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Update on Signaling

Regulation of Cytosolic Calcium in Plants[1]

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Cytosolic Ca^{2+} has been likened to a kind of cellular fire (Rasmussen et al., 1990). The metaphor is apt in an obvious way because it suggests that transient or localized elevations in cytosolic Ca^{2+}—like a fire contained in a hearth—may be useful to plant cells for performing certain tasks, whereas uncontrolled or prolonged elevations in cytosolic Ca^{2+}—like a fire loose on the roof—are destructive of normal cellular processes. The metaphor is also apt in a less obvious way because it suggests that large elevations in cytosolic Ca^{2+}, like the combustion of roofs, are energetically favored, spontaneous events that the cell must constantly work to prevent.

There is now considerable evidence to support both of these views of cytosolic Ca^{2+} in plants. Measurements of cellular Ca^{2+} have shown that plant cells, like those of all other organisms, avoid potentially toxic effects of Ca^{2+} by maintaining levels in the cytosol and nucleus that are 3 to 4 orders of magnitude lower than the levels in other cellular compartments (Felle, 1988b; Bush et al., 1989; Gilroy et al., 1990). There is, therefore, a large gradient for calcium directed into the cytosol across most cellular membranes, a gradient that is potentially useful for amplifying signals that impinge on the cell by transducing them into a regulated increase in cytosolic Ca^{2+}.

At the same time that Ca^{2+} levels and gradients have been measured in plant cells, a number of cellular processes have been identified that depend on changes in cytosolic Ca^{2+} for their activation (Johannes et al., 1991). This has led plant scientists to adopt the theory that Ca^{2+} acts as an intracellular messenger, conveying information about the nature of a particular stimulus or stress impinging on the cell to target proteins that guide the cellular response. Establishing the validity of this theory for plant cells has been the aim of much of the work on Ca^{2+} regulation in recent years. The purpose of this review is to consider what is currently known about Ca^{2+} regulation in plant cells with the aim of understanding, metaphorically speaking, why plant cells play with fire and how they avoid getting burned.

According to the intracellular messenger theory, Ca^{2+} and a small group of other compounds play a pivotal role in signal transduction by communicating signal perception at a localized receptor to other parts of the cell, where the effectors of the cellular response are located (Fig. 1). In animal cells, Ca^{2+}-mediated signal transduction pathways have been shown to be enormously varied and complex in the temporal and spatial organization of the signal, as well as in the proteins that produce and intercept it (Berridge, 1993). Nevertheless, it is possible to sketch the essential features of the pathway using components that are known to exist in various plant cells. The cascade begins with receptor/stimulus interaction that leads, perhaps through a number of alternative steps, to regulation of Ca^{2+} transporters and a change, usually an increase, in intracellular Ca^{2+}. The increased Ca^{2+} level promotes the formation of Ca^{2+} complexes with target proteins that, in turn, regulate the activity of the effector proteins that determine cell response. Although all of the components of a Ca^{2+}-based transduction pathway have not been shown to exist in any one cell or for any one stimulus in plants, the growing number of identified target proteins for Ca^{2+} include several that are likely to have pleiotropic effects on cell function. Calmodulin, Ca^{2+}-dependent protein kinases (Harper et al., 1991), phosphatases (Kauss and Jeblick, 1991), and Ca^{2+}-stimulated phospholipases (Shorrosh and Dixon, 1991) commonly exist in plants, which indicates that changes in Ca^{2+}, whether they occur early or late in the transduction process, are likely to have profound effects on cellular function. As a first step in identifying Ca^{2+}-based transduction pathways in plants, there has been an intense effort to determine the effect of various stimuli on cytosolic Ca^{2+} levels.

