BOTANICAL BRIEFING

Calcium: Just Another Regulator in the Machinery of Life?

CHRISTOPH PLIETH*
Zentrum für Biochemie und Molekularbiologie, Universität Kiel, Am Botanischen Garten 9, 24118 Kiel, Germany

Received: 1 November 2004 Returned for revision: 7 December 2004 Accepted: 1 March 2005

The roles of calcium ions: a reappraisal

When discussing the current debates on Ca$^{2+}$, one has to consider the ‘conundrum’ aspect. On the one hand, Ca$^{2+}$ is an indispensable mineral with many structural and metabolic functions (Hirschi, 2004). On the other hand, Ca$^{2+}$ is a cytotoxic ion because above a critical Ca$^{2+}$ concentration, insoluble calcium phosphate would precipitate in the cytosol, leading to a breakdown of ATP metabolism and to death. Therefore, the free cytosolic Ca$^{2+}$ concentration ([Ca$^{2+}$]$_{cyt}$) is maintained at approximately 100 nM, whereas in luminal or extraprotoplasmic compartments these restrictions are less imperative. Ca$^{2+}$ performs several tasks in living cells. Three classes of function are discussed here.

Ca$^{2+}$ alleviates ionic stress: the emergency brake function

Plants are more susceptible to damage by low pH or high salt when Ca$^{2+}$ is deficient. A lot of evidence suggests that external and apoplastic Ca$^{2+}$ ([Ca$^{2+}$]$_{ext}$, [Ca$^{2+}$]$_{apop}$) directly alleviates symptoms produced by ion stress or mineral toxicity. Different mechanisms are conceivable by which Ca$^{2+}$ is able to prevent the ensuing damage. Ca$^{2+}$ acts as a cross-linking component, stabilizing membranes, and also has a direct impact on ion transport. A few examples are given below.

Proton and Na$^{+}$ toxicity

It is a long-standing observation that Ca$^{2+}$ ‘tightens’ membranes, reducing passive ion-fluxes and rendering membranes more hydrophobic (White and Broadley, 2003). Monovalent ions like H$^{+}$ or Na$^{+}$, however, are able to displace stabilizing Ca$^{2+}$ from a membrane’s phospho- and carboxy-headgroups. Hence, a surplus of Ca$^{2+}$ in the extracellular milieu is always of benefit under ionic stress.

By use of recombinant indicators it was possible to demonstrate that plants increase Ca$^{2+}$ at the sites of stress (Gao et al., 2004). Plants typically respond to a large and potentially dangerous increase in [H$^{+}$]$_{ext}$ or [Na$^{+}$]$_{ext}$ with a release of Ca$^{2+}$ into the cytosol, thus establishing a sustained elevated basal [Ca$^{2+}$]$_{cyt}$. The same is found when H$^{+}$ stress is indirectly initiated by anoxia (Plieth, 2001).

Aluminium toxicity

The protective function of Ca$^{2+}$ also seems to play an important role in lessening aluminium toxicity. Al$^{3+}$ has long been believed to be a highly phytotoxic ion (Kochian, 1995). However, Al$^{3+}$ is poisonous mainly at very low pH. Divalent cations like Ca$^{2+}$ and Mg$^{2+}$ are able to alleviate the Al$^{3+}$ toxicity syndrome in the field. In contrast to Al$^{3+}$ tolerance, the mechanisms of Al$^{3+}$ toxicity are less understood. For the latter, the following proposal has been advanced. Cytosolic pH is under biophysical and biochemical control, and is thus very constant. However, at very low external pH (pH$_{ext}$) the control mechanisms involved cannot prevent a
decrease in cytosolic pH. As mentioned above, the plant actively responds to this by a sustained increase of 
\([\text{Ca}^{2+}]_{\text{cyt}}\) (Plieth et al., 1999b). This counteracts the loss of membrane integrity, and prevents \(H^+\)-mediated
damage in the cytosol. \(Al^{3+}\), however, is a potent blocker of cation channels. It has been demonstrated that in a 
low-pH environment, the presence of \(Al^{3+}\) blocks \(Ca^{2+}\) influx into the cytosol, thus negating its protective effects 
and rendering the plant defenceless against increased \(H^+\) concentrations (Plieth et al., 1999b).

