Temperature sensing by plants: the primary characteristics of signal perception and calcium response

Christoph Plieth1,*, Ulf-Peter Hansen2, Heather Knight1 and Marc R. Knight1
1Department of Plant Sciences, University of Oxford, Oxford, UK, and
2Institute for Applied Physics, Christian-Albrechts-University Kiel, Germany

Summary
Cold elicits an immediate rise in the cytosolic free calcium concentration ([Ca2+]c) of plant cells. We have studied the concerted action of the three underlying mechanisms, namely sensing, sensitisation and desensitisation, which become important when plants in the field are subjected to changes in temperature. We applied different regimes of temperature changes with well-defined cooling rates to intact roots of Arabidopsis thaliana expressing the calcium-indicator, aequorin. Our results indicate that temperature sensing is mainly dependent on the cooling rate, dT/dt, whereas the absolute temperature T is of less importance. Arabidopsis roots were found to be sensitive to cooling rates of less than dT/dt = 0.01°C/s. However, at cooling rates below 0.003°C/s (i.e. cooling 10°C in 1 h) there is no detectable [Ca2+]c response at all. At low temperature, the sensitivity of the plant cold-detection system is increased. This in turn produces greater calcium-induced [Ca2+]c elevations. Prolonged or repeated cold treatment attenuates the [Ca2+]c responses to subsequent episodes of cooling.

Introduction
Many environmental and endogenous stimuli are linked to changes in [Ca2+]c in plants (Gong et al., 1998; Knight et al., 1996; Knight et al., 1997; Knight et al., 1991; Knight et al., 1992; Sedbrook et al., 1996). In particular, it has been demonstrated that plants react to cold-shock (i.e. a temperature drop of several degrees within less than 1 sec) by an immediate and transient rise in cytosolic calcium ([Ca2+]c) (Knight et al., 1991; Knight et al., 1992; Russell et al., 1996). This [Ca2+]c rise has been shown to be initiated by Ca2+ influx through the plasmalemma and by Ca2+ release from internal stores (e.g. vacuole) (Knight et al., 1996).

However, the conditions encountered by plants in the field are quite different. Under natural conditions plants will mostly experience slow cooling rates (i.e. dT/dt < 1°C/s) and hence are likely to respond differently. Thus, it is of value to perform experiments which simulate more realistic situations. Electrophysiological studies have already revealed that ion transport differs depending on the cooling rate (dT/dt) (Minorsky and Spanswick, 1989). It has been hypothesised that plasmalemma associated Ca2+-permeable channels are involved in temperature sensing (Minorsky, 1989; Monroy and Dhindsa, 1995; Pickard, 1984).

The fact that cooling leads to many different cellular responses has been known for more than 150 years (reviewed by Minorsky, 1989). After the primary transient responses to cold, such as membrane depolarisation and [Ca2+]c increase, there is an orchestration of subsequent events in plant physiology. These events include protein phosphorylation (Kawczynski and Dhindsa, 1996; Monroy et al., 1998), altered gene activity (Ishitani et al., 1997; Knight et al., 1996), new gene products (Hayashi et al., 1997; Sabehat et al., 1998), alterations in plant membrane properties (Cossins, 1994; Murata and Los, 1997; Tasaka et al., 1996) and secondary metabolism (Guy, 1990; Steponkus, 1990; Zabotin et al., 1998).

Previous studies have shown that there is a correlation between the [Ca2+]c signal and subsequent cellular events, with [Ca2+]c transients controlling downstream processes. In particular, [Ca2+]c has been implicated in the control of cold-responsive gene expression (Knight et al., 1996; Tahtiharju et al., 1997) and in the acquisition of freezing tolerance (Monroy and Dhindsa, 1995; Monroy et al., 1993). The expression of cold-responsive genes controlled by calcium can be increased in response to natural temperature changes occurring in the wild. Furthermore, the acquisition of freezing tolerance is mediated by long periods at low temperature (days), and is also affected by natural temperature changes encountered in the wild. Therefore, a role for calcium in mediating responses (gene expression and acclimation) to temperature changes occurring in the field is implied. Less is known about the possible role of Ca2+ during chilling, although there are preliminary data suggesting that Ca2+ may be involved in acclimation to chilling temperatures (Kitigawa and Yoshizaki, 1998).