MANY STIMULI ALTER CYTOSOLIC Ca^{2+}

Measurements of cytosolic Ca^{2+} have now been made using a variety of techniques, most notably, fluorescent dyes, Ca^{2+}-sensitive microelectrodes, and photoproteins (for a review of these methods, see Read et al., 1992). All of these approaches have yielded roughly similar estimates of 30 to 200 nM cytosolic Ca^{2+} in unstimulated, i.e. resting, cells. Rapid changes in cytosolic Ca^{2+} from these basal levels occur in response to endogenous stimuli, such as plant hormones, as well as to exogenous stimuli, such as light, heat, salt, elicitors, and touch (Table I). The time course of change in cytosolic Ca^{2+} induced by different stimuli in different cells is, as expected, quite variable, but at least three basic patterns can be discerned: transient and apparently large increases that occur in response to mechanical stimulation, hypotonic shock, and elicitors; steady-state, apparently modest increases/decreases that occur in response to GA, cytokinins, and light; and oscillatory changes with regular or irregular periods that occur in response to auxin and ABA. Two

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Abbreviations: CICR, calcium-induced calcium release; IP_3, inositol-1,4,5-trisphosphate; PM, plasma membrane; TN, tonoplast.

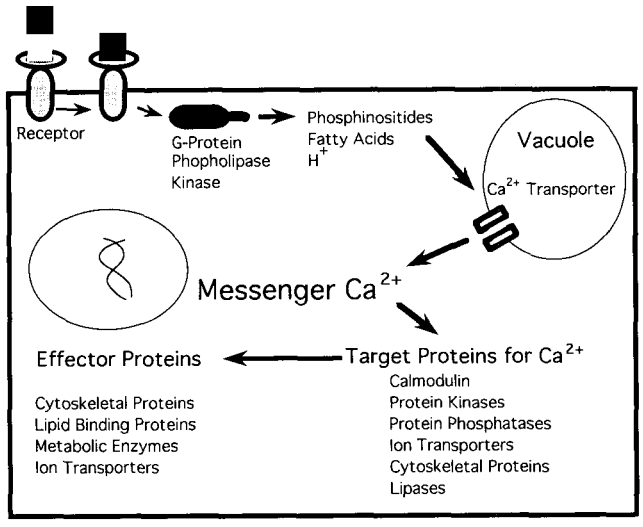

Figure 1. An outline for a hypothetical Ca^{2+}-based messenger system using components that are known to exist in plant cells. Interaction between receptor and stimulus leads to the activation of an associated enzyme (G-protein, phospholipase, or kinase) that produces diffusible messengers (phosphoinositides, fatty acids, H^+) that activate a Ca^{2+} channel on the vacuolar membrane. This messenger Ca^{2+} binds to target proteins, which can then alter the activity of the effector proteins that are responsible for the final cell response. This outline represents only one of many possible ways a Ca^{2+}-based messenger system could be organized in plant cells.

questions of fundamental importance emerge from the observation that stimuli induce Ca^{2+} changes: What do these changes mean to cell function and what is the mechanism by which they occur?

Changes in cytosolic Ca^{2+} that are induced by most of these stimuli cannot yet be placed with certainty in transduction pathways. To do so requires identification of the specific target proteins that are regulated in vivo by Ca^{2+}, the action of which is necessary for cellular response. This has been achieved in only a few cases, as exemplified by Ca^{2+}-regulated salt loss from algae and guard cells (Okazaki et al., 1987; Schroeder and Hagiwara, 1989). In most cases, insight into the position of Ca^{2+} in the transduction chain is provided by the magnitude and timing of the Ca^{2+} change and measurements of cellular responses when Ca^{2+} changes are prevented. It is interesting that among the stimuli that induce sustained responses, only the large, transient changes induced by touch or elicitors closely resemble the common spike and plateau signal response of stimulated mammalian cells (Tsien and Tsien, 1990).

Because the response to touch and elicitors can be induced by other conditions that are likely to elevate cytosolic Ca^{2+} and can be inhibited by treatments that prevent a rise in cytosolic Ca^{2+} (Kauss and Jeblick, 1991; Braam, 1992), it seems likely that the change in Ca^{2+} functions as a signal for these stimuli. It is not yet clear, however, whether the smaller, steady-state changes or oscillations induced by auxins, cytokinins, GA, ABA, and other stimuli serve a signaling function. It has been argued that small and relatively slow changes induced by auxin (Felle, 1988a) or GA (Gilroy and Jones, 1992) are so unlike typical stimulus-induced changes in mammalian cells that they may not serve a signaling function (Felle, 1988a). However, many sustained responses in mammalian cells require small (less than several hundred nanomolar), prolonged elevations in Ca^{2+} (Rasmussen et al., 1990). It is also important to note that in most cases for plant cells, the spatial organization of the Ca^{2+} change is not known. Highly localized or average measurements of cytosolic Ca^{2+}, such as are produced by electrodes or photometry, might not detect larger changes that occur in specific regions of the cell.

