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Temperature Sensing by Plants: Calcium-Permeable Channels as Primary Sensors—A Model

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Abstract. Recently the properties of temperature sensing in plants have been demonstrated experimentally by Plieth et al. (*The Plant Journal* 1999. **18:**491–497). The relevant biophysical parameters are established here by mathematical modeling in order to understand the experimental findings in quantitative terms. A simple one-compartment model is presented, as a preliminary approach to explain how the input signal (i.e., temperature *T*) is perceived and how the information is translated into an output signal in the plant cell (i.e., $[Ca^{2+}]_c$). The model is based on the fact that calcium influx into the cytoplasm is mediated by calcium-permeable channels which are assumed to be solely dependent on cooling rate (*dT/dt*) and calcium efflux is mediated by calcium pumps which have been shown to be dependent on absolute temperature (*T*). Firstly, it is demonstrated that this model is able to meet the demand for a satisfactory interpretation of the experimental data, and secondly that it reproduces the experimentally observed features of the cooling induced $[Ca^{2+}]_c$ changes well. This suggests that the primary temperature sensor in plants might be a Ca^{2+}-permeable channel.

Key words: Calcium permeable channels — Calcium pump — Cytoplasmic calcium — Adaptation — Signal transduction

Introduction

Many environmental and endogenous stimuli are linked to changes in $[Ca^{2+}]_c$ in plants (Gong et al., 1998; Knight, H. et al., 1996; Knight, M.R., Smith & Trewavas, 1992; Knight, M.R. et al., 1991; 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 a second) by an immediate and transient rise in cytosolic calcium ($[Ca^{2+}]_c$) (Knight, M.R. et al., 1991, 1992; Russell et al., 1996). Based on many different experimental findings described in the literature of the last 150 years and on electrophysiological studies, Minorsky (1989) hypothesized that temperature sensing in plants depends on cooling rate (*dT/dt*) rather than on absolute temperature (Minorsky & Spanswick, 1989; Minorsky, 1989). However, 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, Öquist and Sarhan (1998) mentioned the photosynthetic apparatus which might sense changes in temperature through increased energy imbalance and photoinhibition. Finally it has been hypothesised that the cold-induced $[Ca^{2+}]_c$ response is the primary sensing event (Minorsky, 1989; Pickard, 1984) and Ca^{2+}-permeable channels were even proposed to be the primary sensors (Monroy & Dhindsa, 1995). Therefore Plieth et al. (1999) have measured $[Ca^{2+}]_c$ in *Arabidopsis* roots in response to different temperature change regimes, in order to gauge their effects on the cold-sensing system in plant cells. Their results demonstrate directly that the $[Ca^{2+}]_c$ response in plants to cooling is dependent on the cooling rate *dT/dt.* Similar findings have also been reported for animal systems by Nagai and Nakaoka (1998).

A valuable approach to understand the biophysical parameters pertaining to the processes involved is the formulation of a mathematical model (Brook & Wynne, 1988; Sanderson, 1995). Modeling which may help to understand the experimental findings in quantitative terms has to start from the fact that the cytoplasmic calcium concentration $[Ca^{2+}]_c$ in living organisms is balanced by several influx and efflux processes. Buffers (Plieth, Sattelmacher & Hansen, 1997; Plieth & Hansen, 1998) can smooth transitions, but cannot influence the

import-export balance. Thus, the experimental results (Plieth et al., 1999) are interpreted here solely in terms of temperature action on Ca^{2+} influxes (I_{IN}) and effluxes (I_{EX}).

The Model

Due to the prevailing driving forces, influx into the cytosol (I_{IN}) is mediated by calcium-permeable channels (Johannes, Brosnan & Sanders, 1991; Thuleau et al., 1994*a*,*b*). To keep the model as simple as possible we have to restrict our interest to channels in the plasmalemma. However, the iterative algorithm developed below does not exclude the participation of Ca^{2+}-permeable channels of internal stores (Allen & Sanders, 1994*a*,*b;* Johannes, Brosnan & Sanders, 1992*a*,*b;* Knight, Trewavas & Knight, 1996; Plieth et al., 1998) in particular and the participation of other parts of the cell in temperature sensing and acclimation (Kawczinski & Dhindsa, 1996) in general. The Ca^{2+} driving forces also imply that calcium efflux (I_{EX}) has to be mediated by Ca-ATPases (calcium pumps) (Briskin, 1990). Here again, the restriction to plasmalemma pumps does not exclude the participation of pumps in membranes of internal stores. The model presented here is based on the statements above and two main assumptions: (i) The dependence of the cooling-induced $[Ca^{2+}]_c$ increase on the cooling rate *dT/dt* is brought about by the opening of Ca^{2+} permeable influx channels. (ii) If *dT/dt* is zero or even positive (i.e., heating) changes in $[Ca^{2+}]_c$ (i.e., the return of the $[Ca^{2+}]_c$ level to normal steady state) have to be associated with the Ca^{2+} pumps, because under these conditions the cooling-activated channels will be closed.

