals (Taylor and Traynor, 1995). Curiously, neither $InsP_3$- nor cADPR-gated currents across the vacuolar membrane of plants are activated by $[Ca^{2+}]_c$ (Allen and Sanders, 1994b; Leckie et al., 1998), despite the presence of waves, oscillations, and spikes of $[Ca^{2+}]_c$ (see below), which are suggestive of CICR.

The remaining two Ca^{2+}-permeable channel types at the vacuolar membrane are both voltage gated, one by membrane hyperpolarization (Johannes et al., 1992; Gelli and Blumwald, 1993; Allen and Sanders, 1994a) and the other by membrane depolarization (Ward and Schroeder, 1994; Schultz-Lessdorf and Hedrich, 1995; Allen and Sanders, 1996). The second of these channel types is known as the slowly activating vacuolar (SV) channel, and it also is activated by $[Ca^{2+}]_c$ above $\sim$600 nM (Hedrich and Neher, 1987). It has been proposed that SV channel activation might comprise a route for CICR (Ward and Schroeder, 1994), with Ca^{2+} provided by the opening of one or both of the ligand-gated channels acting as the "trigger" for SV activation. This proposal has been contested as a result of the finding that the open probability of the SV channel is highly dependent on $\Delta\bar{\mu}_{Ca}$ and that, at values of $\Delta\bar{\mu}_{Ca}$ favoring mobilization of Ca^{2+} from the vacuole, the open probability of the SV channel becomes vanishingly small (Pottosin et al., 1997). However, this debate might need to be reassessed as a result of the finding that the open probability of the SV channel is markedly enhanced by the presence of cytosolic Mg^{2+} ions (Cantu et al., 1998), which were not present in the experiments of Pottosin et al. (1997).

Despite the prominence of the vacuole as an intracellular store of Ca^{2+} in mature cells, the possibility that other intracellular compartments participate in Ca^{2+} signaling should not be ignored. Indeed, imaging studies have demonstrated intracellular Ca^{2+} mobilization from regions of pollen tubes and root hairs in which no vacuole is apparent (Ehrhardt et al., 1996; Franklin-Tong et al., 1996), and it is not surprising that Ca^{2+} channels have been described at nonvacuolar membranes. A voltage-sensitive Ca^{2+} channel has been identified in the ER from *Brionica dioica* tendrils and functionally characterized in planar lipid bilayers (Klusener et al., 1995). Calcium release elicited by $InsP_3$ furthermore has been demonstrated in sucrose gradient-fractionated vesicles from cauliflower florets, which were considerably denser than the vacuolar fraction and were possibly associated with cortical ER (Muir and Sanders, 1997).

Physiological roles for both $InsP_3$- and cADPR-gated channels are emerging. Microinjection studies and metabolite measurements have suggested roles for $InsP_3$ in stomatal closure (Gilroy et al., 1990), osmoregulation (Srivastava et al., 1989; Cho et al., 1993), modulation of turgor in the motor cells of leaf pulvini by red light (Kim et al., 1996), and cessation of pollen tube growth, possibly during the self-incompatibility response (Franklin-Tong et al., 1996). There

is clear evidence for a role of cADPR-induced Ca^{2+} signaling in ABA responses. Wu et al. (1997) showed that cADPR levels are upregulated by ABA and used a microinjection strategy to demonstrate that cADPR evokes expression of two ABA-regulated genes. Although gene expression is activated by a range of treatments thought to increase $[Ca^{2+}]_c$, such as $InsP_3$ microinjection, the ABA response is blocked selectively by the cADPR antagonist 8-amino-cADPR but not by heparin. Conversely, Ca^{2+}-responsive *cab* gene expression is enhanced by neither cADPR nor $InsP_3$ microinjection. These results suggest considerable specificity in the cADPR-signaling pathway, implying either that cADPR uses a specified pool of Ca^{2+} or that the dynamic properties of the resultant $[Ca^{2+}]_c$ signal are critical for the given response. In a separate study (Leckie et al., 1998), ABA-induced stomatal closure was shown to be both evoked by cADPR and partially blocked by inhibitors of ADP–ribosyl cyclase.

TARGETS OF CALCIUM SIGNALS

The targets of Ca^{2+} signal transduction pathways can be divided into two categories: primary sensors and downstream substrates. Some downstream substrates represent the end point for the signaling pathway and include ion channels. In guard cells, anion channels involved in salt loss during stomatal closure are activated by $[Ca^{2+}]_c$ and by phosphorylation (Schroeder and Keller, 1992; Schmidt et al., 1995). In the case of Ca^{2+} activation of SV channels (discussed above), the effects of Ca^{2+} are mediated by calmodulin (Bethke and Jones, 1994; Schultz-Lessdorf and Hedrich, 1995).

Calcium-dependent protein kinases (CDPKs) are a unique family of kinases, distinguished by a C-terminal calmodulin-like regulatory domain with four calcium binding EF hands. They are likely to be important primary sensors of Ca^{2+} signals and were first identified in plants and protozoa (Harper et al., 1991; Suen and Choi, 1991; Zhao et al., 1993). CDPKs have been found in neither yeast nor animal systems, throughout the course of genome-sequencing projects (J.F. Harper and A.C. Harmon, unpublished results). In plants, on the other hand, divergent subfamilies have been identified with one or more degenerate EF hands (e.g., Lindzen and Choi, 1995; Patil et al., 1995). In Arabidopsis, >12 members of the CDPK superfamily have been identified (Hrabak et al., 1996).

The plethora of CDPK isoforms, along with the comparable paucity of potential gene homologs that encode calmodulin-dependent kinase or protein kinase C–like proteins, has led to the speculation that most calcium-regulated protein kinase activity in plants occurs through CDPK pathways (Satterlee and Sussman, 1998). Some CDPK isoforms appear to regulate distinct signaling pathways. For example, in a transient expression system, only two of eight CDPK isoforms induced expression from the ABA/stress regulated promoter HVA-1 (Sheen, 1996). Biochemical studies, moreover, show distinct substrate specificities for different isoforms (Lee et al., 1998). In addition, different isoforms are activated by different threshold concentrations of calcium, raising the possibility that specific isoforms will decode different Ca^{2+} signals. Although the subcellular localization of specific isoforms has not been completed, it is clear that there are multiple isoforms widely distributed in both soluble and membrane-associated locations (Roberts and Harmon, 1992).

The identification of potential substrates of CDPKs remains a major challenge. Based on in vitro assays, there is a growing list of potential CDPK targets, including chloride and potassium channels in guard cells (Pei et al., 1996; J. Li et al., 1998), the nodulin 26 channel in soybean root nodules (Lee et al., 1995), sucrose phosphate synthase (McMichael et al., 1995), EF1-α/phosphatidylinositol 4-kinase (Yang and Boss, 1994), the plasma membrane proton pump (Lino et al., 1998; Camoni et al., 1998), nitrate reductase (Bachmann et al., 1996), and bZIP-type transcripting factors (Meshi et al., 1998). However, the more difficult task is to demonstrate that a putative substrate is regulated by a particular CDPK pathway within the organism.

A role for phosphatases as primary sensors of Ca^{2+} signals also is emerging. Inward K^+ currents in guard cells, which are downregulated during stomatal closure, are inhibited by calcineurin (protein phosphatase 2B) but activated by immunosuppressants, which antagonize calcineurin activity (Luan et al., 1993). Similarly, SV channels are inactivated by calcineurin, and it has been proposed that if SV channels are involved in CICR, then the physiological significance of this inactivation might be as a negative feedback mechanism to prevent the cytosol from being flooded by the vacuolar Ca^{2+} pool (Allen and Sanders, 1995) or as a regulator of $[Ca^{2+}]_c$ oscillation frequency (McAinsh and Hetherington, 1998). A more defined role for calcineurin is hinted at by the finding (Liu and Zhu, 1998) that the *SOS3* gene of Arabidopsis encodes a homolog of the calcineurin B subunit from yeast (as well as neuronal calcium sensors from animals). *sos3* mutants are hypersensitive to Na^+, and the phenotype is partially suppressed by increasing extracellular Ca^{2+} concentration (Liu and Zhu, 1997). Because salt stress is known to elicit rapid changes in $[Ca^{2+}]_c$ (Knight et al., 1997), SOS3p might be the primary Ca^{2+} sensor in a salt stress signal transduction pathway. However, it should be noted that although the *sos3* phenotype is specific to salt stress, the changes in free Ca^{2+} are not and also are induced by water stress (Knight et al., 1997).

Other signaling components are likely to form primary sensors of Ca^{2+} signals. The finding that plants possess Ca^{2+}-activated phosphoinositide-specific phospholipase C (PI-PLC) activity (e.g., Huang et al., 1995) suggests a role for $InsP_3$ in CICR, because Ca^{2+} mobilization by $InsP_3$ would then trigger further $InsP_3$ production. The recent cloning of plant PI-PLCs has confirmed that they are both Ca^{2+} activated and closely related to the mammalian PLCδ

isoforms (Kopka et al., 1998). In accord with the notion that PI-PLCs participate in Ca^{2+} signaling, Ca^{2+} waves in pollen tubes and ABA-induced oscillations in $[Ca^{2+}]_c$ in guard cells are inhibited by PI-PLC inhibitors (Franklin-Tong et al., 1996; Staxen et al., 1999). In both of these systems, Ca^{2+} appears to participate in determining the dynamics of its own signal.