To understand what information is contained in a Ca^{2+} signal, it is important to obtain a detailed spatial and temporal map of these changes. There is some evidence that information is encoded simply by the magnitude of the change and that cells may be able to distinguish between changes in cytosolic Ca^{2+} above a threshold value. For example, guard cells of *Vicia faba* respond to pulses of Ca^{2+} only when they are greater than 600 nM (Gilroy et al., 1990). In addition to magnitude, other features such as the duration, speed, and spatial organization of the Ca^{2+} signal may contain information. The need for cells to distinguish between different patterns of change in cytosolic Ca^{2+} is underscored by studies of Ca^{2+} changes in a single-cell type in response to multiple kinds of stimuli. Cytosolic-Ca^{2+} changes induced by touch, temperature shifts, and elicitors in tomato differed slightly in magnitude and duration (Knight et al., 1991). The mechanism by which plant cells can distinguish between similarly sized Ca^{2+} signals is not known. One possibility is that changes in Ca^{2+} are accompanied by changes in other messengers whose nature is determined by the stimulus. Bifurcations of the transduction pathway, which produce more than one kind of signal, occur as a result of phospholipase activities or kinase activities, and it is possible that a combination of signals is required for a particular cellular response.

Another way in which plant cells could distinguish between different Ca^{2+} signals is by differences in the spatial organization of the change in cytosolic Ca^{2+}. The relatively high concentrations of Ca^{2+} inside the ER (Bush et al., 1989) and the vacuole (DuPont et al., 1990) and in the extracellular space enable each of these compartments to serve as a source of stimulus-induced increase in cytosolic Ca^{2+}. Calcium influx localized at particular membranes, together with low diffusion rates for Ca^{2+} in the cytosol (Speksnijder et al., 1989), make localized gradients in Ca^{2+} possible. The existence of such gradients in tip-growing cells have long been indicated and, recently, have been shown to be essential for tip growth in pollen tubes (Miller et al., 1992). A similar gradient has been reported in response to GA in barley aleurone protoplasts (Gilroy and Jones, 1992). Localized changes in cytosolic Ca^{2+} may be particularly important for sustained responses such as those induced by GA or tip growth because prolonged elevations in Ca^{2+} throughout the cell may impair normal cellular processes. Although in most cases the spatial organization of the Ca^{2+} change is not known, it is clear that the source of Ca^{2+} differs between stimuli, indicating the possibility of their different spatial organization.

A MULTIPLICITY OF Ca^{2+} TRANSPORTERS REGULATE CYTOSOLIC Ca^{2+}

The ability of cells to maintain low cytosolic Ca^{2+} or to respond to stimuli with changes in cytosolic Ca^{2+} is due to

Table I. *Summary of stimulus-induced changes in cytosolic Ca^{2+} in plant cells*

A partial list of the effect of various stimuli on changes in cytosolic Ca^{2+} was compiled and categorized by direction and length of response (Ca^{2+} Response) and whether the source of the Ca^{2+} change was derived from the extracellular solution or intracellular stores (Ca^{2+} Source). A question mark denotes uncertainty in the Ca^{2+} source.

Stimulus	Ca^{2+} Response	Ca^{2+} Source	Reference
Elicitor	Transient increase	Extracellular (?)	Kauss and Jeblick, 1991; Knight et al., 1991
Mechanical	Transient increase	?	Knight et al., 1991; Braam, 1992
Temperature	Transient increase	?	Knight et al., 1991
Light			
Red	Increase	Intracellular	Chae et al., 1990
UV	Increase	Intracellular	Russ et al., 1991
Hormones			
ABA	Transient increase	Intracellular (?)	Gilroy et al., 1990
	Oscillations	Extracellular	Schroeder and Hagiwara, 1990
Auxin	Oscillations	?	Felle, 1988a
GA	Steady-state increase	Extracellular	Bush and Jones, 1987; Gilroy and Jones, 1992
Cytokinin	Steady-state increase	Extracellular	Hahm and Saunders, 1991