Two additional experimental findings have to be explained by the model: Sensitization (i.e., increase of the $[Ca^{2+}]_c$ response to cooling (*dT/dt*) at lower absolute temperatures (*T*), Fig. 2*e*) and desensitization (i.e., attenuation of the $[Ca^{2+}]_c$ response with time of cold exposure, Fig. 2*a* and *e*). The first effect can be explained by the dependence of the Ca^{2+}-pump turnover rate on the absolute temperature *T* (Caldwell & Haug, 1981). The second effect is introduced by a continuous adjustment of pump capacity to an elevated mean $[Ca^{2+}]_c$ in order to maintain a basic low $[Ca^{2+}]_c$ even at low temperatures. This attenuates the effect of channel opening.

The mathematical background of the model is described in detail below. For reasons of simplicity we consider a one-compartment system in which the $[Ca^{2+}]_c$ is balanced by two calcium flux mechanisms (Fig. 1*a*).

Quantitative Description

Both, Ca^{2+}-permeable channels, and Ca^{2+} pumps are enzymes which are in the following modeled as membrane bound receptor molecules (Bray, 1995) dependent on cooling rate and absolute temperature, respectively. Hence, it makes sense to assume an allosteric behavior, which enables these proteins to perform their computational task (Bray, 1995).

The Calcium Influx into the Cell(I_{IN}) is Mediated by Ca^{2+} Permeable Channels

The activity of calcium influx transporters (channels) is assumed to be only dependent on the cooling rate (*dT/dt*) in a hypersensitive (i.e., sigmoidal) manner:

$$I_{IN} = I_{IN\,0} + \frac{I_{IN\,max} \cdot \left(\frac{dT}{dt}\right)^{n1}}{K_1^{n1} + \left(\frac{dT}{dt}\right)^{n1}} \tag{1}$$

The free parameters are: K_1, n_1, I_{IN0}, I_{INmax}, where I_{INmax} is the maximal ion current which can be achieved. K_1 is the cooling rate which yields half of the maximal current (i.e., a measure of the sensitivity of the channels). n_1 denotes the 'degree of cooperativity'. I_{IN0} is the steady-state leak influx into the resting cell which is usually compensated by a steady state outward-rectified pump current (I_{EX0} *see below*).

Any other dependency of I_{IN} on the Ca^{2+} gradient across the plasma membrane is omitted for simplifying reasons. This is possible because Ca-influx has a huge surplus of driving force. Thus, it can be assumed that the transporter operates in the saturation-region, with flux rate being independent of driving force.

The important feature in Eq. 1 is the dependence of the Ca^{2+} influx on cooling rate *dT/dt* and not on absolute temperature, *T*. Thus, the channel acts as a differentiator. Such a characteristic of a Ca^{2+}-permeable channels is not surprising and has been described earlier (Sachs, Qin & Palade, 1995).

To account for the assumption above that the channel activity is solely dependent on cooling (*dT/dt* < 0), positive temperature changes (i.e., heating) have to be avoided. For computational convenience this is achieved by the following modification of Eq. 1:

$$I_{IN} = I_{IN\,0} + \frac{I_{IN\,max} \cdot \left(-\frac{\left(\left(\frac{dT}{dt}\right) - \left|\frac{dT}{dt}\right|\right)}{2}\right)^{n1}}{K_1^{n1} + \left(-\frac{\left(\left(\frac{dT}{dt}\right) - \left|\frac{dT}{dt}\right|\right)}{2}\right)^{n1}} \tag{2}$$

where $|dT/dt|$ denotes the absolute value.