**Chloride toxicity**

The use of a genetically encoded anion-indicator has allowed the direct visualization of \(Cl^-\) influx into root 
cells under saline conditions (Lorenzen et al., 2004). This influx can be inhibited by an increase in the external 
\([\text{Ca}^{2+}]\). Here, the protective effect of \(Ca^{2+}\) is due to its interaction with ion transport. Since no \(Cl^-\) transporters 
have yet been identified which are directly inhibited by external \(Ca^{2+}\), it is assumed that \(Ca^{2+}\) has an indirect 
effect on \(Cl^-\) transport via the blockade of cation channels. However, further investigations are needed to validate such 
a mechanism.

In summary, a lot of data strongly support the view that the protective effect of \(Ca^{2+}\) is universal, mediated intra-
cellularly, and used extensively by plants. It would thus seem advantageous for a plant cell to respond to a large 
influx of toxic monovalent ions with an active release of 
\(Ca^{2+}\) into both the cytosol and the apoplast in order to 
counteract a loss of membrane and cell wall integrity. These somewhat unspecific roles of \(Ca^{2+}\) can be designated 
as an ‘emergency-brake function’. Other mechanisms, as 
mentioned for \(Cl^-\) toxicity, are effects on ion channels 
and these are discussed below.

**\(Ca^{2+}\) REGULATES ION CHANNELS: THE ION-TRANSPORT CONTROL FUNCTION**

It has been verified many times that \(Ca^{2+}\) controls ion-channel activity (for review, see Sanders et al., 2002). 
\(Ca^{2+}\) can either activate or block ion channels and hence regulate ion-flux. Three examples are given here.

**\(Ca^{2+}\) released from chloroplasts activates \(K^+\) channels**

Photosynthesis acts on ion transport at the plasma mem-
brane via several pathways. Most of them do not seem to have 
an obvious physiological function, but just reflect a disturb-
ance of the ionic milieu as caused by changes in photosyn-
thetic activity. One of these pathways involves calcium: 
\(Ca^{2+}\) released from chloroplasts upon darkening increases 
\([\text{Ca}^{2+}]_{\text{cyt}}\) and thus activates \(K^+\) channels in the plasma 
membrane. This leads to dark-induced changes in the membrane 
voltage (Plieth et al., 1998, and references therein).

**\(Ca^{2+}\) activates ion channels during action potentials**

The bulk current during an action potential (AP) in plant 
cells is conducted via \(Cl^-\) and \(K^+\) channels (reviewed in 
Thiel et al., 1997). An AP usually occurs in parallel with a 
massive transient increase in \([\text{Ca}^{2+}]_{\text{cyt}}\). Based on \(^{45}\text{Ca}^{2+}\)-flux 


studies, it has been suggested that this \([\text{Ca}^{2+}]_{\text{cyt}}\) rise is produced by a \(Ca^{2+}\) influx through \(Ca^{2+}\)-permeable channels in the 
plasma membrane. However, thorough investigations 
using the manganese quench technique revealed that \(Ca^{2+}\) is 


predominantly released from internal stores during an AP 
(Plieth et al., 1999c). These facts support a model proposed 
by Thiel et al. (1993) which implies that a local release of 
\(Ca^{2+}\) from internal stores (possibly the endoplasmic reti-

culum) activate \(Ca^{2+}\)-sensitive \(Cl^-\) channels. In subsequent 


studies it was shown that liberation of \(Ca^{2+}\) from internal 
stores during an AP is very probably triggered by increased 


inositol trisphosphate, which in turn is a consequence of 


membrane depolarization (Wacke et al., 2003, and refer-
ences therein).

**\(Ca^{2+}\) helps to establish a favourable \(K^+:Na^+\) ratio under salt stress**

Another aspect of \(Ca^{2+}\)-mediated ion-flux has already 
been mentioned in the context of salt stress. Voltage-


independent cation channels (VICs) are believed to be the 


major point of entry for \(Na^+\) into the cell, and external 


\(Ca^{2+}\) inhibits VIC-mediated \(Na^+\) currents (Maathuis and 


Ammann, 1999; White and Davenport, 2002). This specific 


modulator function is not simply due to the \(Ca^{2+}\) in the 


extraplastoplasmic matrix but rather a matter of a 


threshold external \(Ca^{2+}\) concentration which has to be main-


tained for effective inhibition of \(Na^+\) influx. Recently, the 


increase of the apoplastic free \(Ca^{2+}\) concentration 


([\(Ca^{2+}\])apo) under salt stress could be demonstrated 


in vivo (Gao et al., 2004). It is likely that this effect also 


helps to establish a favourable intracellular \(K^+:Na^+\) ratio.