the combined activities of many proteins that catalyze the transport of Ca^{2+} across cellular membranes. Although only a few of these Ca^{2+} transport proteins have been purified or sequenced, the activities of a large number have now been characterized. These characterizations have provided considerable insight into the complexity of cytosolic Ca^{2+} regulation. The rapid progress in the characterization of Ca^{2+} transporters has recently been described in several excellent reviews (Evans et al., 1991; Johannes et al., 1991; Schroeder and Thuleau, 1991; Maathuis and Sanders, 1992).

The electrochemical gradients for Ca^{2+} across the PM, ER, TN, and other cellular membranes are large (200–300 mV) and are rarely reversed (see, for example, Miller et al., 1990). Efflux of Ca^{2+} from the cytoplasm into the extracellular solution or into intracellular organelles requires energy, and it is catalyzed by a diverse set of membrane proteins that utilize ATP or a proton motive force to drive Ca^{2+} movement. As a result of active efflux transporters, influx of Ca^{2+} into the cytoplasm is energetically downhill and is mediated by Ca^{2+} channels, pores that can be opened or closed. Thus, in most cellular compartments the pathway for Ca^{2+} entry into the cytosol is separate and can be regulated differently from the pathway for Ca^{2+} exit.

This arrangement provides the cell with great flexibility in both spatial and temporal control over Ca^{2+} flux into the cytosol (Fig. 2). This flexibility is clearly revealed by examining the conventional view that increases in Ca^{2+} must be mediated by altered channel activity (Fig. 1). This view, for which there is abundant evidence in mammalian cells, suggests that changes in cytosolic Ca^{2+} are due to regulation of highly selective Ca^{2+} channels that act in opposition to a relatively constant efflux driven by pumps and carriers. However, an alternative route for altering cytosolic Ca^{2+} would be to regulate efflux activities against a relatively constant background influx. This alternative route could be more important for cells where Ca^{2+} influx is mediated by nonselective Ca^{2+} channels, such as may be the case for the PM of plant cells (see below). Certainly the ability of reduced efflux transporter activity to raise cytosolic Ca^{2+} is demonstrated in mammalian cells by the plant-derived tumor promoter thapsigargan. This compound inhibits the Ca^{2+}-ATPases on the ER and produces rapid changes in cytosolic Ca^{2+} in a number of mammalian cells (Thastrup et al., 1990). Thus, regulation of efflux or influx pathways may lead to changes in cytosolic Ca^{2+}.

The activities of Ca^{2+} transporters that catalyze efflux from the cytoplasm have been characterized in membrane vesicles from a variety of organisms and cell types. Three types of transporters have been found that differ in their intracellular location and in the source of energy used to drive transport (see Evans et al., 1991, and refs. therein). These are P-type ATPases found on the ER, PM, and, possibly, chloroplast membranes (Berkelman et al., 1992); Ca^{2+}/nH^{+} antiporters found principally on the TN; and uniporters/channels associated with chloroplast and possibly mitochondrial membranes. Although most plant cells probably contain all three types of Ca^{2+} transporters, they may differ greatly in the relative abundance of each type. Studies with membrane vesicles indicate that highly vacuolated root cortical cells, for example, have an active TN Ca^{2+}/nH^{+} antiporter and very little of the ER Ca^{2+}-ATPase (DuPont el al., 1990), whereas the reverse appears to be true in GA-stimulated barley aleurone cells (Bush et al., 1989).