The Efflux of Calcium (I_{EX}) is Mediated by the Calcium Efflux Pumps

The enzymatic activity of this Ca^{2+}-ATPase is known to have an exponential dependence on temperature (Caldwell & Haug, 1981). Also for the sake of simplicity a strict linear Arrhenius plot is assumed here with a constant Q_{10} value. The Q_{10} value denotes the proportion to which the enzyme activity increases if the temperature is increased by 10°C. Caldwell and Haug (1981) found factors in the range of $2 < Q_{10} < 20$. Thus:

$$I_{EX} = I_{EX\,0} \cdot \exp(K_Q \cdot (T - T_0)) \quad \text{whereas } K_Q = \frac{\ln(Q)}{10} \tag{3}$$

Table. Model parameter chosen for the iterative calculation of the $[Ca^{2+}]_c$ for different temperature experiments

T-data from Plieth et al. (1999)	Ca^{2+}-model presented here	Influx (channels)				Efflux (pumps)				Desensitization	
		I_{IN0} µM/sec	I_{INmax} µM/sec	K_1 µM	n_1	I_{EXmax} µM/sec	K_M µM	n_2	Q	P_0 µM/sec^2	K_P µM/sec
	Fig. 1*b–h*	0.005	3	1	3	1	0.5	2	10	0.005	0.5
Fig. 1*a*	Fig. 2*a*	0.005	1	1	1.5	1	0.5	2	10	0.01	0.5
Fig. 1*b*	Fig. 2*b*	0.01	1	2	1.5	5	0.5	2	10	0.01	0.5
Fig. 3	Fig. 2*e*	0.005	2	1	1.5	1	0.5	2	10	0.005	0.5
Fig. 7	Fig. 3*a*	0.005	2	1	1.5	1	0.5	2	10	0.005	0.5
Fig. 6	Fig. 3*c*	0.001	1	1	1.5	1	0.5	2	10	0.005	0.5

$[Ca^{2+}]_{c0}$ was always set to 0.1 µM.

I_{EX0} is the current at $T = T_0$. Thus, it becomes obvious that if $T - T_0 = 10$°C then $I_{EX} = Q\ I_{EX0}$. Nevertheless, the pump current is also dependent on the $[Ca^{2+}]_c$ (i.e., the enzyme's substrate) at least in a simple Michaelis-Menten dependent fashion. However, in order to include the option of a cooperative action, a Hill exponent n_2 is introduced, whose value will be determined by fitting the data.

$$I_{EX0} = I_{EX\,max} \cdot \frac{[Ca^{2+}]_c^{n2}}{K_m^{n2} + [Ca^{2+}]_c^{n2}} \tag{4}$$

Introducing Eq. 4 into Eq. 3 yields the temperature dependent pump current:

$$I_{EX} = I_{EX\,max} \cdot \exp(K_Q \cdot (T - T_0)) \cdot \frac{[Ca^{2+}]_c^{n2}}{K_m^{n2} + [Ca^{2+}]_c^{n2}} \tag{5}$$

The free parameters are: I_{EXmax}, Q, K_m, n_2.

Desensitization is Simulated by Increasing Number of Active Pumps

As there is an obvious attenuation of the $[Ca^{2+}]_c$ response during continuous exposure to low temperatures (Plieth et al., 1999, e.g., Fig. 2*a*) we have to consider this in our model. The desensitization mentioned above was assigned to the activation of the pump. This can be modeled in various ways. To keep the whole system as simple as possible it is assumed that a long lasting elevated $[Ca^{2+}]_c$-level stimulates the activation of Ca^{2+}-ATPases, thus, increasing I_{EXmax} to counteract the cold-reduced power of the pumps by increasing the number of active pumps. From the view of a living cell it makes sense when the activation of pumps (dI_{EXmax}/dt) depends on the deviation of $[Ca^{2+}]_c$ from the base level $[Ca^{2+}]_{co}$:

$$\frac{dI_{EX\,max}}{dt} = P_0 \cdot \mathrm{sign}([Ca^{2+}]_c - [Ca^{2+}]_{co}) \cdot \frac{[Ca^{2+}]_c}{K_P + [Ca^{2+}]_c} \tag{6}$$

$\mathrm{sign}([Ca^{2+}]_c - [Ca^{2+}]_{c0}) = -1$ when $[Ca^{2+}]_c < [Ca^{2+}]_{c0}$ otherwise it is +1. Thus, the number of active pumps (as measured by I_{EXmax}) increases when the $[Ca^{2+}]_c$ is increased, and pumps are switched off when $[Ca^{2+}]_c$ falls below $[Ca^{2+}]_{c0}$. The free parameters are: P_0, K_P, $[Ca^{2+}]_{c0}$.