In addition to VICs many other ion transporters are 


controlled by \(Ca^{2+}\), either directly or indirectly, via 


\(Ca^{2+}\)-dependent protein kinases or calcineurin-like proteins 


(Liu and Zhu, 1998; Pardo et al., 1998). The currently 


proposed regulatory pathway for intracellular \(Na^+\) and \(K^+\) 


homeostasis and \(Na^+\) tolerance in plants assumes that the 


increase in \([\text{Ca}^{2+}]_{\text{cyt}}\) occurring under \(Na^+\) stress is needed 

to switch protein kinases and phosphatases related to salt 


tolerance (Halfter et al., 2000; Xiong and Zhu, 2002) as 


well as phosphoinositol turnover (Hirayama et al., 1995).


Hence, it seems justified to assign \(Ca^{2+}\) an ‘ion-transport 


control’ function.

**\(Ca^{2+}\) ACTIVATES ENZYMES: THE CHEMICAL SWITCH FUNCTION**

\(Ca^{2+}\) controls the activity not only of ion transporters but 
also of many other proteins, either directly or indirectly (via 
\(Ca^{2+}\)-binding factors). Chloroplasts, for example, harbour 
a large number of proteins (e.g. photosystem II, the water-


splitting enzyme, a calmodulin-stimulated NAD-kinase, 
thioredoxins, ferredoxin, and stromal enzymes of the Calvin 


cycle) that need \(Ca^{2+}\) to function properly.

The experiment shown in Fig. 1 is an example of the 


function of \(Ca^{2+}\) as a chemical switch in mitochondrial 


energy conversion; the external NADH dehydrogenase
(NDX) of plant mitochondria is dependent on the presence of Ca\(^{2+}\). It supplies electrons to the quinone pool of the electron-transfer chain (Fig. 1A). When mitochondria are fed with NADH, oxygen is consumed owing to NDX activity (Fig. 1B; \(t = 5\) min, 15 min). This effect is stopped when Ca\(^{2+}\) concentration is restored, the remaining NADH is metabolized (\(t = 65\) min). Other complexes of the electron-transfer chain are independent of Ca\(^{2+}\) (e.g. complex II = succinate dehydrogenase) and continue to function in the presence of EGTA (\(t = 40\) min).

There are many other examples where Ca\(^{2+}\) modulates protein function. Frequently Ca\(^{2+}\)-binding proteins such as calmodulins (Snedden and Fromm, 2001) or CDPKs (Romeis et al., 2001) serve as amplifying elements.

Owing to the correlation between environmental stimuli, specific changes in [Ca\(^{2+}\)]\(_{cyt}\) and selective activation of enzymes or genes (i.e. physiological activity), it is tempting to assume that Ca\(^{2+}\) has a ‘chemical-switch function’. However, this assumption has to be seen in the context of simultaneous activities of other cell components (Scrase-Field and Knight, 2003), and conclusions should be drawn cautiously. Experiments such as the above (Fig. 1) do not allow us to conclude that Ca\(^{2+}\)-dependent proteins are also switched by Ca\(^{2+}\) \textit{in vivo}. Therefore, experiments are needed to provide evidence that a specific [Ca\(^{2+}\)]\(_{cyt}\) transient is necessary \textit{and} sufficient to switch a protein \textit{in vivo}. This is discussed in more detail below.

**SENSING AND RESPONDING: IS Ca\(^{2+}\) A GENERAL INFORMATION CARRIER?**

In plants, the term ‘sensing’ is often used with a broad definition; sensing is the reception of a stimulus that is relayed into a cellular signal and then translated to allow the appropriate physiological response. There are many findings suggesting that Ca\(^{2+}\) is indispensable for this.
Apart from the functions discussed above (i.e. protectant, and switch of ion transport and other cellular activities), Ca\(^{2+}\) is believed to be a more general key player in cellular activity. It has often been stated that—as so-called second messengers—calcium ions carry specific information and thus mediate the connections from ‘sensing’ to ‘responding’. Some important findings which led to this commonly accepted notion will now be discussed. Data and views questioning this concept are presented later.