Among the efflux transporters, the P-type ATPases show the highest and the chloroplast uniport shows the lowest affinity for Ca^{2+} (Evans et al., 1991). Typical values for the

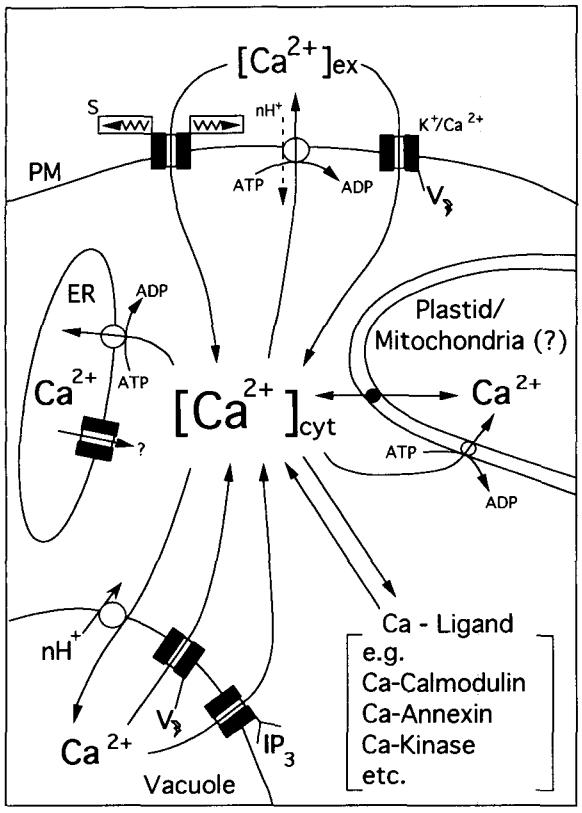

Figure 2. The ensemble of Ca^{2+} transporters that regulate cytosolic Ca^{2+} levels in plants cells. The principal transporters that drive Ca^{2+} efflux from the cytosol ($[Ca^{2+}]cyt$) into the extracellular solution ($[Ca^{2+}]ex$) are Ca ATPases (large circles with ATP) found on the PM, ER, a Ca^{2+}/nH^+ antiporter on the tonoplast membrane (circle), and a uniport on the plastid membrane (small filled circle). Ca^{2+} influx into the cytosol is regulated by Ca^{2+} channels (squares) that are controlled by voltage (V), stretch (S), or IP_3 concentration (IP_3). Cytosolic Ca^{2+} established by this ensemble is in equilibrium with cytosolic proteins (Ca-Ligand) that reversibly bind Ca^{2+}.

K_m for Ca^{2+} of the P-type ATPases vary between 0.5 and 5 μM, compared with 1 to 50 μM for the antiporters and 100 μM for the uniporters. Because affinity of the P-type transporters for Ca^{2+} is closest to the observed levels of cytosolic Ca^{2+}, it has been proposed that they are primarily responsible for setting resting levels of cytosolic Ca^{2+} (Evans et al., 1991). This is not to say that the lower-affinity carriers do not contribute to establishing resting levels of cytosolic Ca^{2+}. There is, in fact, evidence that Ca^{2+} transport activity on chloroplast (Miller and Sanders, 1987) and TN (Miller et al., 1990; Felle, 1991) membranes alters resting levels of Ca^{2+} in the cytosol. Nevertheless, despite the lack of direct evidence, it is usually assumed that the high-affinity P-type pumps are primarily responsible for Ca^{2+} efflux in resting cells, whereas the lower-affinity transporters may be more important during periods or locations of increased cytosolic Ca^{2+}.

Attempts to distinguish biochemically between the diverse types of P-type Ca^{2+} transporters have shown that they differ in their ability to be stimulated by lipids, cations, or exogenous calmodulin, and in their sensitivity to inhibition by vanadate or by erythrosin B (Evans et al., 1991, and refs. therein). These differences, which imply subtle alterations in regulation or structure of the transporter, vary greatly between species and cell type. An additional level of complexity is that an organelle may have more than one of the P-type Ca^{2+} transporters. In carrot suspension cells, for example, two P-type transport activities are associated with the ER, one that is stimulated by calmodulin and is insensitive to cyclopiazonic acid, and another that has the opposite characteristics (Hsieh et al., 1991).

The functional significance of multiple kinds of P-type transporters on a single membrane is not yet known. It is possible that they may exist in different regions of the ER and form differentially regulated Ca^{2+} stores. In any case, these observations point to an underlying genetic diversity in the P-type Ca^{2+} transporters just as there is in the P-type H^+-ATPases (Harper et al., 1989). Recently, complete and partial coding sequences (Wimmers et al., 1992) for a putative P-type Ca^{2+} transporter have been obtained by homology with transporters in mammalian and yeast cells. Information of this type should greatly accelerate our understanding of the structure and function of this group of transporters by leading to the development of molecular tools for studying their regulation and localization in living cells.