Iterative Calculation of the Model

The cytosol is a reservoir of $[Ca^{2+}]_c$ filled and depleted by influx and efflux (Fig. 1*a*). The temporal behavior of $[Ca^{2+}]_c$ is obtained from an iterative calculation (i.e., calculating Ca_{i+1} at time $t_{i+1} = t_i + dt$ from Ca_i at time t_i) with respect to the given temperature T_i and the temperature change $(dT/dt)_i = T_i - T_{i-1}$

$$Ca_{i+1} = Ca_i + (I_{INi} - I_{EXi}) \cdot dt \tag{7}$$

Introducing Eqs. 2, 5, 6 into the iteration Eq. 7 yields:

$$Ca_{i+1} = Ca_i + dt \cdot \left(I_{IN0} + \frac{I_{IN\,max} \cdot \dot{T}_i^{n1}}{K_1^{n1} + \dot{T}_i^{n1}} - I_{Ex\,max(i+1)} \cdot \exp(K_Q \cdot (T_i - T_0)) \cdot \frac{Ca_i^{n2}}{K_m^{n2} + Ca_i^{n2}} \right) \tag{8}$$

with $\dot{T}_i = -½ \cdot ((dT_i/dt) - |dT_i/dt|)$, according to Eq.2 and

$$I_{EX\,max(i+1)} = I_{EX\,max(i)} + dI_{EX\,max(i)} \tag{9}$$

and $dI_{EX\,maxi} = dt \cdot (P_0 \cdot \mathrm{sign}(Ca_i - Ca_{co}) \cdot Ca_i/(K_P + Ca_i))$, according to Eq. 6. All iterative calculations were performed on an Excel worksheet (vers. 7.0, MicroSoft) which can be obtained from the author.

Choosing Appropriate Parameters

The whole model consists of eleven free parameters: Four for the influx (K_1, n_1, I_{IN0}, I_{INmax}), four for the efflux (I_{EXmax}, Q, K_m, n_2) and three for the desensitization ($[Ca^{2+}]_{c0}$, P_0, K_P). Because of numerical reasons all parameters are given in terms of µM concentrations of $[Ca^{2+}]_c$ (Table). Many different parameter sets had to be tested for their ability to fit the experimental findings. However, previous publications and certain considerations restricted most parameters to a reasonable range of physiological meaningful and relevant values limiting the number of fits to be performed.

Many previous studies showed that the steady-state cytoplasmic calcium concentration $[Ca^{2+}]_{c0}$ in plants is usually in the range of 80 nM $\leq [Ca^{2+}]_{c0} \leq$ 250 nM (Bauer et al., 1998; Knight, H. et al., 1996; Plieth et al., 1998, 1999). The maximum of the $[Ca^{2+}]_c$ peak during cold shock is as high as 2 µM and reached within less of two seconds (Knight, H. et al., 1996). Thus a maximum inward current of $I_{INmax} \geq$ 1 µM/sec seems to be an appropriate estimate. The maximal outward

current (I_{EXmax}) is assumed to be of the same order of magnitude (about 1 μM/sec), since a calcium clearance after cold shock is achieved within about 20 sec (Knight, H. et al., 1996) although the pumps are inhibited at the much lower temperature T by a factor of $Q_{10} = 10$ (*see below*). Compared to this the steady state influx leakage through the plasma membrane has to be some orders of magnitude lower ($I_{IN0} \leq 0.01$ μM/sec) because it would otherwise affect the energy balance (ATP-consumption) of the cell. The Ca^{2+} affinities (K_M) of Ca-ATPases have been shown to be in the range of $0.07\ \mu M < K_M < 12\ \mu M$ (Bush, 1995). Thus, physiological meaningful values are within this interval. Findings of Caldwell and Haug (1981) are used for a first estimate of Q_{10}. They found that the activity (V_{max}) of the Ca-pump is decreased about one order of magnitude when a 10°C lower temperature was adjusted. For reasons of simplicity a linear Arrhenius plot and thus an exponential dependency of $V_{max} = I_{EX0}$ on the temperature with $Q_{10} = 10$ is assumed here.

