Oscillations in plant membrane transport: model predictions, experimental validation, and physiological implications

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Abstract

Although oscillations in membrane-transport activity are ubiquitous in plants, the ionic mechanisms of ultradian oscillations in plant cells remain largely unknown, despite much phenomenological data. The physiological role of such oscillations is also the subject of much speculation. Over the last decade, much experimental evidence showing oscillations in net ion fluxes across the plasma membrane of plant cells has been accumulated using the non-invasive MIFE technique. In this study, a recently proposed feedback-controlled oscillatory model was used. The model adequately describes the observed ion flux oscillations within the minute range of periods and predicts: (i) strong dependence of the period of oscillations on the rate constants for the H+ pump; (ii) a substantial phase shift between oscillations in net H+ and K+ fluxes; (iii) cessation of oscillations when H+ pump activity is suppressed; (iv) the existence of some ‘window’ of external temperatures and ionic concentrations, where non-damped oscillations are observed: outside this range, even small changes in external parameters lead to progressive damping and aperiodic behaviour; (v) the frequency encoding of environmental information by oscillatory patterns; and (vi) strong dependence of oscillatory characteristics on cell size. All these predictions were successfully confirmed by direct experimental observations, when net ion fluxes were measured from root and leaf tissues of various plant species, or from single cells. Because oscillatory behaviour is inherent in feedback control systems having phase shifts, it is argued from this model that suitable conditions will allow oscillations in any cell or tissue. The possible physiological role of such oscillations is discussed in the context of plant adaptive responses to salinity, temperature, osmotic, hypoxia, and pH stresses.

Key words: Adaptation, encoding, feedback, ion flux, membrane, rhythms, stress.

Introduction

Membranes have often been postulated as a central component of cellular oscillators (Scott, 1957; Jenkinson and Scott, 1961; Njus et al., 1974; Buschmann and Gradmann, 1997; Gradmann and Buschmann, 1997). Oscillations in membrane-transport activity are ubiquitous in the plant kingdom. Examples include rhythmical changes in surface (Scott, 1957; Newman, 1963; Hecks et al., 1992) and plasma membrane potential (Felle, 1988; Blatt and Thiel, 1994; Grabov and Blatt, 1998; Tyerman et al., 2001), vacuolar potential and current oscillations (Vucinic et al., 1978; Miedema et al., 2000), concentration changes in the apoplast (Engelmann and Antkowiak, 1998), oscillations in cytosolic pH (Felle, 1988) and Ca2+ (Bauer et al., 1998; McAinsh et al., 1995; Ehrhardt et al., 1996; Blatt, 2000; Holdaway-Clarke and Hepler, 2003), and net ion flux oscillations across the plasma membrane of various cell types (Feijo et al., 2001; Holdaway-Clarke et al., 1997;
Shabala and Newman, 1997a, 1998; Shabala et al., 1997; Shabala and Knowles, 2002; Zonia et al., 2002; Holdaway-Clarke and Hepler, 2003). These oscillations are observed at various levels of structural organization, from the molecular to the whole plant, and they cover an extremely broad range of periods, from several milliseconds (oscillations in the single channel conductance; Markevich and Sel’kov, 1986) to circadian and diurnal rhythms (Webb, 2003; Macduff and Dhanoa, 1996). However, despite decades of intensive research, little is known about the biochemical or biophysical nature of the oscillators, except for the broad generalization that membranes and ion fluxes are involved, directly or indirectly (Satter and Galston, 1981). With a possible exception of oscillations in cytosolic free Ca\(^{2+}\) in guard cells (McAinsh et al., 1995; Hetherington et al., 1998; Blatt, 2000; Allen et al., 2000; Evans and Hetherington, 2001) and pollen tubes (Holdaway-Clarke et al., 1997; Feijo et al., 2001; Holdaway-Clarke and Hepler, 2003), many plant physiologists still treat oscillations in membrane-transport activity as a ‘curiosity’. Why?