Progress has also recently been made using the patch-clamp technique to identify Ca^{2+} channels that regulate the influx of Ca^{2+} into the cytosol. These influx transporters, which could rapidly raise cytosolic Ca^{2+} levels in response to stimuli, have been found on both the PM and TN, which are, not coincidentally, the membranes that can be most easily patch-clamped. At least three types of Ca^{2+}-permeable channels have been identified: voltage-operated channels on both the PM and TN (Schroeder and Hagiwara, 1990; Fairley-Grenot and Assmann, 1992; Johannes et al., 1992; Pantoja et al., 1992); IP_3-operated channels on the TN (Alexandre et al., 1990); and stretch-operated channels on the PM (Cosgrove and Hedrich, 1991). Like the efflux transporters, there are large differences between these channels, not only in cellular location but also in regulation (Fig. 2). As their names imply, these channels are gated open at permissive voltages, IP_3 concentrations, or membrane tensions. Thus, Ca^{2+} influx into the cytosol could be induced by a wide range of stimuli that affect membrane polarization, phospholipase activity, or turgor.

The rate of discovery of Ca^{2+} channels has increased dramatically in the last year or two, and this seems certain to continue as more cell types are examined. Nevertheless, it is surprising that no efflux channel has yet been identified on the ER and, in particular, that there is no evidence for an IP_3-induced release of Ca^{2+} from the ER. Studies of efflux of Ca^{2+} from preloaded ER vesicles show an exponential loss that is not affected by any of the many compounds, such as ryanodine, IP_3, caffeine, and NADH, that alter channel activity on the ER of animal cells (Bush et al., 1993; for a review of the mammalian literature, see Tsien and Tsien, 1990). Although messenger-operated channels may eventually be discovered on the ER of plant cells, current data indicate that the primary routes of stimulus-induced Ca^{2+} influx into the cytosol may occur across the PM and TN. Certainly, many experiments have shown that both Ca^{2+} influx across the PM

and cellular responses to stimuli could be inhibited with pharmacological agents that block the voltage-operated channels in animal cells.

Although a highly selective, voltage-operated Ca^{2+} channel at the PM has not yet been reported, Ca^{2+} permeation through nonselective channels and through K^+ channels at the PM has been shown by several groups (Schroeder and Hagiwara, 1990; Fairley-Grenot and Assmann, 1992). It is possible that this is one of the main routes of entry of Ca^{2+} across PM because extracellular K^+ and Ca^{2+} have been shown to have antagonistic effects on cytosolic Ca^{2+} in *Riccia* (Felle, 1991). The mechanosensitive, i.e. stretch, Ca^{2+} channel provides another route for stimulus-induced Ca^{2+} entry across the PM. These channels, so far reported only from guard cells (Cosgrove and Hedrich, 1991), may play a very important role in turgor sensing in other cell types as well.

In contrast to the PM, at least three types of selective Ca^{2+} channels have been found on the TN. Some evidence for an IP_3-operated channel has been obtained (Alexandre et al., 1990), and the ability of IP_3 to affect Ca^{2+} influx has been demonstrated in living cells and in isolated vacuoles and membranes (Gilroy et al., 1990, and refs. therein). These observations point to a role of an IP_3-operated channel in stimulus-induced cytosolic Ca^{2+} changes. The function of the other two types of channels found on the vacuolar membrane is less clear. Both of them are voltage operated but are gated open at different membrane potentials. One of them is open only at very negative, seemingly unphysiological, potentials and allows Ba^{2+} (Pantoja et al., 1992) or Ca^{2+} (Ping et al., 1992) entry into the vacuole. One of the discoverers of this channel type has suggested that it may function in Ca^{2+} accumulation by the vacuole following IP_3-induced Ca^{2+} loss (Pantoja et al., 1992). Serious objections to this proposal have been raised, however, and their function remains something of a mystery (Maathuis and Sanders, 1992).