DECODING CALCIUM SIGNALS: THE PROBLEM OF SPECIFICITY

The involvement of Ca^{2+} in a wide variety of stimulus–response pathways in plant cells raises several questions concerning how different responses can be regulated by the same messenger. Moreover, a variety of graded Ca^{2+}-mediated responses to stimuli of varying strengths is a hallmark of plant responses to environmental changes (McAinsh et al., 1997; Malhó et al., 1998; McAinsh and Hetherington, 1998). Which features of the Ca^{2+} signaling machinery are relevant to understanding how a single messenger can carry multiple messages? Key considerations include amplitude, duration, frequency, and location of the Ca^{2+} signal, as well as the interactions with other cellular components and signaling pathways that reflect the "physiological address" of the given cell (McAinsh et al., 1997).

In animal cells, differential responses to Ca^{2+} signals varying in amplitude, duration, frequency, and localization have been clearly demonstrated. The amplitude and duration of a Ca^{2+} signal in B lymphocytes, for instance, induce differential activation of transcription factors (Dolmetsch et al., 1997). Changes in the frequency of electrically induced Ca^{2+} spikes, moreover, differentially affect nerve cell differentiation (Gu and Spitzer, 1995). Using a membrane-permeant form of caged $InsP_3$, W.H. Li et al. (1998) showed that the frequency of Ca^{2+} spikes can determine the degree of gene expression via a nuclear factor of T cells, lending support to the hypothesis that information can be encoded into the frequency of repetitive Ca^{2+} signals (Tang and Othmer, 1995; Berridge, 1997). Additionally, in mouse pituitary cells, elevations of nuclear $[Ca^{2+}]$ can elicit changes in gene expression via cAMP response elements, whereas changes in $[Ca^{2+}]_c$ affect gene expression by means of serum response elements (Hardingham et al., 1997). The recent demonstration that a human transcriptional repressor is directly modulated by Ca^{2+} indicates that Ca^{2+} can regulate gene expression without involving protein phosphorylation cascades (Carrión et al., 1999).

Calcium Transients and Downstream Responses

Evidence that different types of Ca^{2+} signal can differentially influence gene expression is less direct for plant cells. There are several examples of single Ca^{2+} transients in response to defined stimuli. These include rapid rises in $[Ca^{2+}]_c$ in response to touch or cold shock, involving both vacuolar and plasma membrane Ca^{2+} fluxes (Knight et al., 1991, 1996), and transient increases in $[Ca^{2+}]$ of varying duration in response to oxidative stress (Price et al., 1994), elicitors (Knight et al., 1991), systemin (Moyen et al., 1998), and hypo-osmotic treatments (Tazawa et al., 1995; Taylor et al., 1996; Takahashi et al., 1997).

The simplest hypothesis linking Ca^{2+} signals to graded downstream responses is that the magnitude of the Ca^{2+} transient reflects the magnitude of the stimulus and determines the degree of the response. Malhó et al. (1998) have collated Ca^{2+} transient data pertaining to 11 different stimuli and point out that with respect to lag phase, rise time, and duration, each $[Ca^{2+}]_c$ signal possesses distinct characteristics that could be viewed as an identifying fingerprint of the stimulus. For example, the lag phase in response to wind/touch is imperceptible, the rise time is <1 sec, and the duration of the transient only 15 sec, whereas hypo-osmotic shock in tobacco cells produces a lag phase of 30 sec, followed by a rise time of 60 sec and signal duration of 150 sec. The magnitude of hypo-osmotically induced Ca^{2+} transients has been shown to depend on the size of the hypo-osmotic shock (Takahashi et al., 1997; Brownlee et al., 1999). In tobacco, the hypo-osmotic Ca^{2+} transient showed biphasic behavior with a slow, small elevation preceding a larger, faster transient elevation. Both phases were dependent on the presence of extracellular Ca^{2+}, although they showed differential sensitivities to the vacuolar-type H^+-ATPase inhibitor bafilomycin and the protein kinase inhibitor K-252a, suggesting the involvement of different mechanisms for elevating Ca^{2+}. The data also suggested that Ca^{2+}-independent phosphorylation was required as part of the osmosensing mechanism. Recently, it was shown that the initial phase resulted from influx from the external medium, whereas the larger transient elevation reflected release of Ca^{2+} from intracellular stores (Cessna et al., 1998). In Arabidopsis, the magnitude of Ca^{2+} transients arising from drought or oxidative stress could be influenced by prior drought or oxidative treatments (Knight et al., 1997, 1998). These different Ca^{2+} signals correlated well with changes in osmotic stress–induced patterns of gene expression and the acquisition of osmotic stress tolerance.

However, a more complex relationship appears to exist between Ca^{2+} signals and the downstream responses in plants. Thus, osmotic (mannitol) and salt stress can each induce Ca^{2+} transients of similar magnitude and duration, with different levels of expression of the *p5cs* gene, which encodes for an enzyme involved in proline synthesis (Knight et al., 1997). These findings suggest that factors other than Ca^{2+} are involved in discriminating between the two response pathways. A further complication in interpreting Ca^{2+} signals in terms of a simple response has been demonstrated in soybean in response to UV and red light. Specifically, phytochrome control of the gene encoding chalcone synthase is

regulated positively by cGMP and negatively by Ca^{2+}/calmodulin, whereas the response to UV light was regulated positively by Ca^{2+}/calmodulin (Frohnmeyer et al., 1998). An explanation as to how Ca^{2+}/calmodulin could have opposing effects on the expression of the same gene in the same cell appears to lie in the observation that the responses to UV and red light were temporally separated.

Ca^{2+} Oscillations and Repetitive Spikes

Several plant cell types respond to a variety of physiological and nonphysiological stimuli with repetitive increases in $[Ca^{2+}]_c$ (oscillations) (Subbaiah et al., 1994; McAinsh et al., 1995; Ehrhardt et al., 1996; Bauer et al., 1997, 1998; Schonknecht et al., 1998). Whether these represent true Ca^{2+} signals or are the result of perturbations of normal Ca^{2+} homeostasis requires the demonstration of clear downstream responses dependent on the frequency, duration, or amplitude of the oscillations. Downstream responses to repetitive Ca^{2+} signals have been characterized in only a few cases in plants. Repetitive elevations in root hair $[Ca^{2+}]$ occur in response to Nod factors (Ehrhardt et al., 1996). Anoxic gene expression is preceded by repetitive Ca^{2+} spikes in Arabidopsis roots in anaerobic conditions (Subbaiah et al., 1994). Oscillations in the tip-focused Ca^{2+} gradient correlate with growth rate in pollen tubes (see below). In guard cells, Ca^{2+}-induced oscillations can be induced by treatments such as changes in external $[Ca^{2+}]$ and ABA (McAinsh et al., 1995; Staxen et al., 1996). The frequency and magnitude of Ca^{2+} oscillations have been shown to influence the stomatal aperture. Both the amplitude and dynamics of the Ca^{2+} transients, moreover, were dependent on the magnitude of the imposed external Ca^{2+} concentration. Transfer to high concentrations of Ca^{2+} (1.0 mM) produced large (up to 859 nM) elevations in $[Ca^{2+}]$, whereas lower external $[Ca^{2+}]$ gave rise to smaller symmetrical elevations up to 600 nM with a shorter period.

A mechanism for generating specificity from different patterns of Ca^{2+} oscillations has been proposed (Goldbeter et al., 1990; McAinsh and Hetherington, 1998) that involves the action of a Ca^{2+}-dependent phosphatase and a Ca^{2+}-independent protein kinase. Changes in the pattern of Ca^{2+} oscillations would be reflected in the degree of phosphorylation of target proteins leading to different responses.

Spatially Localized Ca^{2+} Signals

A further mechanism by which Ca^{2+} signals can exert specific effects, and one that is poorly understood in many aspects of plant signal transduction, involves the localization of the signal to a specific subcellular region. Repetitive Ca^{2+} signals elicited by Nod factors localized to the nuclear region of root hairs (Ehrhardt et al., 1996), thereby revealing spatial separation from the apical Ca^{2+} signals involved in root hair growth (see below). The use of aequorin, targeted separately to the chloroplast or cytosol, has revealed the presence of circadian oscillations of chloroplast and cytosolic Ca^{2+} after transfer from light to darkness (Johnson et al., 1995). In contrast, heat shock was shown to induce prolonged elevations of cytosolic, but not chloroplast, $[Ca^{2+}]$ in tobacco (Gong et al., 1998).