To understand the role of each facet of these complex processes, it is necessary to first understand the stimulus perception and early signal transduction which eventually
leads to the mentioned orchestrated alterations in plants. The sensor for temperature perception has not yet been found. Murata and Los (1997) emphasised the role of membrane fluidity. They speculate that the sensor is located in microdomains of the membrane and able to detect physical phase transitions which then lead to conformational changes and/or phosphorylation dephosphorylation cycles due to changes in temperature. Huner et al. (1998) mentioned the photosynthetic apparatus which might sense changes in temperature through increased energy imbalance and photoinhibition. Monroy and Dhindsa (1995) proposed a calcium permeable channel as primary sensor. Similarly, Minorsky (1989) proposed that the cold-induced \([Ca^{2+}]_c\) response was the primary sensing event. Thus, we have measured \([Ca^{2+}]_c\) in Arabidopsis roots in response to different temperature change regimes in order to gauge the effects of these regimes on the cold-sensing system in plant cells.

We designed a new experimental set-up to enable long-term measurements of the cytoplasmic free calcium concentration \([Ca^{2+}]_c\) in whole intact tissues in parallel with temperature \((T)\) measurements. \([Ca^{2+}]_c\) was measured in intact root systems of transgenic Arabidopsis expressing the photoprotein aequorin which produces luminescence which is directly related to \([Ca^{2+}]_c\) (Knight et al., 1991; Knight et al., 1993; Knight and Knight, 1995).

Results

Rapid cooling pulses (mostly described as ‘cold shocks’) to non-injurious temperatures cause strong transient increases in \([Ca^{2+}]_c\). (Figure 1). This finding is in line with many previous studies where the \([Ca^{2+}]_c\) response to cold shock was investigated (Knight et al., 1996; Knight et al., 1991; Knight et al., 1993; Russell et al., 1996).

The most striking feature of the responses in Figure 1 is the spike-like \([Ca^{2+}]_c\) kinetics which suggests a ‘high-pass filter behaviour’ of the system (Brook and Wynne, 1988; Pearson, 1992). Making this assumption implies that the system (i.e. the plant) responds like a differentiator, which means that the cooling rate \(dT/dt\) and not the absolute value of the temperature \(T\) is the important parameter which determines the response.

In order to give a quantitative basis for this statement, the dependence of the response (the \([Ca^{2+}]_c\)-peak value) on the cooling rate is shown in Figure 2. Here data from 13 different and comparable experiments, where only the response of the very first cooling event is taken into account (i.e. zero cold-history of the specimen) and where the cooling started in the range of \(14°C < T < 18.5°C\) (i.e. similar sensitivity to the specimen), are summarised. The good correlation \((r = -0.91)\) supports our view that the main factor which influences the \([Ca^{2+}]_c\) response is \(dT/dt\).

Another interesting feature of the cooling-induced \([Ca^{2+}]_c\) response is shown in Figure 3. In this experiment, temperature was decreased in a stepwise fashion. Again, as in Figure 1, the spikes in \([Ca^{2+}]_c\) demonstrate the role of \(dT/dt\). Consequently, there is no obvious increasing of \([Ca^{2+}]_c\) during the horizontal parts of the \(T\)-signal (when \(T\) is not decreasing but is maintained at a particular value). However, in this experiment we decreased the mean value of temperature. This caused a strong increase in sensitivity (as measured by increasing \([Ca^{2+}]_c\) amplitudes with decreasing temperature) which even overrides the attenuation (as measured by decreasing \([Ca^{2+}]_c\) amplitudes with time of cold exposure) seen in Figure 1(a).

The experiment in Figure 3 also shows that the \([Ca^{2+}]_c\) response is almost suppressed when the plant has already had prolonged exposure to low temperatures. The \([Ca^{2+}]_c\)
responses to a second set of stepwise temperature drops in Figure 3 confirm that the attenuation mechanism was operative in this experiment. However, a subsequent cooling at a higher cooling rate still elicited the typical $[\text{Ca}^{2+}]_c$ spike, demonstrating that this attenuation could be overcome by increasing the magnitude of the primary signal $dT/dt$.