The function of the second type of voltage-gated channel is somewhat easier to understand because it is gated open at normal, i.e. slightly positive, transvacuolar potentials and it allows Ca^{2+} efflux from the vacuole (Johannes et al., 1992). Because there is in vivo regulation of this channel, it may function as a pathway for Ca^{2+} influx into the cytosol in response to stimuli. One may speculate that another possible function for this channel is to prevent excessive accumulation of Ca^{2+} in the vacuole by the Ca^{2+}/nH^+ antiporter. It might be wrong to interpret all transporter activities as mechanisms for regulating Ca^{2+} levels in the cytosol. There is accumulating evidence that regulation of Ca^{2+} levels in organelles might be important for their function as well (Bush et al., 1993).

Ca^{2+} HOMEOSTASIS IN LIVING CELLS

The characterization of Ca^{2+} transporters in membrane vesicles and membrane patches is necessary but not sufficient to understand how cytosolic Ca^{2+} is regulated in living cells. The shear number of different types of Ca^{2+} transporters clearly points to a high degree of flexibility and redundancy in the control of cytosolic Ca^{2+}. How, then, is cytosolic Ca^{2+} actually regulated in living cells? Studies of cytosolic Ca^{2+} in plants are only just beginning to appear in significant numbers, so a complete answer to this question is not yet possible. Nevertheless, the available evidence supports the idea that levels of cytosolic Ca^{2+} are established by a "pump and leak" mechanism in which both influx and efflux transporters are active and the balance established between them sets cytosolic Ca^{2+}. In this way, cytosolic Ca^{2+} can be thought of as a state of poise or tension between two antagonistic processes. Observations of stimulus-induced changes in cytosolic Ca^{2+} indicate that there is both short-term and long-term regulation of this pump and leak mechanism. Short-term regulation leads to abrupt and usually transient changes in response to stimuli that suddenly and preferentially alter the activity of an influx or an efflux transporter. Long-term regulation, on the other hand, leads to the establishment of resting or steady-state levels of cytosolic Ca^{2+} and the restoration of poise in the pump/leak balance. The change in cytosolic Ca^{2+} induced by most stimuli is transient and involves short-term regulation. However, some stimuli, such as GA, which appear to permanently alter steady-state cytosolic Ca^{2+}, may alter long-term regulation.

Currently, there is considerable effort being made to understand the molecular basis of short-term regulation of cytosolic Ca^{2+}. Work of this type, described in the previous section, has focused on identifying influx channels whose activity can be rapidly modulated by specific stimuli. It seems possible but not proven that the stretch- and IP_3-operated channels are responsible for generating the Ca^{2+} changes induced by ABA or hypotonic treatment (Gilroy et al., 1990; Cosgrove and Hedrich, 1991). Other mechanisms for short-term regulation of cytosolic Ca^{2+} will certainly be uncovered. In the case of elicitation, for example, there is indirect evidence that transporters on the PM are stimulated by a mechanism involving protein phosphorylation or dephosphorylation (Kauss and Jeblick, 1991). Although there is still no direct evidence of regulation of Ca^{2+} channels in plants by phosphorylation state, it has long been suspected. Nor is there yet any evidence of CICR in plants, a phenomenon documented in animal cells in which elevated levels of cytosolic Ca^{2+} induce the release of yet more Ca^{2+} from intracellular stores. CICR provides a means of amplifying stimulus-induced calcium changes both temporally and spatially, and it has been hypothesized to be one possible mechanism for generating oscillations in cytosolic Ca^{2+} (Tsien and Tsien, 1990). Oscillations in cytosolic Ca^{2+} are known to exist in plants (Felle, 1988a; Schroeder and Hagiwara, 1990), but the mechanism by which they are generated is not known.