In animal cells, many Ca^{2+} signals have been shown to propagate as waves of elevated $[Ca^{2+}]$. These initiate at a defined location and propagate as release of Ca^{2+} from intracellular stores (Jaffe, 1995). In contrast, there are few examples of Ca^{2+} waves in plants. The hypo-osmotically induced Ca^{2+} transient in *Fucus* rhizoid cells propagates as a wave of elevated $[Ca^{2+}]$ that initiates at the extreme rhizoid apex and involves Ca^{2+} influx at apical regions and Ca^{2+} release from subapical intracellular stores (Taylor et al., 1996). The rate of propagation is at least 5 to 10 $\mu m\ sec^{-1}$, comparable to the propagation velocities of waves of intracellular Ca^{2+} release observed in a variety of animal systems (Jaffe, 1995). No similar physiologically induced Ca^{2+} waves have been characterized yet in other plant systems, although a wave of intracellular Ca^{2+} release can be induced in *Papaver* pollen tubes in response to photorelease of caged $InsP_3$. Preliminary evidence also exists for the occurrence of a centripetally directed Ca^{2+} wave in electrically stimulated guard cells (Grabov and Blatt, 1998). The activation voltage, threshold amplitude, and duration of this Ca^{2+} signal were affected by the presence of ABA. In animal cells, Ca^{2+} waves propagate via release of Ca^{2+} from the ER. The domination of many higher plant cells by a large central vacuole might be one reason why few Ca^{2+} waves have been observed. Accordingly, in both *Fucus* and pollen tubes, Ca^{2+} waves propagate through regions that contain ER but no large vacuole. Evidence for the involvement of different intracellular Ca^{2+} pools that may reflect spatially distinct Ca^{2+} signals comes from pharmacological studies of aequorin-transformed plants and the use of aequorin targeted to different cellular locations. As seen in Figure 2, for example, both vacuolar and plasma membrane Ca^{2+} fluxes are implicated in cold shock and drought Ca^{2+} signals. In contrast, a different Ca^{2+} pool appears to be involved in response to oxidative signals (Price et al., 1994). Figure 2 provides additional examples in which the source or location of a specific Ca^{2+} signal is known.

Gradients in Apically Growing Systems

Tip-focused Ca^{2+} gradients have been described in several types of pollen tubes (Rathore et al., 1991; Miller et al., 1992; Franklin-Tong et al., 1997), root hairs (Hermann and Felle, 1995; Felle and Hepler, 1997; Wymer et al., 1997), and algal rhizoids (Brownlee and Wood, 1986; Brownlee and Pulsford, 1988; Shaw and Quatrano, 1996; Taylor et al., 1996). Ca^{2+} at the growing apex can reach mi-

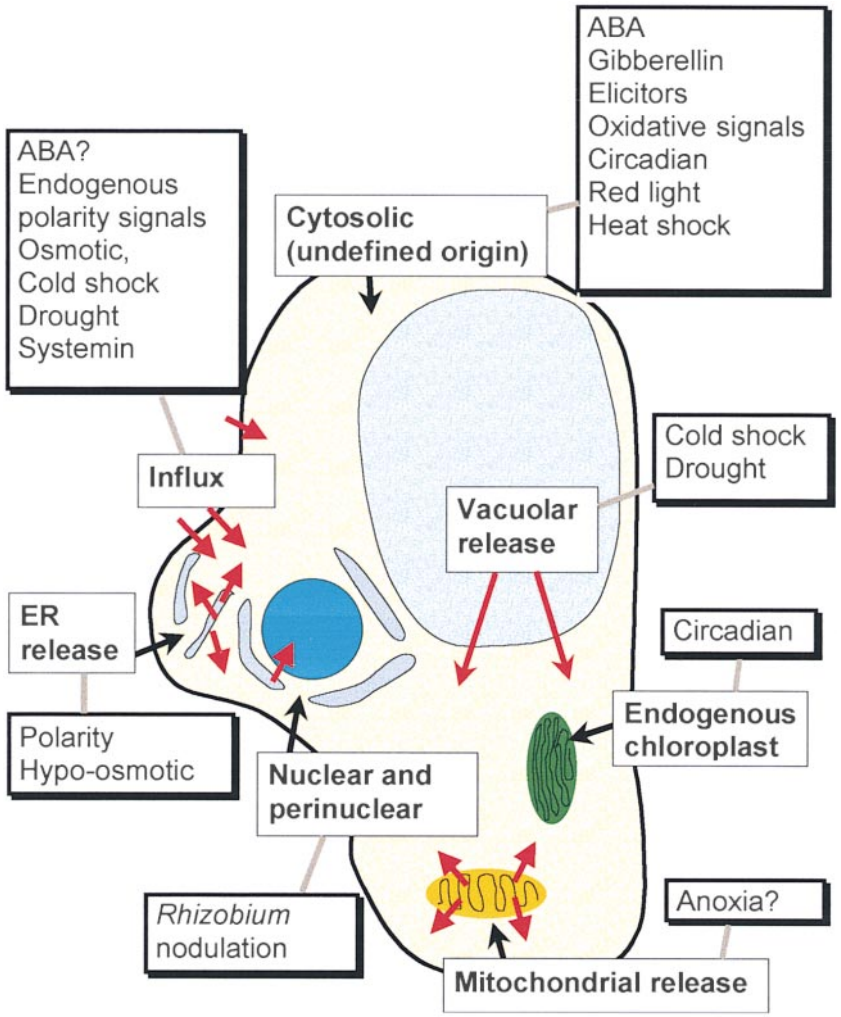

Figure 2. Schematic Representation of the Origins and Locations of Calcium Signals in Response to Specific Stimuli.

Signals originate from the external medium, ER, mitochondrion, chloroplast, and vacuole and can be localized or global, cytosolic, nuclear and perinuclear, or chloroplastic. Red arrows indicate the principal Ca^{2+} fluxes. See the text and Table 1 for discussion and references.

cromolar levels. The Ca^{2+} gradient in pollen tubes correlates closely with growth (Rathore et al., 1991; Miller et al., 1992). Accordingly, microinjection of the Ca^{2+} buffer dibromo-BAPTA into pollen tubes prevented growth and concomitantly abolished the Ca^{2+} gradient (Miller et al., 1992). *Agapanthus undulatus* pollen tubes could be oriented according to localized elevations of $[Ca^{2+}]$ induced by treatments such as photorelease of caged Ca^{2+} at the pollen tube apex (Malhó et al., 1994, 1996). Tip-localized Ca^{2+} pulses were shown to coincide with oscillations in growth rate (Messerli and Robinson, 1997). It is tempting to speculate that the magnitude and frequency of the Ca^{2+} oscillations provide information that regulates growth rate (Holdaway-Clarke et al., 1997). Oscillatory increases in growth rate were accompanied by in-phase increases in Ca^{2+} at the pollen tube apex. However, measurements of Ca^{2+} influx at the growing apex, by using an extracellular Ca^{2+}-selective microelectrode, showed that oscillations in Ca^{2+} influx occurred with the same frequency as, but out of phase with, growth and $[Ca^{2+}]_c$. This could reflect Ca^{2+} influx associated with the refilling of a store, such as the ER, although the ER of pollen tubes is not particularly concentrated at the apex (Lancelle and Hepler, 1992). Alternatively, the observed Ca^{2+} influx could reflect changes in Ca^{2+} binding to different cell wall components laid down during oscillatory growth pulses (Holdaway-Clarke et al., 1997). The significance of Ca^{2+} oscillations and pulsatile

pollen tube growth is not clear inasmuch as pollen tubes can grow normally without manifesting Ca^{2+} oscillations (Holdaway-Clarke et al., 1997; Messerli and Robinson, 1997).

Indirect evidence for the involvement of Ca^{2+}-permeable channels in facilitating Ca^{2+} influx at the pollen tube apex comes from the use of the Mn^{2+}-quench technique (Malhó et al., 1995), whereby entry of Mn^{2+} through putative Ca^{2+}-permeable channels quenches Ca^{2+} dye fluorescence. Unfortunately, attempts to apply the patch–clamp technique to pollen tubes for definitive demonstration of Ca^{2+}-permeable channels so far have been unsuccessful, because protoplasts from the pollen tube apex remain hypersecretory, thereby preventing Gigaohm seal formation between the membrane and the patch–clamp electrode. Vibrating Ca^{2+}-selective microelectrode studies also have shown preferential Ca^{2+} influx at the root hair apex (Hermann and Felle, 1995; Jones et al., 1995). Both the apically localized $[Ca^{2+}]$ elevation and root hair growth were prevented by verapamil, suggesting that Ca^{2+}-permeable channels might be involved in maintenance of the gradient (Wymer et al., 1997). Moreover, Mn^{2+}-quench experiments indicated that a significant component of the apical Ca^{2+} gradient was due to Ca^{2+} influx. The root hair system should be more tractable than is the pollen tube for patch–clamp studies of Ca^{2+} channels and signaling. Ca^{2+} influx in *Fucus* rhizoids, which might involve the activity of mechanosensitive Ca^{2+}-permeable channels, is involved in maintaining the apical Ca^{2+} gradient (Taylor et al., 1996). The apical Ca^{2+} gradient in *Fucus* correlates well with the presence of actin and calmodulin at the rhizoid apex (Henry et al., 1996; Love et al., 1997). Moreover, actin has been shown to localize to sites of attachment of the plasma membrane to the cell wall (Henry et al., 1996). Whereas earlier Ca^{2+} imaging experiments suggested that $[Ca^{2+}]$ becomes elevated at the rhizoid pole before germination of the rhizoid cell (Berger and Brownlee, 1993), it was not clear whether this occurred before or during fixation of the polar axis. A potential problem with imaging Ca^{2+} gradients during the early stages of polarization is the buffering of small localized Ca^{2+} elevations by the dye. Pu and Robinson (1998) used an indirect method to investigate early Ca^{2+} gradients in polarizing *Pelvetia* zygotes by ratio imaging of zygotes injected with low concentrations of the nonratiometric indicator Calcium Crimson against images of zygotes loaded with rhodamine B. $[Ca^{2+}]$ elevations could be detected within 1 hr of illumination with unilateral light, suggesting that the Ca^{2+} gradient could form before polar axis fixation.