The answer lies partially in the ‘unpredictability’ of such oscillations. Quite often, the oscillations are strongly damped and, therefore, are not easily observed. For example, a strong association was found between oscillations in root H\(^{+}\) and Ca\(^{2+}\) fluxes and root growth rate (Shabala et al., 1997; Shabala and Newman, 1997a), with no oscillations found in roots growing slower than 2 \(\mu\)m min\(^{-1}\). These oscillations were always observed in the elongation and meristematic regions of plant roots, but only occasionally in the mature root zone (Shabala and Knowles, 2002). What specific properties of cells make this difference? Also, as measuring membrane potential in fast-growing tissues is a great methodological challenge, the lack of experimental observations is not surprising. Another complication is the strong dependence of such oscillations on plant developmental stage (Shabala et al., 2001), temperature (Macduff and Dhanoa, 1996; Erdei et al., 1998), light (Zivanovic and Vunic, 1996), nutrient availability (McAinsh et al., 1995; Buer et al., 2000), etc. Are the oscillations an exception rather than the rule?

Scott (1957) argued that electric oscillations at broad bean root tips required a feedback system for their explanation. He suggested a three-component loop: electric field, auxin supply and ‘wall’ (= membrane) permeability. The roots had a natural period of \(~6\) min and showed resonance at the same period in response to applied auxin or osmotic oscillations (Jenkinson and Scott, 1961). Today, membrane-transport processes in plants are believed to be controlled by a large number of positive and negative feedbacks (Hansen, 1978; Fisahn et al., 1986; Felle, 1988). Electric field and membrane permeability remain key components. The foundations of systems theory suggest that such a feedback-controlled system will oscillate, with some characteristic period, under certain conditions. What are these conditions?

If the relationship between several parameters within the systems is described by linear relationships, such a system should eventually reach its stable state (Stucki and Somogyi, 1994). If perturbed, it will eventually return to a new stable state through a series of damped oscillations. This is often observed for plant electrophysiological characteristics (Lefebvre et al., 1970; Gradmann and Slayman, 1975). The story is quite different if the system is governed by non-linear mechanisms. In that case, a limited cycle (a two-dimensional attractor), rather than a singular point, will be a stable condition (Stucki and Somogyi, 1994). Thus, self-sustained oscillations are expected to be found in such non-linear systems.

There is no doubt that membrane-transport processes are governed by non-linear mechanisms. Thus, as soon as the disturbance to cell homeostasis (caused by either internal or external stimuli) is beyond the linear range, non-damping self-sustained oscillations are expected to be seen. Physiologically, it means that there is a certain environmental ‘window’, within which oscillations may be observed. How can it be quantified?

There is no shortage of models describing various types of cellular oscillators (Chay, 1981; Antkowiak and Engelmann, 1995; Homble, 1996; Grabov and Blatt, 1998; Miedema et al., 2000). The simplest case is a two-component system. This may be either the inward and outward current mechanisms, interacting through changes in intracellular Ca\(^{2+}\) (Berridge and Rapp, 1979), or a carrier-type transporter (e.g. electrogenic pump) and a channel, coupled through membrane voltage (Fisahn et al., 1986). The necessary condition is that these transporters have different equilibrium voltages with one of them providing positive feedback (Buschmann and Gradmann, 1997). Even these, obviously oversimplified, models, predict complex oscillatory behaviour in plant membrane-transport activity. More complex models (Gradmann, 2001) suggest that such ensembles of coupled oscillators might produce a range of responses, from the steady-state to oscillatory or even chaotic responses.

Despite numerous attempts to quantify oscillatory processes at plant membranes, no direct comparison between model predictions and experimental observations has been made. In this paper, a five-component model of coupled membrane oscillators was used (Gradmann, 2001) to compare model predictions with experimental observations in various plant systems. Oscillatory kinetics of net ion fluxes across the plasma membrane of root, leaf, and fungal cells were measured using a non-invasive, slowly vibrating ion-selective microelectrode probe, under various environmental conditions. These results were then compared with the model predictions. A striking similarity is shown between model predictions and actual experimental data and the physiological implications of such oscillatory behaviour at plant membranes are discussed.
Materials and methods

Plant material

Most experiments on plant roots were performed using 3-d-old corn (Zea mays L.) or barley (Hordeum vulgare L.) seedlings. Seeds of several commercially available corn (Terrific, Aussie Gold, and SR073; all from Snowy River Co-operative Ltd, Orbost, Victoria, Australia; and cv. Gritz, Maïsadour Semences, France) and barley (cv. Franklin, TIAR, Launceston, Australia) cultivars were surface-sterilized in 1% NaOCl for 10 min, thoroughly rinsed in running distilled water, and germinated in the dark between two layers of wet filter paper in 90 mm Petri dishes at 25 °C for 2 d. Uniformly germinated seedlings were suspended in a vertical position over the surface of the growth solution (0.5 mM KCl, 0.1 mM CaCl₂, pH 5.5 unbuffered) and grown for another 24 h in the conditions described above. Measurements were taken when the root length was between 60 mm and 80 mm.