Responses of $[Ca^{2+}]_c$ were iteratively calculated on the basis of the above equations (Eqs. 1–9) and with different temperature protocols as input variables. Sets of parameters which generated $[Ca^{2+}]_c$ time series close to the experimental findings are listed in the Table.

Results and Discussion

A first step to prove the validity of the model is its application to experiments where single exponentially decaying cooling steps with different initial cooling rates (i.e., different time constants) are applied (Fig. 1*b–h*).

A single $[Ca^{2+}]_c$ peak is obtained when a 'cold shock' is applied (Fig. 1*b*). At very low cooling rates however the response lacks the initial peak completely (Fig. 1*g*). The deprivation of pump power by low temperature leads to the second slow phase of the $[Ca^{2+}]_c$ increase because the Ca^{2+} leak influx current is maintained. A biphasic response to a single cooling step is thus obtained when the sensitizing action on the pumps by low temperature (T) reaches a similar magnitude as the response of the channels to the changes in temperature (dT/dt) (Fig. 1*c–e*). The responses predicted by the model here (Fig. 1) reflect all characteristics of the measured ones (Plieth et al., 1999).

In Figs. 2 and 3 the temperature protocols from Plieth et al. (1999) were used to calculate the predicted responses. The parameters employed in the calculation of the responses in Figs. 1–3 are shown in the Table. The comparison of measured data (grey traces) and predicted changes in $[Ca^{2+}]_c$ (bold lines) shows that the basic features of the experimental results are predicted correctly.

Basically, the accuracy of the predictions does not verify that the underlying model is true, but it shows that the assumptions are not in contradiction to the experimental results. This indicates that the model gives a possible explanation for the observations.

The time series modeled in Figs. 2 and 3 provide an interesting representation of the cooperation of the different mechanisms involved in the cooling response of

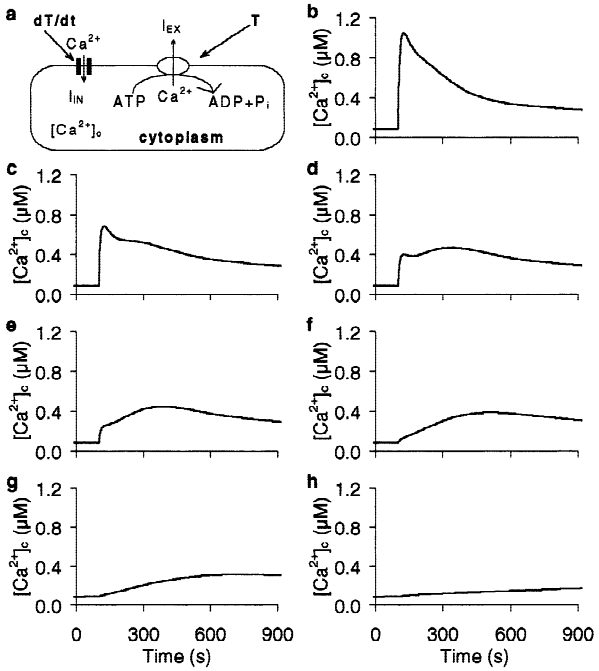

Fig. 1. Responses of $[Ca^{2+}]_c$ to single cooling steps at $t = 100$ sec with different initial rates of cooling predicted by a simple one-compartment model. (*a*) Scheme of a one-compartment model with passive Ca^{2+} influx transporters which are modulated by the cooling rate, dT/dt, and active efflux transporters which are directly affected by the absolute temperature, T. $[Ca^{2+}]_c$ = cytoplasmic free calcium concentration. (*b–h*) Numerical simulation of $[Ca^{2+}]_c$ responses to different rates of cooling from 18°C down to 4°C. The underlying model and its iterative equations are given in the text. The parameter values employed here are displayed in the Table. The cooling rate is given by the time constant of the applied exponential temperature decay and its amplitude. The time constants τ correspond to different initial cooling rates as given in the following:

Figure	b	c	d	e	f	g	h
τ (sec)	30	35	45	60	120	240	900
Initial cooling Rate (°C/sec)	−0.47	−0.40	−0.31	−0.23	−0.12	−0.06	−0.016

$[Ca^{2+}]_c$. Thus, the time courses of the parameters responsible for sensitization (i.e., $\exp(K_Q(T - T_0))$, a measure of the actual pump power) and desensitization (i.e., I_{EXmax}, a measure of the number of active pumps) are displayed in Fig. 4 in addition to the predicted and measured time courses of $[Ca^{2+}]_c$ shown in Fig. 2*e*. Here it becomes obvious how the interplay of sensitizing and desensitization of the pump changes the magnitude of the transient $[Ca^{2+}]_c$ responses and the value of the $[Ca^{2+}]_c$ level.