The Ca^{2+} gradient in root hairs could be detected only during growth and not during root hair initiation (Wymer et al., 1997), implying a role for Ca^{2+} in growth after initiation. Although pollen tube orientation can be manipulated by treatments that cause asymmetric distribution of Ca^{2+}, root hairs tend to extend along a predefined polar axis. Perturbations in growth direction induced by asymmetric photorelease of a caged Ca^{2+} ionophore (A23187) induced only transient changes in the direction of growth, after which elongation resumed along the original axis (Bibikova and Gilroy, 1997). Cytoskeletal architecture might underlie the inherent polarity and morphogenesis of the extending root hair. In contrast to the pollen tube (Miller et al., 1996), actin localizes to the growing root hair apex (Jiang et al., 1997). However, it is also clear that root hairs tend to grow at fixed angles relative to the main root, indicating that some reference signal is used to monitor the position of the growing tip with respect to the main root axis.

FUTURE PROSPECTS

Research is still needed to identify and clone the different carriers and pumps involved in Ca^{2+} efflux from the cytosol. For example, there is biochemical evidence for a calmodulin-regulated Ca^{2+} pump in the plasma membrane (e.g., Askerlund, 1997; Hwang et al., 1997), but the corresponding gene has not been identified. An even larger gap in our knowledge concerns the absence of insight into the molecular identity of plant Ca^{2+}-permeable channels. The low abundance and (probable) large size of Ca^{2+} channels make them poor candidates for identification from cDNA libraries. However, candidate genes for both Ca^{2+} transporters and channels that are both expected and novel will likely emerge from genome-sequencing projects in the near future.

The greatest challenge ahead is to understand the isoform-specific functions of different transporters and channels. We have entered the era of "pathway quantification" (Koshland, 1998), where fundamental advances will require a detailed investigation into how Ca^{2+} fluxes are controlled in specific regions of the cell. Toward that goal, research is needed to determine the subcellular location, biochemical activity, and regulatory properties of each transporter and channel.

It is clear that genetics will be required to achieve an integrated understanding of each transporter in cellular and whole-plant physiology. Two important genetic strategies already have begun. The first is a "reverse genetic" approach to identify mutations in known Ca^{2+} transporters in Arabidopsis. This approach has yielded the first genetic mutation of a Ca^{2+} transporter in higher plants, identified as a T-DNA insertion into the *ACA3/ECA1* gene (Krysan et al., 1996). The second approach is to engineer transgenic plants with altered transporters designed to activate or block specific Ca^{2+} transport pathways. For example, the ACA2p pump has been engineered with a mutation that makes it constitutively active (Harper et al., 1998). Such genetic tools are providing us with new strategies to modify specific transport pathways in transgenic plants, without the inherent problems associated with modifying calcium transients with potentially nonspecific inhibitors. The use of fluorescent indicators and nontargeted aequorin to monitor global changes in $[Ca^{2+}]_c$ can provide only limited data on the spa-

tiotemporal characteristics of Ca^{2+} signals. Information about localized [Ca^{2+}] changes can come only from highly resolved confocal imaging studies or the use of targeted indicators. The application of new indicators, such as ratioable fluorescent cameleons (Miyawaki et al., 1997), is likely to provide much-needed information on Ca^{2+} signaling. This indicator offers exciting possibilities for combined studies of gene expression and interaction of Ca^{2+} signals with specific components of signaling pathways.

ACKNOWLEDGMENTS

We thank Dr. Helen V. Coombs for assistance with manuscript preparation.

REFERENCES

Alexandre, J., Lassalles, J.P., and Kado, R.T. (1990). Opening of Ca^{2+} channels in isolated red beet root vacuole membrane by inositol 1,4,5-trisphosphate. Nature **343,** 567–570.

Allen, G.J., and Sanders, D. (1994a). Two voltage-gated calcium release channels coreside in the vacuolar membrane of guard cells. Plant Cell **6,** 685–694.

Allen, G.J., and Sanders, D. (1994b). Osmotic stress enhances the competence of *Beta vulgaris* vacuoles to respond to inositol 1,4, 5-trisphosphate. Plant J. **6,** 687–695.

Allen, G.J., and Sanders, D. (1995). Protein phosphatase 2B (calcineurin) modulates the Ca^{2+} selective slow vacuolar ion channel of stomatal guard cells. Plant Cell **7,** 1473–1483.

Allen, G.J., and Sanders, D. (1996). Control of ionic currents in guard cell vacuoles by cytosolic and lumenal calcium. Plant J. **10,** 1055–1067.

Allen, G.J., and Sanders, D. (1997). Vacuolar ion channels of higher plants. Adv. Bot. Res. **25,** 218–252.

Allen, G.J., Muir, S.R., and Sanders, D. (1995). Release of Ca^{2+} from individual plant vacuoles by both $InsP_3$ and cyclic ADP-ribose. Science **268,** 735–737.

Askerlund, P. (1997). Calmodulin-stimulated Ca^{2+} ATPases in the vacuolar and plasma membranes in cauliflower. Plant Physiol. **114,** 999–1007.

Axelsen, K.B., and Palmgren, M.G. (1998). Evolution of substrate specificities in the P-type ATPase superfamily. J. Mol. Evol. **46,** 84–101.

Bachmann, M., Shiraishi, N., Campbell, W.H., Yoo, B.C., Harmon, A.C., and Huber, S.C. (1996). Identification of the major regulatory phosphorylation site as Ser-543 in spinach leaf nitrate reductase and its phosphorylation by a calcium-dependent protein kinase in vitro. Plant Cell **8,** 505–517.

Bauer, C.S., Plieth, C., Hansen, U.-P., Sattlemacher, B., Simonis, W., and Schonknecht, G. (1997). Repetitive Ca^{2+} spikes in a unicellular green alga. FEBS Lett. **405,** 390–393.

Bauer, C.S., Plieth, C., Bethmann, B., Popescu, O., Hansen, U.P., Simonis, W., and Schonknecht, G. (1998). Strontium-induced repetitive calcium spikes in a unicellular green alga. Plant Physiol. **117,** 545–557.

Berger, F., and Brownlee, C. (1993). Ratio confocal imaging of free cytoplasmic calcium gradients in polarising and polarised *Fucus* zygotes. Zygote **1,** 9–15.

Berridge, M.J. (1997). The AM and FM of calcium signaling. Nature **386,** 759–760.

Bethke, P.C., and Jones, R.L. (1994). Ca^{2+}-calmodulin modulates ion channel activity in storage protein vacuoles of barely aleurone cell. Plant Cell **6,** 277–285.

Bhogal, M.S., and Colyer, J. (1998). Depletion of Ca^{2+} from the sarcoplasmic reticulum of cardiac muscle prompts phosphorylation of phospholamban to stimulate store refilling. Proc. Natl. Acad. Sci. USA **95,** 1484–1489.

Bibikova, T.N., and Gilroy, S. (1997). Root hair growth in *Arabidopsis thaliana* is directed by calcium and an endogenous polarity. Planta **203,** 495–505.

Blackford, S., Rea, P.A., and Sanders, D. (1990). Voltage sensitivity of H^+/Ca^{2+} antiport in higher plant tonoplast suggests a role in vacuolar calcium accumulation. J. Biol. Chem. **265,** 9617–9620.

Bowler, C., Neuhaus, G., Yamagata, H., and Chua, N.-H. (1994). Cyclic-GMP and calcium mediate phytochrome transduction. Cell **77,** 73–81.

Brinley, F.J., Tiffert, T., and Scarpa, A. (1977). Mitochondria and other calcium buffers of squid axon studied *in situ.* J. Gen. Physiol. **70,** 355.

Brownlee, C., and Pulsford, A.L. (1988). Visualization of the cytoplasmic Ca^{2+} gradient in *Fucus serratus* rhizoids: Correlation with cell ultrastructure and polarity. J. Cell Sci. **91,** 249–256.

Brownlee, C., and Wood, J.W. (1986). A gradient of cytoplasmic free calcium in growing rhizoid cells of *Fucus serratus.* Nature **320,** 624–626.