If a much lower temperature than in Figure 1 is maintained after initial cooling, the resulting $[\text{Ca}^{2+}]_c$ spike, demonstrating that this attenuation could be overcome by increasing the magnitude of the primary signal $dT/dt$. The cold shock at the end of the experiment shows that the plant is still able to respond in a normal fashion. A representative curve is shown.

Both phases show attenuation when the cooling procedure is repeated with the same plant (Figure 4). This biphasic behaviour can also be seen in Nicotiana plumbaginifolia (data not shown) and therefore is not unique to Arabidopsis.

In order to obtain a clearer picture of the influence of the cooling rate ($dT/dt$) and the absolute temperature ($T$) on the kinetics of the $[\text{Ca}^{2+}]_c$ response, we conducted cooling experiments with a single cooling event (from $T_0=18^\circ C$ down to $T_f=4^\circ C$), but with different initial cooling rates (Nagai and Nakaoka, 1998). The experimental results (Figure 5a–d) show again that the cooling rate is the main parameter which determines the magnitude and form of the $[\text{Ca}^{2+}]_c$ increase; with high cooling rates only one single $[\text{Ca}^{2+}]_c$ peak is observed (Figure 5a). Lower cooling rates (Figure 5b,c) reveal the biphasic response already shown in Figure 4. An interesting finding here is that at very low cooling rates the $[\text{Ca}^{2+}]_c$ trace is lacking the first peak completely (Figure 5d) and only the second, prolonged slow response is observed. With extremely low cooling rates ($dT/dt<0.003^\circ C/s$, i.e. cooling by $10^\circ C$ in more than 1 h) no $[\text{Ca}^{2+}]_c$ response was observed at all (Figure 5d – lower trace).

However, we always found that the $[\text{Ca}^{2+}]_c$ response started almost simultaneously with the onset of cooling. This means that even a drop of $dT<1^\circ C$ is able to elicit a response as long as a sufficient cooling rate, $dT/dt$, is provided.

The fact that an increase in cooling sensitivity (i.e. sensitisation as measured by increasing $[\text{Ca}^{2+}]_c$ amplitudes with decreasing temperature) and attenuation of the $[\text{Ca}^{2+}]_c$ response (i.e. desensitisation as measured by decreasing $[\text{Ca}^{2+}]_c$ amplitudes with time of cold exposure) have opposite effects is demonstrated by the experiments shown in Figure 6. Here, a periodic cycling

A pattern of cold stimuli was given. From Figure 1 it would be expected that the amplitude of responses would decrease with time due to attenuation. However, the superimposed steady decrease of the mean temperature $T$ (Figure 6c) increases sensitivity and just compensates the effect of attenuation. This leads to permanent $[Ca^{2+}]_c$ oscillations where the amplitude of the $[Ca^{2+}]_c$ signal (Figure 6a) is correlated to the cooling rate amplitude (Figure 6b).

Finally, Figure 7 presents an experimental protocol which clearly demonstrates the operation of the three mechanisms (i.e. sensing, sensitisation and desensitisation) revealed above. Again, a stepwise decrease in temperature was applied as in Figure 3. However, now the cooling rate $dT/dt$ of the steps was increased from step to step (Figure 7). This increase in steepness together with the sensitising effect of the decrease in absolute temperature overcompensates attenuation. Thus, the $[Ca^{2+}]_c$ trace (Figure 7a) appears to be almost a mirror image of the cooling rate (Figure 7b) with increasing amplitudes. Concomitant with this increase in amplitude is an increase in the baseline of $[Ca^{2+}]_c$. This is most probably related to the mechanism of sensitisation as it is not observed when only attenuation is active (Figure 1a–c). After $T$ has levelled off to a steady value (in Figure 7 at $t=24\,\text{min}$), $[Ca^{2+}]_c$ starts to return to the previous baseline value. This indicates that sensitisation depends on the absolute temperature but that the degree of attenuation increases with the length of time of exposure to cold.