As already mentioned above, the sensitizing characteristic (deprivation of pump power) of the system is in parallel with the temperature T (Fig. 2*f vs.* Fig. 4*a*)

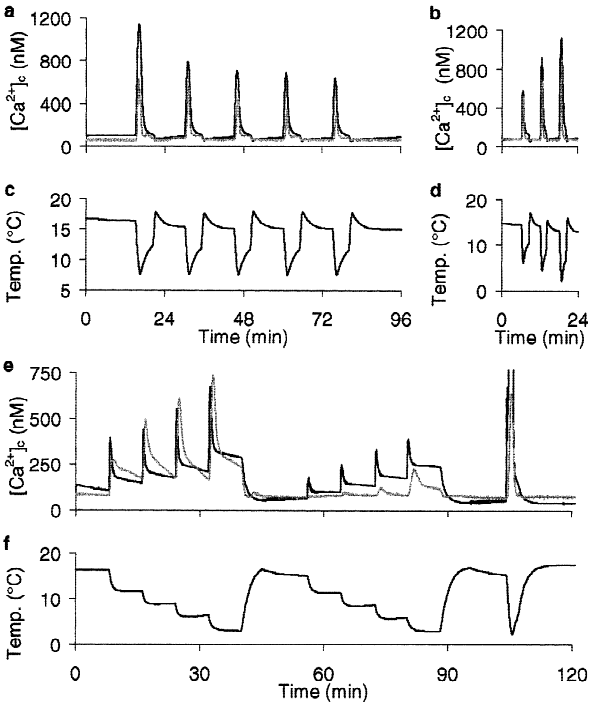

Fig. 2. Time series of $[Ca^{2+}]_c$ iteratively calculated from the temperature time courses on the basis of the model shown in Fig. 1*a*. The iterative equations are derived in the text. The parameters of the simulations are given in the Table. The measured $[Ca^{2+}]_c$-time series (Plieth et al., 1999) are overlaid here in *a*, *b*, and *e* as grey lines for comparison. (*a–d*) Effect of repetitive cold shocks on $[Ca^{2+}]_c$. (*a*) Responses to cold shocks with constant temperature amplitudes (*c*). (*b*) Responses to cold shocks with increasing temperature amplitudes (*d*). (*e*) Responses to stepwise decrease in the temperature (*f*) as input signal.

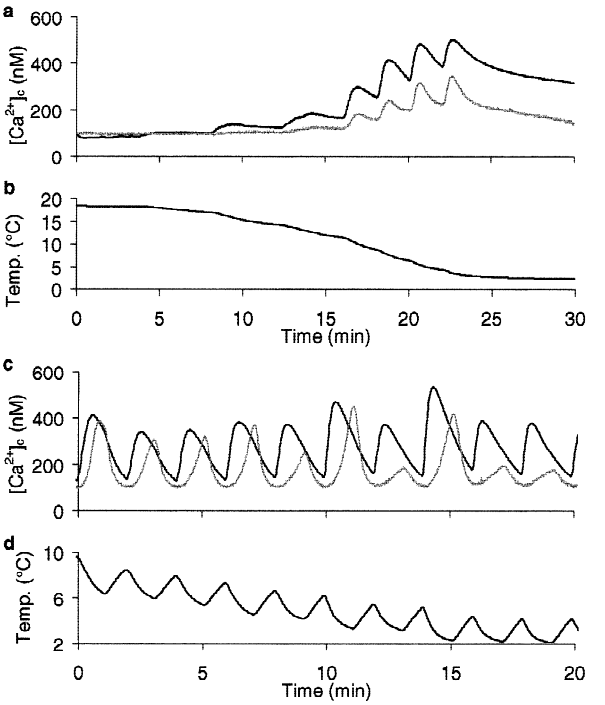

Fig. 3. Time series of $[Ca^{2+}]_c$ iteratively calculated from the temperature time courses on the basis of the model shown in Fig. 1*a*. The iterative equations are derived in the text. The parameters of the simulations are given in the Table. The measured $[Ca^{2+}]_c$-time series (Plieth et al., 1999) are overlaid as grey lines for comparison in *a* and *c*. (*a* and *b*) $[Ca^{2+}]_c$ responses to cooling with changes in cooling rate; (*c* and *d*) $[Ca^{2+}]_c$ responses to an oscillating cooling regime (*d*) as input signal.