Brownlee, C., Goddard, H., Hetherington, A.M., and Peake, L.-A. (1999). Specificity and integration of responses: Ca^{2+} as a signal in polarity and osmotic regulation. J. Exp. Bot., in press.

Bush, D.S. (1995). Calcium regulation in plant cells and its role in signaling. Annu. Rev. Plant Physiol. Plant Mol. Biol. **46,** 95–122.

Bush, D.S., and Jones, R.L. (1988). Cytoplasmic calcium and α-amylase secretion from barley aleurone protoplasts. Eur. J. Cell Biol. **46,** 466–469.

Camacho, P., and Lechleiter, J.D. (1993). Increased frequency of calcium waves in *Xenopus laevis* oocytes that express a calcium-ATPase. Science **260,** 226–229.

Camoni, L., Fullone, M.R., Marra, M., and Aducci, P. (1998). The plasma membrane H^+-ATPase from maize roots is phosphorylated in the C-terminal domain by a calcium-dependent protein kinase. Physiol. Plant. **104,** 549–555.

Cantu, A.M., Carpento, A., and Gambale, F. (1998). Cytoplasmic magnesium modulates slow vacuolar currents in plant vacuoles. Abstract from the 11th International Workshop on Plant Membrane Biology, Cambridge, UK.

Carafoli, E. (1997). Plasma membrane calcium pump: Structure, function and relationships. Basic Res. Cardiol. **92,** 59–61.

Carrión, A.M., Link, W.A., Ledo, F., Mellstrom, B., and Naranjo, J.R. (1999). DREAM is a Ca^{2+}-regulated transcriptional repressor. Nature **398,** 80–84.

Cessna, S.G., Chandra, S., and Low, P.S. (1998). Hypo-osmotic shock of tobacco cells stimulates Ca^{2+} fluxes deriving first from external and then internal Ca^{2+} stores. J. Biol. Chem. **273,** 27286–27291.

Chen, X., Chang, M., Wang, B., and Wu, B. (1997). Cloning of a Ca^{2+}-ATPase gene and the role of cytosolic Ca^{2+} in the gibberellin-dependent signaling pathway in aleurone cells. Plant J. **11,** 363–371.

Cho, M.H., and Spalding, E.P. (1996). An anion channel in *Arabidopsis* hypocotyls activated by blue light. Proc. Natl. Acad. Sci. USA **93,** 8134–8138.

Cho, M.H., Shears, S.B., and Boss, W.F. (1993). Changes in phosphatidylinositol metabolism in response to hyperosmotic stress in *Daucus carota* L. cells grown in suspension culture. Plant Physiol. **103,** 637–647.

Clemens, S., Antosiewicz, D.M., Ward, J.M., Schachtman, D.P., and Schroeder, J.I. (1998). The plant cDNA *LCT1* mediates the uptake of calcium and cadmium in yeast. Proc. Natl. Acad. Sci. USA **95,** 12043–12048.

Cubitt, A.B., Reddy, I., Lee, S., McNally, J.G., and Firtel, R.A. (1998). Coexpression of a constitutively active plasma membrane calcium pump with green fluorescence protein identifies roles for intracellular calcium in controlling cell sorting during morphogenesis in Dictyostelium. Dev. Biol. **196,** 77–94.

Cunningham, K.W., and Fink, G.R. (1996). Calcineurin inhibits VCX1-dependent H^+/Ca^{2+} exchange and induces Ca^{2+} ATPases in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **16,** 2226–2237.

Dolmetsch, R.E., Lewis, R.S., Goodnow, C.C., and Healy, J.I. (1997). Differential activation of transcription factors induced by Ca^{2+} response amplitude and duration. Nature **386,** 855–858.

Ehrhardt, D.W., Atkinson, E.M., and Long, S.R. (1992). Depolarization of alfalfa root hair membrane potential by *Rhizobium meliloti* Nod factors. Science **256,** 998–1000.

Ehrhardt, D.W., Wais, R., and Long, S.R. (1996). Calcium spiking in plant root hairs responding to *Rhizobium* nodulation signals. Cell **85,** 673–681.

Ermolayeva, E., Hohmeyer, H., Johannes, E., and Sanders, D. (1996). Calcium-dependent membrane depolarization activated by phytochrome in the moss *Physcomitrella patens.* Planta **199,** 352–358.

Ermolayeva, E., Sanders, D., and Johannes, E. (1997). Ionic mechanism and role of phytochrome-mediated membrane depolarization in caulonemal side branch initial formation in the moss *Physcomitrella patens.* Planta **201,** 109–118.

Evans, D.E., and Williams, L.E. (1998). P-type calcium ATPases in higher plants—Biochemical, molecular and functional properties. Biochim. Biophys. Acta **1376,** 1–25.

Felle, H. (1989). Ca^{2+}-selective microelectrodes and their application to plant cells and tissues. Plant Physiol. **91,** 1239–1242.

Felle, H.H., and Hepler, P.K. (1997). The cytosolic Ca^{2+} concentration gradient of *Sinapis alba* root hairs as revealed by Ca-selective microelectrode tests and fura-dextran ratio imaging. Plant Physiol. **114,** 39–45.

Ferrol, N., and Bennett, A.B. (1996). A single gene may encode differentially localized Ca^{2+}-ATPases in tomato. Plant Cell **8,** 1159–1169.

Franklin-Tong, V.E., Drobak, B.K., Allan, A.C., Watkins, P.A.C., and Trewavas, A.J. (1996). Growth of pollen tubes of *Papaver rhoeas* is regulated by a slow-moving calcium wave propagated by inositol 1,4,5-trisphosphate. Plant Cell **8,** 1305–1321.

Franklin-Tong, V.E., Hackett, G., and Hepler, P.K. (1997). Ratio-imaging of Ca^{2+} in the self-incompatibility response of *Papaver rhoeas.* Plant J. **12,** 1375–1386.

Frohnmeyer, H., Bowler, C., Zhu, J.-K., Yamagata, H., Schäfer, E., and Chua, N.-H. (1998). Different roles for calcium and calmodulin in phytochrome- and UV-regulated expression of chalcone synthase. Plant J. **13,** 763–772.

Gelli, A., and Blumwald, E. (1993). Calcium retrieval from vacuolar pools. Plant Physiol. **102**, 1139–1146.

Gill, D.L., Waldron, R.T., Rys-Sikora, K.E., Ufret-Vincenty, C.A., Graber, M.N., Favre, C.J., and Alfonso, A. (1996). Calcium pools, calcium entry, and cell growth. Biosci. Rep. **16,** 139–157.

Gilroy, S., Read, N.D., and Trewavas, A.J. (1990). Elevation of cytoplasmic calcium by caged calcium or caged inositol trisphosphate initiates stomatal closure. Nature **346,** 769–771.

Goldbeter, A., Dupont, G., and Berridge, M.J. (1990). Minimal model for signal-induced Ca^{2+} oscillations and for their frequency encoding through protein phosphorylation. Proc. Natl. Acad. Sci. USA **87,** 1461–1465.

Gong, M., Van de Luit, A.H., Knight, M.R., and Trewavas, A.J. (1998). Heat-shock-induced changes in intracellular Ca^{2+} level in tobacco seedlings in relation to thermotolerance. Plant Physiol. **116,** 429–437.

Gorman, A.L.F., and Thomas, M.V. (1980). Intracellular calcium accumulation during depolarization in a molluscan neurone. J. Physiol. **308,** 259–285.

Grabov, A., and Blatt, M.R. (1997). Parallel control of the inward rectifier K^+ channel by cytosolic free Ca^{2+} and pH in *Vicia* guard cells. Planta **201,** 84–95.

Grabov, A., and Blatt, M.R. (1998). Membrane voltage initiates Ca^{2+} waves and potentiates Ca^{2+} increases with abscisic acid in stomatal guard cells. Proc. Natl. Acad. Sci. USA **95,** 4778–4783.

Gu, X., and Spitzer, N.C. (1995). Distinct aspects of neuronal differentiation encoded by frequency of spontaneous Ca^{2+} transients. Nature **375,** 784–787.

Hardingham, G.E., Chawla, S., Johnson, C., and Balding, H. (1997). Distinct functions of nuclear and cytoplasmic calcium in the control of gene expression. Nature **385,** 260–264.

Harper, J.F., Sussman, M.R., Schaller, G.E., Putnam-Evans, C., Charbonneau, H., and Harmon, A.C. (1991). A calcium-dependent protein kinase with a regulatory domain similar to calmodulin. Science **252,** 951–954.

Harper, J.F., Hong, B., Hwang, I., Guo, H.Q., Stoddard, R., Huang, J.F., Palmgren, M.G., and Sze, H. (1998). A novel calmodulin-regulated Ca^{2+}-ATPase (ACA2) from *Arabidopsis* with an N-terminal autoinhibitory domain. J. Biol. Chem. **273,** 1099–1106.