The physiological response can be inhibited by \('Ca^{2+}\' inhibitors\)

Experiments have shown that a physiological response (e.g. activation of ‘stress genes’) can be suppressed by so-called \(Ca^{2+}\) inhibitors (i.e. blockers of \(Ca^{2+}\)-permeable channels such as \(La^{3+}\), or \(Ca^{2+}\)-chelators). Hence, inhibiting the typical internal \(Ca^{2+}\) response after a given stimulus also blocks the subsequent physiological response (e.g. Polisenski and Braam, 1996). This provoked the idea that the cellular \(Ca^{2+}\) signal is essential for producing the final response. However, it is important to keep in mind that inhibitors and blockers are not specific. \(La^{3+}\), for instance, is also a potent inhibitor of anion channels (Lorenzen et al., 2004) and a blocker of \(K^{+}\) channels (Terry et al., 1992). Furthermore, \(La^{3+}\) on its own can produce \([Ca^{2+}]_{cyt}\) transients in plants (Plieth, 2001) and is able to up-regulate gene expression in otherwise unstimulated cells (Polisensky and Braam, 1996). Thus, the caveat in inhibitor experiments is that the full spectrum of side-effects is not known.

\([Ca^{2+}]_{cyt}\) transients and gradients occur with almost any kind of stimulus

Another fundamental finding is that almost any stimulus is able to elicit a \([Ca^{2+}]_{cyt}\) transient with a typical, unique time course (tables and graphs in McAinsh et al., 1997; Plieth, 2001; Scrase-Field and Knight, 2003). Even seemingly similar stimuli like wind and gravity (both of which are purely mechanical stimuli) produce completely different time courses (Plieth and Trewavas, 2002).

\([Ca^{2+}]_{cyt}\) transients are involved in the activity of auxin and ABA (abscisic acid)

It has been suggested that auxin is the genuine primary effector in the gravitropic response. \([Ca^{2+}]_{cyt}\) transients induced by gravitropic stimulation are altered by auxin-transport inhibitors such as triiodobenzoic acid and naphthylphthalamic acid (Plieth and Trewavas, 2002). This is compatible with the view that \([Ca^{2+}]_{cyt}\) may be somehow involved but suggests that auxin is upstream of any \([Ca^{2+}]_{cyt}\) involvement in the gravity-stimulated signalling cascade.

In the case of stomatal functioning, it has become clear that the phytohormone ABA is the important primary intercellular signal linking the stimulus with stomatal closure. Since ABA has been found to produce \([Ca^{2+}]_{cyt}\) transients and oscillations (e.g. Schroeder et al., 2001b), it has been concluded that these \([Ca^{2+}]_{cyt}\) kinetics transduce the ABA signal and trigger stomatal closure. However, since there are data showing that stomatal closure is not necessarily preceded by a \([Ca^{2+}]_{cyt}\) transient (Allan et al., 1994), the suggestion that \([Ca^{2+}]_{cyt}\) transients are always involved in eliciting appropriate responses (McAinsh et al., 1997; Schroeder et al., 2001a) is highly speculative. Nevertheless, it has raised a lot of questions and triggered intense research activities.

Open questions

The most intriguing questions in need of an answer are:

1a) If a \([Ca^{2+}]_{cyt}\) transient is essential, is it also in itself sufficient to produce a response?

1b) Vice versa: if a \([Ca^{2+}]_{cyt}\) transient is in itself sufficient, is it also essential to produce a response?

2) How can a single cellular component like \(Ca^{2+}\) specifically channel a plethora of external signals into the right cellular destinations downstream so that a proper physiological response is produced?

\([Ca^{2+}]_{cyt}\) transients can induce physiological responses

In efforts to answer the first two questions, reverse experiments were designed to induce artificially \([Ca^{2+}]_{cyt}\) transients able to produce a normal physiological response without the appropriate stimulus. By means of \(Ca^{2+}\)-ionophores or \(Ca^{2+}\)-channel antagonists, various studies have successfully elevated \([Ca^{2+}]_{cyt}\) in the absence of a stimulus, and produced physiological responses (e.g. Monroy and Dhindsa, 1995; Sheen, 1996). However, the artificially induced \([Ca^{2+}]_{cyt}\) transients were not monitored in parallel. Photo-activation of caged \(Ca^{2+}\) and caged inositol trisphosphate also yielded a physiological response, namely stomatal closure and reorientation of pollen-tube growth (Gilroy et al., 1990; Malhó and Trewavas, 1996). This showed that the induced \([Ca^{2+}]_{cyt}\) peak alone is sufficient for certain cellular responses. When micro-injected caged ABA was released in guard cells by photolysis, the subsequent \([Ca^{2+}]_{cyt}\) transient appeared to be dependent on the temperature regime the plants had experienced 2 d before the experiment, whereas the physiological response (i.e. closure) was independent of this (Allan et al., 1994). It was concluded that \([Ca^{2+}]_{cyt}\) is not the only route through which ABA can trigger stomatal closure.