Oscillations in cytosolic Ca^{2+} and long-term regulation of resting Ca^{2+} levels demonstrate the existence of a feedback mechanism that senses elevation in cytosolic Ca^{2+}. Although the complete nature of this feedback mechanism is not known, it is clear that elevated cytosolic Ca^{2+} alone, and not some additional stimulus, is sufficient to induce the regulatory process that restores resting levels of cytosolic Ca^{2+}. A pulse of extracellular Ca^{2+} or microinjection of Ca^{2+} into cells transiently raises cytosolic Ca^{2+} and also activates Ca^{2+}-efflux transporters that rapidly lower cytosolic Ca^{2+}, sometimes below the initial resting levels (Felle, 1988a, 1988b; Gilroy et al., 1990). Part of this activation of the efflux transporters must occur through an increase in substrate (i.e. Ca^{2+}) levels. The K_m for Ca^{2+} reported for most efflux transporters is greater than resting levels of cytosolic Ca^{2+}, indicating that a

large increase in efflux rate will accompany elevations in cytosolic Ca^{2+} (Evans et al., 1991). However, Ca^{2+} efflux transporters are probably also stimulated by elevated cytosolic Ca^{2+} through the action of the Ca^{2+}-binding protein calmodulin. Inhibitors of calmodulin action have been shown to raise cytosolic Ca^{2+} in protoplast suspensions (Gilroy et al., 1987). This observation is consistent with the finding that, in many plant cells, Ca^{2+} efflux transporters can be stimulated by exogenous calmodulin (Evans et al., 1991, and refs. therein; Hsieh et al. 1991). The stimulation by calmodulin of the P-type ATPases may be a direct inactivation of an autoinhibitory domain as in mammalian cells (Evans et al., 1991, and refs. therein). The mechanism of action of calmodulin on the Ca^{2+}/nH^+ antiporter is not known.

In addition to cytosolic Ca^{2+} and calmodulin, a number of other cellular factors have been identified that are important for determining the poise or resting levels of cytosolic Ca^{2+}. Chief among these is cellular pH. Diminution of the pH gradient across the PM or TN by pH changes on either side of the membrane results in increases in cytosolic Ca^{2+} (Bush and Jones, 1987; Felle, 1988b). In *Riccia*, for example, neutral red, which causes a slight alkalinization of the vacuole without significantly altering cytosolic pH, results in large increases in cytosolic Ca^{2+} (Felle, 1988b). The increase in cytosolic Ca^{2+} could be due to the dependence of Ca^{2+} on H^+ as the energy source for Ca^{2+} transport at the TN via the Ca^{2+}/nH^+ antiporter, and at the PM via the Ca^{2+}-ATPase (Felle, 1988b; Miller et al., 1990). However, it could also be due to the activation of a Ca^{2+} channel with a pH dependence similar to that reported for the highly selective, voltage-gated channel on the TN (Johannes et al., 1992). This channel is sensitive to vacuolar pH and remains open at elevated pH, which could lead to a prolonged influx of cytosolic Ca^{2+} from the vacuole. A rise in cytosolic or vacuolar pH could, therefore, lead to the observed increase in cytosolic Ca^{2+} either through inhibition of an efflux transporter or stimulation of a Ca^{2+}-influx channel. These interactions between cellular pH and cytosolic Ca^{2+} illustrate how long-term regulation could be influenced by small changes in the activity of cellular processes. Identifying those factors that are most important for long-term regulation is one of the goals of future research.

FUTURE PROSPECTS

Our understanding of Ca^{2+} regulation and its significance for cell function has expanded greatly within the last few years. This progress has been possible because several technologies have developed that allow regulation to be addressed at a number of levels. Molecular cloning of ion channels and pumps, in vitro characterization of isolated transporter activity, and measurements of cytosolic Ca^{2+} in living cells are being successfully pursued in a number of cell types. We can reasonably expect that important work on these fronts will progress rapidly, in part because we have the benefit of paradigms and homologs that have already been described in other organisms, and in part because of progress in related areas of plant signal transduction that will undoubtedly intersect with cytosolic Ca^{2+} regulation. Experimentally, the most challenging questions may lie in understanding how the ensemble of Ca^{2+} transporters functions as a system to generate Ca^{2+} signals and in understanding how information is encoded in the signal. An important part in answering these questions will be to establish how well the transporter composition of a particular plant cell resembles the model we have pieced together from partial descriptions of a number of different cell types (Fig. 2). Beyond this, it will be important to obtain detailed maps of the temporal and spatial character of the signal and identify the target proteins that intercept it.

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