Hedrich, R., and Neher, E. (1987). Cytoplasmic calcium regulates voltage dependent ion channels in plant vacuoles. Nature **329,** 833–836.

Henry, C.A., Jordan, J.R., and Kropf, D.L. (1996). Localized membrane-wall adhesion in *Pelvetia* zygotes. Protoplasma **190,** 39–52.

Hermann, A., and Felle, H.H. (1995). Tip growth in root hair cells of *Sinapis alba* L.: Significance of internal and external Ca^{2+} and H^+. New Phytol. **129,** 523–533.

Hirschi, K.D., Zhen, R.G., Cunningham, K.W., Rea, P.A., and Fink, G.R. (1996). CAX1, an H^+/Ca^{2+} antiporter from Arabidopsis. Proc. Natl. Acad. Sci. USA **93,** 8782–8786.

Holdaway-Clarke, T., Feijó, J.A., Hackett, G.R., Kunkel, J.G., and Hepler, P.K. (1997). Pollen tube growth and the intracellular cytosolic calcium gradient oscillates in phase while extracellular calcium influx is delayed. Plant Cell **9,** 1999–2010.

Holdaway-Clarke, T.L., Hackett, G.R., Kunkel, J.G., Hepler, P.K., and Feijó, J.A. (1998). Oscillations of cell expansion rate, cytoplasmic calcium, and calcium influx in the pollen tube. J. Gen. Physiol. **112,** 94.

Hong, B.A., Ichida, S., Wang, Y., Gens, J.S., Pickard, B.G., and Harper, J.F. (1999). Identification of a calmodulin-regulated calcium ATPase in the ER. Plant Physiol. **119,** 1165–1176.

Hrabak, E.M., Dickmann, L.J., Satterlee, J.S., and Sussman, M.R. (1996). Characterization of eight new members of the calmodulin-like domain protein kinase gene family from *Arabidopisis thaliana.* Plant Mol. Biol. **31,** 405–412.

Huang, C.-H., Tate, B.F., Crain, R.C., and Cote, G.G. (1995). Multiple phosphoinositide-specific phospholipases C in oat roots: Characterization and partial purification. Plant J. **8,** 257–267.

Huang, L., Berkelman, T., Franklin, A.E., and Hoffman, N.E. (1993). Characterization of a gene encoding a Ca^{2+}-ATPase-like protein in the plastid envelope. Proc. Natl. Acad. Sci. USA **90,** 10066–10070.

Hwang, I., Ratterman, D.M., and Sze, H. (1997). Distinction between endoplasmic reticulum–type and plasma membrane–type Ca^{2+} pumps. Plant Physiol. **113,** 535–548.

Jaffe, L.F. (1995). Calcium waves and development. CIBA Found. Symp. **188,** 4–12.

Jenkins, G.I. (1998). Signal transduction networks and the integration of responses to environmental stimuli. Adv. Bot. Res. **29,** 55–75.

Jiang, C.J., Weeds, A.G., and Hussey, P.J. (1997). The maize actin depolymerizing factor, ZmADF3, redistributes to the growing tip of elongating root hairs and can be induced to translocate into the nucleus with actin. Plant J. **12,** 1035–1043.

Johannes, E., Brosnan, J.M., and Sanders, D. (1992). Calcium channels in the vacuolar membrane of plants: Multiple pathways for intracellular Ca^{2+} mobilization. Philos. Trans. R. Soc. Lond. B **338,** 105–112.

Johnson, C., Knight, M.R., Kondo, T., Masson, P., Sedbrook, J., Haley, A., and Trewavas, A.J. (1995). Circadian oscillations in cytosolic and chloroplast free calcium in transgenic luminous plants. Science **269,** 1863–1866.

Jones, D.L., Shaff, J.E., and Kochian, L.V. (1995). Role of calcium and other ions in directing root hair tip growth in *Limnobium stoloniferum.* Inhibition of tip growth by aluminium. Planta **197,** 672–680.

Kasai, N., and Muto, S. (1990). Ca^{2+} pump and Ca^{2+}/H^+ antiporter in plasma membrane vesicles isolated by aqueous two-phase partitioning from maize leaves. J. Membr. Biol. **114,** 133–142.

Kim, H.Y., Cote, G.G., and Crain, R.C. (1996). Inositol 1,4,5-trisphosphate may mediate closure of K^+ channels by light and darkness in *Samanea saman* motor cells. Planta **198,** 279–287.

Klusener, B., Boheim, G., Liss, H., Engleberth, J., and Weiler, E.W. (1995). Gadolinium-sensitive, voltage-dependent calcium release channels in the endoplasmic reticulum of a higher plant mechanoreceptor organ. EMBO J. **14,** 2708–2714.

Knight, H., Trewavas, A.J., and Knight, M.R. (1996). Cold calcium signaling in Arabidopsis involves two cellular pools and a change in calcium signature after acclimation. Plant Cell **8,** 489–503.

Knight, H., Trewavas, A.J., and Knight, M.R. (1997). Calcium signaling in *Arabidopsis thaliana* responding to drought and salinity. Plant J. **12,** 1067–1078.

Knight, H., Brand, S., and Knight, M.R. (1998). A history of stress alters drought calcium signaling pathways in *Arabidopsis.* Plant J. **16,** 681–687.

Knight, M.R., Campbell, A.K., Smith, S.M., and Trewavas, A.J. (1991). Transgenic plant aequorin reports the effects of cold shock and elicitors on cytoplasmic calcium. Nature **352,** 524–526.

Kopka, J., Pical, C., Gray, J.E., and Muller-Rober, B. (1998). Molecular and enzymatic characterization of three phosphoinositide-specific phospholipase C isoforms from potato. Plant Physiol. **116,** 239–250.

Koshland, D.E., Jr. (1998). The era of pathway quantification. Science **280,** 852–853.

Kreimer, G., Melkonian, M., and Latzko, E. (1985). An electrogenic uniport mediates light-dependent Ca^{2+} influx into intact spinach chloroplasts. FEBS Lett. **180,** 253–258.

Krysan, P.J., Young, J.C., Tax, F., and Sussman, M.R. (1996). Identification of transferred DNA insertions within *Arabidopsis* genes involved in signal transduction and ion transport. Proc. Natl. Acad. Sci. USA **93,** 8145–8150.

Kuchitsu, K., Kikuyama, M., and Shibuya, N. (1993). N-acetylchito-oligosaccharides, biotic elicitors for phytoalexin production, induce transient membrane depolarization in suspension-cultured rice cells. Protoplasma **174,** 79–81.

Lancelle, S.A., and Hepler, P.K. (1992). Ultrastructure of freeze-substituted pollen tubes of *Lilium longiflorum.* Protoplasma **167,** 215–230.

Lechleiter, J.D., John, L.M., and Camacho, P. (1998). Ca^{2+} wave dispersion and spiral wave entrainment in *Xenopus laevis* oocytes overexpressing Ca^{2+} ATPases. Biophys. Chem. **72,** 123–129.

Leckie, C.P., McAinsh, M.R., Allen, G.J., Sanders, D., and Hetherington, A.M. (1998). Abscisic acid–induced stomatal closure mediated by cyclic ADP-ribose. Proc. Natl. Acad. Sci. USA **95,** 15837–15842.

Lee, J.W., Zhang, Y.X., Weaver, C.D., Shomer, N.H., Louis, C.F., and Roberts, D.M. (1995). Phosphorylation of nodulin-26 on serine-262 affects its voltage-sensitive channel activity in planar lipid bilayers. J. Biol. Chem. **270,** 27051–27057.

Lee, J.Y., Yoo, B.C., and Harmon, A.C. (1998). Kinetic and calcium-binding properties of three calcium-dependent protein kinase isoenzymes from soybean. Biochemistry **37,** 6801–6809.

Li, J., Lee, Y.R., and Assmann, S.M. (1998). Guard cells possess a calcium-dependent protein kinase that phosphorylates the KAT1 potassium channel. Plant Physiol. **116,** 785–795.

Li, W.H., Llopis, J., Whitney, M., Zlokarnik, G., and Tsien, R.Y. (1998). Cell-permeant caged $InsP_3$ ester shows that Ca^{2+} spike frequency can optimise gene expression. Nature **392,** 936–941.

Liang, F., Cunningham, K.W., Harper, J.F., and Sze, H. (1997). ECA1 complements yeast mutants defective in Ca^{2+} pumps and encodes an endoplasmic reticulum–type Ca^{2+}-ATPase in *Arabidopsis thaliana.* Proc. Natl. Acad. Sci. USA **94,** 8579–8584.

Lindzen, E., and Choi, J.H. (1995). A carrot cDNA encoding an atypical protein kinase homologous to plant calcium-dependent protein kinases. Plant Mol. Biol. **28,** 785–797.

Lino, B., Baizabal-Aguirre, V.M., and Gonzalez de la Vara, L.E. (1998). The plasma-membrane H^+-ATPase from beet root is inhibited by a calcium-dependent phosphorylation. Planta **204,** 352–359.