**Discussion**

*The cooling rate-dependent $[Ca^{2+}]_c$ influx*

The important feature of the data presented here is the dependence of the $[Ca^{2+}]_c$ increase on the cooling rate $dT/dt$ rather than the absolute temperature $T$. This fact also substantiates the hypothesis of Minorsky (1989) who investigated this phenomenon in cucumber seedlings by means of electrophysiological measurements (Minorsky and Spanswick, 1989). It is also in line with a recent study showing the cooling response of $[Ca^{2+}]_c$ in animal cells (Nagai and Nakaoka, 1998). Hence, the first conclusion from our present study is that the $Ca^{2+}$ influx mediating channels are dependent on the cooling rate, $dT/dt$, and not on the absolute temperature, $T$.

*Attenuation to cold is induced by prolonged or repeated cold exposure*

Another interesting feature in Figure 1(a) is that the amplitudes of the $[Ca^{2+}]_c$ spikes decrease when the temperature stimuli are repeated (Figure 1a,c). The dependence on past low temperature episodes that the plant has previously encountered (Figure 1a,c) indicate that the plant $[Ca^{2+}]_c$ response to cooling can be attenuated (Knight et al., 1996). This attenuation, which can also be termed as ‘desensitisation’, is a property of the time of cold exposure given. This feature can also be seen in Figure 3. Figure 1(b,d), however, shows that the attenua-
tion of the $[Ca^{2+}]_c$ responses can be (over)compensated by an increasing $dT/dt$ in subsequent cold shocks. This finding is also in line with what is known about cold-induced action potentials in *Cabomba australis* and *Cucurbita pepo* (Minorsky, 1989).

The overall sensitivity to cold is increased at lower absolute temperatures

Figure 3 provides information about the sensitivity of the temperature-sensing system. With decreasing absolute temperature ($T$) the responses to cooling are increased although the intensity of the stimulation (i.e. the cooling rate, $dT/dt$) is the same. Thus, sensitivity is dependent on the absolute temperature, $T$. As it seems likely that the Ca$^{2+}$ influx mediating channels are dependent on $dT/dt$ (Figures 1 and 2) we conclude that sensitivity acts on another mechanism (i.e. the Ca$^{2+}$-extrusion mechanism) which counteracts the Ca$^{2+}$-influx. Ca$^{2+}$ extrusion is achieved by Ca$^{2+}$-ATPases (Briskin, 1990; Bush, 1995) which have been shown to be strongly dependent on the absolute temperature, $T$ (Caldwell and Haug, 1981). At low temperatures the power of pumps is reduced to a certain fraction of full capacity defined by the $Q_{10}$ value of the pumps. Thus, the increased response at lower $T$ can be explained in terms of cold-induced inhibition of pump activity.

The biphasic character of the cold $[Ca^{2+}]_c$ response

Further information regarding the mechanisms of attenuation and sensitivity is shown in Figures 4 and 5. On the basis of the findings in Figures 1 and 3, the time course of the $[Ca^{2+}]_c$ responses in Figures 4 and 5 can be described as follows. The steep temperature slope at the beginning of the cold period causes the initial $[Ca^{2+}]_c$ peak which would return to the baseline under the conditions of Figure 1. However, in Figures 4 and 5 the horizontal part of the temperature kinetic occurs at a lower temperature. This increases the sensitivity which causes a long-lasting increase in $[Ca^{2+}]_c$ observed directly after the initial sharp peak. As the Ca$^{2+}$-influx is dependent on $dT/dt$ it cannot be active when $dT/dt$ is almost zero and thus cannot be responsible for the second phase. Therefore, a possible explanation for the second phase in the $[Ca^{2+}]_c$ kinetic is that the steady-state Ca$^{2+}$-leak current remains unaffected whereas the pumps become unable to counteract at low temperatures. A new higher $[Ca^{2+}]_c$ is thus established. The third mechanism, attenuation, comes into play thus bringing the $[Ca^{2+}]_c$ back to the baseline, as demonstrated in Figures 1 and 3.

At very low cooling rates which plants naturally undergo in the field, the attenuation mechanism is strong enough to suppress any $[Ca^{2+}]_c$ increase completely during the cooling process (Figure 5d). We therefore assume that the attenuation process is related to an increase in the number of active pumps which may be induced by the elevated Ca$^{2+}$-influx and which occurs in order to counteract the cold-induced inhibition of pump activity.