whereas the desensitizing characteristic (number of active pumps) increases in response to the duration of elevated cytosolic Ca^{2+} during low temperature exposure (Fig. 4*b*). The latter decays slowly as soon as $[Ca^{2+}]_c$ is back to the previous base level (i.e., $[Ca^{2+}]_{c0}$).

Deviations Between Predicted and Measured Curves

The modeling above shows that the experimental data can be well described by the model depicted in Fig. 1*a*. Deviations between predicted and measured time courses (Figs. 2 and 3) should not be taken too seriously as this first approach was aimed solely at modeling the basic mechanisms. The main reason that the model does not match the obtained data exactly is that many simplifications have been made in order to keep the mathematics as uncomplicated as possible. Several features are neglected:

(i) Internal stores (e.g., vacuole; Knight, H. et al.,

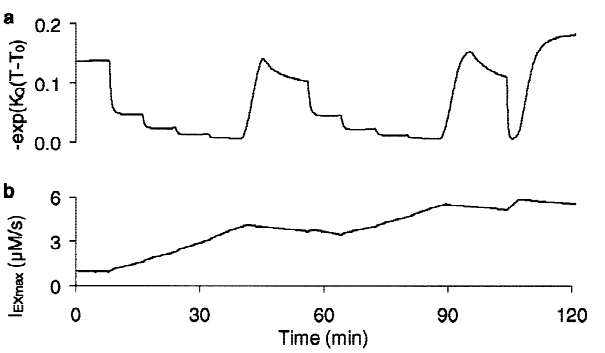

Fig. 4. Time series of sensitization (*a*) and desensitization (*b*) from the experiment shown in Fig. 2*e, f*. Sensitization (i.e., deprivation of pump power given by the term $\exp(K_Q(T - T_0))$, with $T_0 = 25°C$) and desensitization (i.e., number of active pumps given by I_{EXmax}) were iteratively calculated from the temperature time course in Fig. 2*f* on the basis of the model shown in Fig. 1*a* and on the basis of the equations given in the text. The corresponding traces of modeled and measured $[Ca^{2+}]_c$ are shown in Fig. 2*e*. The parameters of the simulations are given in the Table.

1996) participate in the $[Ca^{2+}]_c$ increase in response to low temperature by release of their Ca^{2+} ions. This would render the model much more complicated as the internal stores are depletable and certainly have pumps and channels of different biophysical and biochemical properties (Bush, 1995). (ii) Limitations of speed and kinetics of $[Ca^{2+}]_c$ signaling by diffusion processes (Neher & Augustine, 1992) as influenced by buffer capacity and buffer mobility (Plieth et al., 1997; Plieth & Hansen, 1998; Zhou & Neher, 1993). (iii) Nonlinear Arrhenius plot of the temperature dependency of the pump activity which ought to be approximated by several linear lines of different slopes as shown by Caldwell and Haug (1981). (iv) Further mechanisms which may contribute to the cold acclimation phenomena in *Arabidopsis* such as change of the receptor sensitivity to cooling rate (i.e., K_1, Eq. 1) or change of membrane lipid composition and general membrane properties after cooling as shown by Murata and Los (1997) and Lynch (1990).

Direct determinations of channel and pump rates which are not accessible to experimental recordings in vivo at the present stage will have to be performed in the future to evaluate the interference by the latter mechanisms mentioned above. At present the above conclusions can be used as a basis for the discussion of the physiological importance of the cooling induced $[Ca^{2+}]_c$ response in general and of the importance of Ca^{2+}-permeable channels as possible primary cold sensors in particular. However, even if the primary mechanism for cooling-evoked changes in cytosolic $[Ca^{2+}]$ is produced by Ca^{2+} permeable channels, the sensor itself could still be a regulatory element upstream of the channels.

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