Liu, J., and Zhu, J.-K. (1997). An *Arabidopsis* mutant that requires increased calcium for potassium nutrition and salt tolerance. Proc. Natl. Acad. Sci. USA **94,** 14960–14964.

Liu, J., and Zhu, J.-K. (1998). A calcium sensor homolog required for plant salt tolerance. Science **280,** 1943–1945.

Love, J., Brownlee, C., and Trewavas, A.J. (1997). Ca^{2+} and calmodulin dynamics during photopolarization in *Fucus serratus* zygotes. Plant Physiol. **115,** 249–261.

Luan, S., Li, W., Rusnak, F., Assman, S.M., and Schreiber, S.L. (1993). Immunosuppressants implicate protein-phosphatase regulation of K^+ channels in guard cells. Proc. Natl. Acad. Sci. USA **91,** 2202–2206.

MacRobbie, E.A.C. (1997). Signalling in guard cells and regulation of ion channel activity. J. Exp. Bot. **48,** 515–528.

Malhó, R., and Trewavas, A.J. (1996). Localized apical increases of cytosolic free calcium control pollen tube orientation. Plant Cell **8,** 1935–1949.

Malhó, R., Read, N.D., Pais, M.S., and Trewavas, A.J. (1994). Role of cytoplasmic free calcium in the reorientation of pollen tube growth. Plant J. **5,** 331–341.

Malhó, R., Read, N.D., Trewavas, A.J., and Pais, M.S. (1995). Calcium channel activity during pollen tube growth and reorientation. Plant Cell **7,** 1173–1184.

Malhó, R., Moutinho, A., van der Lui, A., and Trewavas, A.J. (1998). Spatial characteristics of calcium signaling: The calcium wave as a basic unit in plant cell calcium signaling. Philos. Trans. R. Soc. Lond. B **353,** 1463–1473.

Malmstrom, S., Askerlund, P., and Palmgren, M.G. (1997). A calmodulin-stimulated Ca^{2+}-ATPase from plant vacuolar membranes with a putative regulatory domain at its N-terminus. FEBS Lett. **400,** 324–328.

McAinsh, M.R., and Hetherington, A.M. (1998). Encoding specificity in Ca^{2+} signaling systems. Trends Plant Sci. **3,** 32–36.

McAinsh, M.R., Brownlee, C., and Hetherington, A.M. (1990). Abscisic acid–induced elevation of guard cell cytosolic Ca^{2+} precedes stomatal closure. Nature **343,** 186–188.

McAinsh, M.R., Webb, A.A.R., Taylor, J.E., and Hetherington, A.M. (1995). Stimulus-induced oscillations in guard cell cytoplasmic free calcium. Plant Cell **7,** 1207–1219.

McAinsh, M.R., Brownlee, C., and Hetherington, A.M. (1997). Calcium ions as second messengers in guard cell signal transduction. Physiol. Plant. **100,** 16–29.

McMichael, R.W., Jr., Bachmann, M., and Huber, S.C. (1995). Spinach leaf sucrose-phosphate synthase and nitrate reductase are phosphorylated/inactivated by multiple protein kinases in vitro. Plant Physiol. **108,** 1077–1082.

McPhalen, C.A., Strynadka, N.C., and James, M.N.G. (1991). Calcium-binding sites in proteins: A structural perspective. Adv. Prot. Chem. **42,** 77–144.

Meshi, T., Moda, I., Minami, M., Okanami, M., and Iwabuchi, M. (1998). Conserved Ser residues in the basic region of the bZIP-type transcription factor HBP-la(17): Importance in DNA binding and possible targets for phosphorylation. Plant Mol. Biol. **36,** 125–136.

Messerli, M., and Robinson, K.R. (1997). Tip localized Ca^{2+} pulses are coincident with peak pulsatile growth rates in pollen tubes of *Lilium longiflorum.* J. Cell Sci. **110,** 1269–1278.

Miller, A.J., and Sanders, D. (1987). Depletion of cytosolic free calcium induced by photosynthesis. Nature **326,** 397–400.

Miller, A.J., Vogg, G., and Sanders, D. (1990). Cytosolic calcium homeostasis in fungi: Roles of plasma membrane transport and intracellular sequestration of calcium. Proc. Natl. Acad. Sci. USA **87,** 9348–9352.

Miller, D., Callaham, D.A., Gross, D.J., and Hepler, P.K. (1992). Free Ca^{2+} gradient in growing pollen tubes of *Lilium.* J. Cell Sci. **101,** 7–12.

Miller, D.D., Lancelle, S.A., and Hepler, P.K. (1996). Actin microfilaments do not form a dense meshwork in *Lilium longiflorum* pollen tube tips. Protoplasma **195,** 123–132.

Miyawaki, A., Llopis, J., Heim, R., McCaffery, J.M., Adams, J.A., Ikura, M., and Tsien, R.Y. (1997). Fluorescent indicators for Ca^{2+} based on green fluorescent proteins and calmodulin. Nature **388,** 882–887.

Mogami, H., Tepikin, A.V., and Petersen, O.H. (1998). Termination of cytosolic Ca^{2+} signals: Ca^{2+} reuptake into intracellular stores is regulated by the free Ca^{2+} concentration in the store lumen. EMBO J. **17,** 435–442.

Moyen, C., Hammond-Kosack, K.E., Jones, J., Knight, M.R., and Johannes, E. (1998). Systemin triggers an increase of cytoplasmic calcium in tomato mesophyll cells: Ca^{2+} mobilization from intra- and extracellular compartments. Plant Cell Environ. **21,** 1101–1111.

Muir, S.R., and Sanders, D. (1996). Pharmacology of Ca^{2+} release from red beet microsomes suggests the presence of ryanodine receptor homologs in higher plants. FEBS Lett. **395,** 39–42.

Muir, S.R., and Sanders, D. (1997). Inositol 1,4,5-trisphosphate–sensitive Ca^{2+} release across non-vacuolar membranes in cauliflower. Plant Physiol. **114,** 1511–1521.

Neuhaus, G., Bowler, C., Hiratsuka, K., Yamagata, H., and Chua, N.-H. (1997). Phytochrome-regulated repression of gene expression requires calcium and cGMP. EMBO J. **16,** 2554–2564.

Patil, S., Takezawa, D., and Poovaiah, B.W. (1995). Chimeric plant calcium/calmodulin-dependent protein-kinase gene with a neural visinin-like calcium-binding domain. Proc. Natl. Acad. Sci. USA **92,** 4897–4901.

Pei, Z.M., Ward, J.M., Harper, J.F., and Schroeder, J.I. (1996). A novel chloride channel in *Vicia faba* guard cell vacuoles activated by the serine/threonine kinase, CDPK. EMBO J. **15,** 6564–6574.

Penniston, J.T., and Enyedi, A. (1998). Modulation of the plasma membrane Ca^{2+} pump. J. Membr. Biol. **165,** 101–109.

Pineros, M., and Tester, M. (1995). Characterization of a voltage-dependent Ca^{2+}-selective channel from wheat roots. Planta **195,** 478–488.

Pineros, M., and Tester, M. (1997). Calcium channels in plant cells: Selectivity, regulation and pharmacology. J. Exp. Bot. **48,** 551–557.

Ping, Z., Yabe, I., and Muto, S. (1992). Voltage-dependent Ca^{2+} channels in the plasma membrane and vacuolar membrane of *Arabidopsis thaliana*. Biochim. Biophys. Acta **1112,** 287–290.

Pottosin, I.I., Tikhonova, L.I., Hedrich, R., and Schonknecht, G. (1997). Slowly activating vacuolar channels cannot mediate Ca^{2+}-induced Ca^{2+} release. Plant J. **12,** 1387–1398.

Price, A.H., Taylor, A., Ripley, S.J., Griffiths, A., Trewavas, A.J., and Knight, M.R. (1994). Oxidative signals in tobacco increase cytosolic calcium. Plant Cell **6,** 1301–1310.

Pu, R., and Robinson, K.R. (1998). Cytoplasmic calcium gradients and calmodulin in the early development of the fucoid alga *Pelvetia compressa.* J. Cell Sci. **111,** 3197–3207.

Rathore, K.S., Cork, R.J., and Robinson, K.R. (1991). A cytoplasmic gradient of Ca^{2+} is correlated with the growth of lily pollen tubes. Dev. Biol. **148,** 612–619.

Read, N.D., Shacklock, P.S., Knight, M.R., and Trewavas, A.J. (1993). Imaging calcium dynamics in living plant cells and tissues. Cell Biol. Int. Rep. **17,** 111–125.

Roberts, D.M., and Harmon, A.C. (1992). Calcium-modulated proteins: Targets of intracellular calcium signals in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **43,** 375–414.

Rudolph, H.K., Antebi, A., Fink, G.R., Buckley, C.M., Dorman, T.E., LeVitre, J., Davidow, L.S., Mao, J.I., and Moir, D.T. (1989). The yeast secretory pathway is perturbed by mutations in PMR1, a member of a Ca^{2+} ATPase family. Cell **58,** 133–145.

Santella, L., and Carafoli, E. (1997). Calcium signaling in the cell nucleus. FASEB J. **11,** 1091–1109.