Many experiments were designed to mimic the \([Ca^{2+}]_{cyt}\) transients that have been observed after ABA or external \(Ca^{2+}\) treatment. In guard cells, artificial repetitive transients were produced by superfusion of epidermal strips with depolarizing and hyperpolarizing buffers (Allen et al., 2001). This indeed triggered stomatal closure without the natural abiotic stimulus needed to induce this response. Moreover, the number, frequency and duration of induced transients were found to be important for the final stomatal aperture. All this supported the so-called \(Ca^{2+}\) signature hypothesis discussed below.
THE Ca\(^{2+}\) SIGNATURE HYPOTHESIS: \([\text{Ca}\(^{2+}\)]_{\text{cyt}}\) KINETICS ENCODE INFORMATION

The second question mentioned above concerning Ca\(^{2+}\) specificity was answered by the so-called signature hypothesis (McAinsh et al., 1997; McAinsh and Hetherington, 1998), which states that the specificity of Ca\(^{2+}\) is given by the time course of the \([\text{Ca}\(^{2+}\)]_{\text{cyt}}\) transient and the location of the \([\text{Ca}\(^{2+}\)]_{\text{cyt}}\) increase (both of which constitute the ‘Ca\(^{2+}\) signature’).

This hypothesis implies that if each stimulus gives a unique \([\text{Ca}\(^{2+}\)]_{\text{cyt}}\) transient, then the information necessary to produce the correct response is encoded in the temporal (amplitude, duration, frequency) and spatial (site, dimension, spread) behaviour of the transient. Thus, each specific transient is proposed to switch a different sub-set of Ca\(^{2+}\)-dependent cellular components and, in this way, specifically to induce further downstream activities, which are then integrated to produce the physiological response (e.g. stomatal closure). This hypothesis was very attractive and was consequently adopted by many others and refined to models proposing that the \([\text{Ca}\(^{2+}\)]_{\text{cyt}}\) signal is frequency- or amplitude-modulated, thus somehow ‘digitally’ encoding information (Allen and Schroeder, 2001; Sanders et al., 2002; Ng and McAinsh, 2003).

Is the calcium signature hypothesis valid?

The Ca\(^{2+}\) signature hypothesis is very much influenced by the animal literature. Data on plant responses show some similarities with \([\text{Ca}\(^{2+}\)]_{\text{cyt}}\) events in animals and have been interpreted in the context of the molecular machinery in the cytoplasm of animal cells. Now, more and more doubts are emerging about the theoretical concept of the \([\text{Ca}\(^{2+}\)]_{\text{cyt}}\) signature and the encoding of cellular information in plants (Scrase-Field and Knight, 2003). The pros and cons of the alleged universality of \([\text{Ca}\(^{2+}\)]_{\text{cyt}}\) as an information carrier have already been reviewed in detail (Plieth, 2001). Further findings making the signature concept even more controversial are presented in the following section.

All that glitters is not gold

The above experiments (Allen et al., 2001) were performed with the fluorescence resonance energy transfer (FRET)-based Ca\(^{2+}\)-indicator protein, cameleon, to monitor \([\text{Ca}\(^{2+}\)]_{\text{cyt}}\) during buffer treatment. However, data obtained from identical experiments with Arabidopsis expressing the FRET-based, Cl\(^{-}\)-sensitive, anion-indicator protein, clomeleon (Lorenzen et al., 2004), differ only slightly from those produced with cameleon (Fig. 2). These findings demonstrate that some facts had been overlooked. First, cameleon contains a FRET acceptor (yellow fluorescent protein) similar to that of clomeleon (Lorenzen et al., 2004). Hence, changes in cameleon’s fluorescence ratio may be adulterated by Cl\(^{-}\)-flux (Fig. 2). Secondly, there are no means to distinguish Ca\(^{2+}\) influx from Cl\(^{-}\) efflux in guard cells by use solely of the 530:480 nm emission ratio of cameleon. Thirdly, since the Cl\(^{-}\) concentration and probably many other cellular parameters are also shifted in guard cells during washing procedures with different buffers, it is questionable whether one can conclude that Ca\(^{2+}\) is a key regulator determining stomatal aperture.