Thus, we suggest that attenuation and sensitisation both act on the Ca$^{2+}$-extrusion mechanism which operates when the Ca$^{2+}$-influx mechanism is inoperative. (This statement is drawn from Figures 4 and 5 and is further supported by Figures 6 and 7).

Conclusion

Cold-induced increases in $[Ca^{2+}]_c$ are mainly a function of the cooling rate, $dT/dt$. Thus, under persistent low temperature, $[Ca^{2+}]_c$ returns to normal resting levels.

Sensitivity increases (i.e. sensitisation occurs) with lower absolute temperature, $T$. Thus, increased $[Ca^{2+}]_c$ responses to cooling occur at lower $T$ even when the cooling rate, $dT/dt$, is the same.

Attenuation (i.e. desensitisation) occurs with increasing time of low $T$ exposure. Thus, the $[Ca^{2+}]_c$ responses to cooling decrease with time upon repeated exposure to low temperatures under identical regimes.

Sensitisation and desensitisation operate on the Ca$^{2+}$-extrusion mechanism whereas the cooling rate operates on the Ca$^{2+}$-influx mechanism.

Experimental procedures

All experiments were carried out in unbuffered standard medium (SM) containing KNO$_3$, CaCl$_2$ and MgCl$_2$, 0.1 mM each.

Plant material

Roots of transgenic *Arabidopsis thaliana* (biotype RLD1) expressing cytosolic apoaequorin were used for $[Ca^{2+}]_c$ measurements (Knight et al., 1997b) instead of whole plants because of their higher transparency and lack of air-filled apoplast which would hinder fast and reproducible heat exchange. Plants were grown in 9 cm Petri dishes on vertical 1.2% agar plates with half-strength MS medium (Gamborg et al., 1968; Murashige and Skoog, 1962) at 21°C with 16 h photoperiod. After 2 weeks, plants were individually transferred to sterile 50 ml falcon tubes and cultivated hydroponically on 0.5 × MS for another 2-4 weeks under the same conditions.

Preparation of roots

Whole root systems were dissected from plants and used when 4- to 6-weeks-old. For $[Ca^{2+}]_c$ measurements reconstitution of aequorin was performed in vivo essentially as described previously (Knight et al., 1991). Briefly, roots were incubated in water containing 2.5 μM coelenterazine (Prolume Ltd, Pittsburgh, PA, USA) in the dark overnight at 21°C in order to allow formation of functional aequorin.
The experiments were performed by placing a root on wet filter paper fixed on a glass microscope slide. The slide with the root was fixed in front of a purpose-built heat exchange coil which could be placed in a 50 ml falcon tube. During the experiments the tube was permanently perfused with fresh SM. The SM was always aerated in order to avoid anoxic stress which can also cause changes in \([\text{Ca}^{2+}]_c\). (Sedbrook et al., 1996). The pH of the unbuffered aerated SM was always in the range between 5.4 and 5.7.

\([\text{Ca}^{2+}]_c\) measurements

The tube with the root and heat exchanger was placed in purpose-built light tight sample housing in front of a digital chemiluminometer. Luminescence counts were integrated every 2 sec and calibrated in terms of \([\text{Ca}^{2+}]_c\)-values as described previously (Knight et al., 1997b; Knight and Knight, 1995). The temperature was measured in parallel by means of a NTC-resistor which was placed near the roots as described previously (Plieth and Hansen, 1992). The A/D converted thermosensor voltage was fed into a second computer which simultaneously triggered the photon counter. This experimental set-up enabled us to measure temperature (T) with a resolution of less than 0.01°C simultaneously with the calcium-dependent luminescence emitted from the Arabidopsis roots expressing aequorin, with a sample frequency of 0.5 Hz.

Temperature adjustments

Cooling or heating of the roots was performed by drawing ice-cold or pre-warmed water through the heat exchange coil. The rate of temperature changes (dT/dt) was determined by the flux through the coil. Regression analysis was performed using MicroCal Origin vers. 2.8 (MicroCal, Northampton, MA, USA).

Acknowledgements

We thank Mr M. Brix (Physics Department Workshop, University Kiel, Kiel, Germany) for manufacturing the luminometer sample housing. This study was supported by the Deutsche Forschungsgemeinschaft (C.P. was funded by PL253/1-1). M.R.K. is a Royal Society University Research Fellow.

References