Satterlee, J.S., and Sussman, M.R. (1998). Unusual membrane-associated protein kinases in higher plants. J. Membr. Biol. **164,** 205–213.

Schachtman, D.P., Kumar, R., Schroeder, J.I., and Marsh, E.L. (1997). Molecular and functional characterization of a novel low-affinity cation transporter (LCT1) in higher plants. Proc. Natl. Acad. Sci. USA **94,** 11079–11084.

Schmidt, C., Schelle, T., Liao, Y.J., and Schroeder, J.I. (1995). Strong regulation of slow anion channels and abscisic acid signaling in guard cells by phosphorylation and dephosphorylation events. Proc. Natl. Acad. Sci. USA **92,** 9535–9539.

Schonknecht, G., Bauer, C.S., and Simonis, W. (1998). Light-dependent signal transduction and transient changes in cytosolic Ca^{2+} in a unicellular green alga. J. Exp. Bot. **49,** 1–11.

Schroeder, J.I., and Hagiwara, S. (1990). Repetitive increases in cytosolic Ca^{2+} of guard cells by abscisic acid activation of non-selective Ca^{2+} permeable channels. Proc. Natl. Acad. Sci. USA **87,** 9305–9309.

Schroeder, J.I., and Keller, B.U. (1992). Two types of anion channel currents in guard cells with distinct voltage regulation. Proc. Natl. Acad. Sci. USA **89,** 5025–5029.

Schultz-Lessdorf, B., and Hedrich, R. (1995). Protons and calcium modulate SV-type channels in the vacuolar–lysosomal compartment–channel interaction with calmodulin inhibitors. Planta **197,** 655–671.

Schumaker, K.S., and Sze, H. (1986). Calcium transport into the vacuole of oat roots. Characterization of H^+/Ca^{2+} exchange activity. J. Biol. Chem. **261,** 12172–12178.

Schumaker, K.S., and Sze, H. (1987). Inositol 1,4,5-trisphosphate releases Ca^{2+} from vacuolar membrane vesicles of oat roots. J. Biol. Chem. **262,** 3944–3946.

Shacklock, P.S., Read, N.D., and Trewavas, A.J. (1992). Cytosolic free calcium mediates red light induced photomorphogenesis. Nature **358,** 153–155.

Shaw, S.L., and Quatrano, R.S. (1996). The role of targeted secretion in the establishment of cell polarity and the orientation of the division plane in *Fucus* zygotes. Development **122,** 2623–2630.

Sheen, J. (1996). Ca^{2+}-dependent protein kinases and stress signal transduction in plants. Science **274,** 1900–1902.

Spalding, E.P., and Cosgrove, D.J. (1989). Large plasma-membrane depolarization precedes rapid blue-light-induced growth inhibition in cucumber. Planta **178,** 407–410.

Srivastava, A., Pines, M., and Jacoby, B. (1989). Enhanced potassium uptake and phosphatidylinositol-phosphate turnover by hypertonic mannitol shock. Physiol. Plant. **77,** 320–325.

Staxen, I., Montgomery, L., Hetherington, A.M., and McAinsh, M.R. (1996). Do oscillations in cytoplasmic free calcium encode the ABA signal in stomatal guard cells? Plant Physiol. **111** (suppl.), 151.

Staxen, R., Pical, C., Montgomery, L.T., Gray, J.E., Hetherington, A.M., and McAinsh, M.R. (1999). Abscisic acid induces oscillations in guard-cell cytosolic free calcium that involve phosphoinositide-specific phospholipase C. Proc. Natl. Acad. Sci. USA **96,** 1779–1784.

Stokes, D.L. (1997). Keeping calcium in its place: Ca^{2+}-ATPase and phospholamban. Curr. Opin. Struct. Biol. **7,** 550–556.

Subbaiah, C.C., Bush, D.S., and Sachs, M.M. (1994). Elevation of cytosolic calcium precedes anoxic gene expression in maize suspension cultured cells. Plant Cell **6,** 1747–1762.

Suen, K.L., and Choi, J.H. (1991). Isolation and sequence analysis of a cDNA clone for a carrot calcium-dependent protein kinase: Homology to calcium/calmodulin-dependent protein kinases and to calmodulin. Plant Mol. Biol. **17,** 581–590.

Takahashi, K., Isobe, M., Knight, M.R., Trewavas, A.J., and Muto, S. (1997). Hypo-osmotic shock induces increases in cytosolic Ca^{2+} in tobacco suspension culture cells. Plant Physiol. **113,** 587–594.

Tang, Y.H., and Othmer, H.G. (1995). Frequency encoding in excitable systems with applications to calcium oscillations. Proc. Natl. Acad. Sci. USA **92,** 7869–7873.

Taylor, A.R., Manison, N.F.H., Fernandez, C., Wood, J.W., and Brownlee, C. (1996). Spatial organization of calcium signaling involved in cell volume control in the *Fucus* rhizoid. Plant Cell **8,** 2015–2031.

Taylor, C.W., and Traynor, D. (1995). Calcium and inositol trisphosphate receptors. J. Membr. Biol. **145,** 109–118.

Tazawa, M., Shimada, K., and Kikuyama, M. (1995). Cytoplasmic hydration triggers a transient increase in cytoplasmic Ca^{2+} concentration in *Nitella flexilis.* Plant Cell Physiol. **36,** 335–340.

Thiel, G., and Blatt, M.R. (1994). Phosphatase antagonist okadaic acid inhibits steady-state K^+ currents in guard cells of *Vicia faba.* Plant J. **5,** 727–733.

Thiel, G., MacRobbie, E.A.C., and Blatt, M.R. (1992). Membrane transport in stomatal guard cells: The importance of voltage control. J. Membr. Biol. **126,** 1–18.

Thion, L., Mazars, C., Thuleau, P., Graziana, A., Rossignol, M., Moreau, M., and Ranjeva, R. (1996). Activation of plasma membrane voltage-dependent calcium-permeable channels by disruption of microtubules in carrot cells. FEBS Lett. **340,** 45–50.

Thomas, M.V. (1982). Techniques in Calcium Research. (London: Academic Press).

Thuleau, P., Graziana, A., Ranjeva, R., and Schroeder, J.I. (1993). Solubilized proteins from carrot (*Daucus carota* L.) membranes bind calcium channel blockers and form calcium-permeable ion-channels. Proc. Natl. Acad. Sci. USA **90,** 765–769.

Trewavas, A.J., and Mahlo, R. (1998). Ca^{2+} signaling in plant cells: The big network! Curr. Opin. Plant Biol. **1,** 428–433.

Ward, J.M., and Schroeder, J.I. (1994). Calcium activated K^+ channels and calcium-induced calcium release by slow vacuolar ion channels in guard cell vacuoles implicated in the control of stomatal closure. Plant Cell **6,** 669–683.

Ward, J.M., Pei, Z.-M., and Schroeder, J.I. (1995). Roles of ion channels in initiation of signal transduction in higher plants. Plant Cell **7,** 833–844.

White, P.J. (1993). Characterization of a high-conductance, voltage-dependent cation channel from the plasma membrane of rye roots in planar lipid bilayers. Planta **191,** 541–551.

White, P.J. (1994). Characterization of a voltage-dependent cation channel from the plasma membrane of rye (*Secale cereale* L.) roots in planar lipid bilayers. Planta **193,** 186–193.

White, P.J. (1998). Calcium channels in the plasma membrane of root cells. Ann. Bot. **81,** 173–183.

Wimmers, L.E., Ewing, N.N., and Bennett, A.B. (1992). Higher plant Ca^{2+}-ATPase: Primary structure and regulation of mRNA abundance by salt. Proc. Natl. Acad. Sci. USA **89,** 9205–9209.

Wu, Y., Kuzma, J., Marechal, E., Graeff, R., Lee, H.C., Foster, R., and Chua, N.-H. (1997). Abscisic acid signaling through cyclic ADP-ribose in plants. Science **278,** 2126–2130.

Wymer, C.L., Bibikova, T.N., and Gilroy, S. (1997). Cytoplasmic free calcium distributions during the development of root hairs of *Arabidopsis thaliana.* Plant J. **12,** 427–439.

Yang, W., and Boss, W. (1994). Regulation of phosphatidylinositol 4-kinase by the protein activator PIK-A49. Activation requires phosphorylation of PIK-A49. J. Biol. Chem. **269,** 3852–3857.

Zhao, Y., Kappes, B., and Franklin, R.M. (1993). Gene structure and expression of an unusual protein kinase from *Plasmodium falciparum* homologous at its carboxyl terminus with the EF hand calcium-binding proteins. J. Biol. Chem. **268,** 4347–4354.

Zimmermann, S., Nürnberger, T., Frachisse, J.-M., Wirtz, W., Guern, J., Hedrich, R., and Scheel, D. (1997). Receptor-mediated activation of a plant Ca^{2+}-permeable ion channel involved in pathogen defense. Proc. Natl. Acad. Sci. USA **94,** 2751–2755.