Another tool that often leads to misinterpretation of data is aequorin, a widely used luminescent [Ca\(^{2+}\)] indicator (Plieth, 2001). This luciferase system probably evolved from early antioxidant reactions by a functional shift of

\[\text{530:480 nm ratio} = \frac{\text{Fluorescence at 530 nm}}{\text{Fluorescence at 480 nm}}\]
the cofactor coelenterazine (CTZ) from a general antioxidant to a special luciferin (Wergifosse et al., 1999). CTZ is currently on offer as a chemiluminescent indicator for reactive oxygen species (ROS). Hence, in experiments with transgenic plants expressing apoaequorin, it is essential to keep in mind that CTZ luminescence can also be triggered by ROS, e.g. H₂O₂, without any increase in [Ca²⁺] (Fig. 3).

Thus, if reconstituted and luminescent plants are exposed to H₂O₂, or to stress situations in which ROS are released, then CTZ chemiluminescence (a ROS-triggered light signal) might interfere with the bioluminescence from aequorin (a [Ca²⁺]-triggered light signal) and lead to false interpretations. Furthermore, circadian luminescence recordings from apoaequorin-expressing plants (e.g. Love et al., 2004) do not necessarily represent pure [Ca²⁺] oscillations and can also be interpreted in terms of circadian rhythms of photosynthetic activity, respiration, or ROS release. Luminescence data can only be interpreted in terms of [Ca²⁺] changes when control experiments have shown that the light signal is not perturbed by luminescence from unbound CTZ.

**Temperature-sensing and [Ca²⁺] cyt, response: the signature of the input signal**

Another often-overlooked argument that also weakens the Ca²⁺ signature hypothesis is the fact that the [Ca²⁺] cyt signature is closely related to the time course of the applied stimulus. This has been demonstrated in the case of the cold response in plants. Since the cold-induced [Ca²⁺] cyt increase is the very first measurable cellular response, it is believed to switch metabolism in favour of gaining cold-hardiness or freezing tolerance (Knight et al., 1996). More detailed experiments have demonstrated that plants perceive a decline in temperature (i.e. dT/dt) very much more sensitively than the actual temperature (T) (Plieth et al., 1999a). Accordingly, [Ca²⁺] cyt depends on dT/dt rather than on T itself. The plant seems to sense its environment differentially, i.e. the rate of change and not the absolute values are detected. This seems to be true also for drought (i.e. declining relative humidity in air or soil; Bray, 1997). Thus, rapid changes are translated first and acclimation is optimized. Here, two points emerge which are at odds with the Ca²⁺ signature hypothesis:

1. When low cooling rates are applied (i.e. cooling rates of <0-001 °C s⁻¹) then no [Ca²⁺] cyt transient at all is detected in plants. These cooling rates, however, are in the physiologically relevant range which normally produces cold adaptation in the wild. Therefore, if there is no natural [Ca²⁺] cyt change, how can [Ca²⁺] cyt govern cold adaptation?

2. The [Ca²⁺] cyt signature is completely dependent on the time course (signature) of the applied temperature drop. In other words, [Ca²⁺] cyt transients of any shape—including long-lasting oscillations—can be mimicked in plants simply by application of an appropriate cooling protocol. This, however, contradicts the assumption that the [Ca²⁺] cyt kinetics encode information on the type and strength of an applied stimulus, since amplitude or frequency modulation (i.e. analogue or digital information encoding) need at least one invariant dynamic component in the signal (i.e. the reference signal), which is then modulated. Such invariant [Ca²⁺] cyt signals encoding the type of stimulus received have not yet been found.

**Feedback: [Ca²⁺] cyt transients depend on the physiological state**

In each cell a decision has to be made as to whether or not an incoming signal (stimulus) is useful enough to be fully relayed into the cell and processed. This requires an efficient intracellular feedback system modulating the receptor sensitivity. [Ca²⁺] cyt transients are often attenuated in the plant by its stress history (Knight et al., 1998). In terms of the [Ca²⁺] cyt signature concept, this would mean a loss of information about the type or strength of the current stimulus. Hence, it is very likely that the cell determines the kinetics of [Ca²⁺] cyt appropriate for the final response and not the [Ca²⁺] cyt kinetics that dictate the final response of the cell.

**Does a [Ca²⁺] cyt transient necessarily produce a response?**

In animal systems, [Ca²⁺] cyt oscillations have been reported to be responsible and necessary for meiosis, mitosis, fertilization and secretion. The function of [Ca²⁺] cyt oscillations in plants, however, remains largely obscure. [Ca²⁺] cyt oscillations (spiking) can be observed in root hairs of certain legumes when treated with nodulation factors. In mutants defective in nodule biogenesis, this [Ca²⁺] cyt spiking is absent (Wais et al., 2000; Walker et al., 2000), an observation suggesting that [Ca²⁺] cyt...
oscillations are involved in the nodulation response. However, in other cases a conclusive correlation between [Ca$^{2+}$]$_{cyt}$ and a physiological response is lacking.

The most regular and reproducible [Ca$^{2+}$]$_{cyt}$ oscillations reported so far from plants have been produced in the alga *Eremosphaera viridis* (Bauer et al., 1998). However, there are no means of altering the frequency or amplitude of these oscillations nor methods to detect any relevant physiological responses. Hence, there is no dependency on the strength of the stimulus that would be a pre-requisite for the signature hypothesis.

[Ca$^{2+}$]$_{cyt}$ oscillations have been repeatedly reported from pollen tubes and correlated to oscillations in tube growth rate. However, pollen-tubes can develop and grow quite normally without any detectable [Ca$^{2+}$]$_{cyt}$ oscillations (R. Malhö, pers. comm.).

Regulatory networks with amplifying pathways and feedback loops often tend to oscillate. Hence, [Ca$^{2+}$]$_{cyt}$ oscillations in plant cells may reflect natural regulation and ion-transport mechanisms based on antagonistic pump and channel activity rather than signal transduction-mediating development, tip growth rate or growth direction. Another example which has already been mentioned above is pertinence in this context: transients in [Ca$^{2+}$]$_{cyt}$, brought about by light–dark transitions, are apparently less involved in signal transduction but rather reflect inevitable disturbances caused by the enormous requirement of the photosynthetic machinery for Ca$^{2+}$ and H$^+$ when switched on by light.

Also, alkalinization of the cytosol leads to a prominent [Ca$^{2+}$]$_{cyt}$ transient regardless of whether the alkalinization was produced by NH$_4$Cl treatment, by washing pH-clamped roots, by re-oxygenation after a period of anoxia, or by bringing acidified tissue back to normal pH (Plieth, 2001, and references therein).

**CONCLUSIONS**

[Ca$^{2+}$]$_{cyt}$ signatures following a particular stimulus may be inevitable but tolerated side-effects of metabolic processes which may be neither necessary nor employed by the organism to encode information for signal transduction. It is probable that in only very few cases a [Ca$^{2+}$]$_{cyt}$ transient is sufficient and necessary to form a cellular response. One could speculate that evolution used inevitable changes in [Ca$^{2+}$]$_{cyt}$ to signal to other functional units that a primary process is in operation. In this way, [Ca$^{2+}$]$_{cyt}$ could have started to participate in cellular messenger networks. The same could be true for other ‘signalling components’ like pH, NO, ROS, cyclic nucleotides, sugars, etc.

Nevertheless, it must be kept in mind that a ‘cellular signal’ recorded by a certain device may not necessarily constitute a relevant ‘signal’ perceived by the plant and can occur without communicating any information to enzymes or structures. A simple correlation of changes occurring before or simultaneously with a certain physiological response is no satisfactory evidence for a general signalling function of the recorded component. In particular, calcium is not the be-all and end-all, and much more detailed research is needed to separate real Ca$^{2+}$ signalling from pure drive in a plant cell.

**ACKNOWLEDGEMENTS**

I am grateful to Lee Shaw (Biochemistry Institute, University of Kiel), Hartmut Kaiser (Botany Institute, University of Kiel) and Ulrike Hunold (Kiel) for critically reading the manuscript.

**LITERATURE CITED**


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