For protoplast experiments, oat seedlings (Avena sativa L. cv. Victory, Svalof, Sweden) were grown using the above protocol. Protoplasts were isolated enzymatically from 4–5-d old coleoptiles essentially as described by Shabala et al. (1998).

Experiments on leaf tissues were performed using corn (Zea mays L. SR073, Snowy River Co-op, Orbost, Australia) and broad bean (Vicia faba L. cv. Coles Dwarf, Cresswell’s Seeds, New Norfolk, Australia) plants. Both species were grown from seeds, in 2.01 plastic pots containing standard potting mix, in the glasshouse (16/8 h light/dark). For all details on potting mix composition, watering, and growth conditions please refer to Shabala et al. (2000). Small leaf segments (5×8 mm), for use in MIFE experiments, were excised from the third leaf of 14–16-d-old corn plants and from the youngest fully expanded leaf of 20–30-d-old bean plants. Mesophyll tissue was isolated essentially as described by Shabala and Newman (1999), and measurements on leaf epidermis were conducted following the protocol described by Zivanovic et al. (2005).

For pollen experiments, the pollen was collected from greenhouse-grown tomato (Lycopersicon esculentum var. esculentum cv. Chandler’s English, Chandlers Nursery, Hobart, Australia) plants 40–60 d after planting. For all details of pollen germination and ion flux measurements from growing pollen tubes see Tegg et al. (2005).

Single cell measurements were conducted on a marine protist Thraustochytrium sp (TAS’C’), kindly provided by Dr T Lewis (University of Tasmania). For all details on growth conditions and experimental media, see Shabala et al. (2001).

Electrophysiology

Net ion fluxes were measured using non-invasive microelectrode vibrating probe techniques. In most experiments, the MIFE® (University of Tasmania, Hobart, Australia) system was used. For all details on microelectrode fabrication, calibration and ion flux measuring procedures, please see previous publications (for root measurements: Shabala et al., 1997; Shabala and Knowles, 2002; for leaf measurements: Shabala, 2000; Zivanovic et al., 2005; for pollen tube measurements: Tegg et al., 2005; for measurements on Thraustochytrium sp: Shabala et al., 2001). The only exception was measurements of K⁺ fluxes from corn roots; these measurements were performed using a vibrating-microelectrode system essentially as described by Mancuso et al. (2000). In these experiments, recordings were made in the transition zone of the root apex, with microelectrodes oscillating in a square wave manner, with a frequency of 0.1 Hz between two positions (10 and 30 μm) above the root surface.

Oxygen flux measurements

Net O₂ fluxes were measured using non-invasive microelectrode vibrating probe techniques. For all details on microelectrode fabrication, calibration, and oxygen flux measuring procedures, see Mancuso et al. (2000) or Mancuso and Boselli (2002).

O₂ concentration of the solution in the measuring chamber was varied from 0 mg l⁻¹ to about 8 mg l⁻¹, covering the whole range of O₂ concentration that can occur in soils. The root system of the plant and the measuring chamber were placed in a glove-bag and the different oxygen concentrations were obtained using pressurized gases containing 0, 1, 2, 3⋯21% O₂, 1 ml l⁻¹ CO₂, and the balance N₂. Bulk solution oxygen concentration in the measuring chamber was recorded polarographically using a Clark type electrode for pO₂ (ECD, mod. 0225, Italy) connected to an oxygen monitor (ECD, mod. 8602, Italy), in turn connected via the multi-channel A-D converter card to the computer.

Data analysis

Spectral analysis of ion flux oscillations was typically performed by applying the Discrete Fourier Transform (DFT) using EXCEL (MS Office 2000) package essentially as described in Shabala and Newman (1998). The ‘data window’ contained 256 or 512 data points (either 21.3 or 42.6 min intervals). Using the IMABS tool in EXCEL, the moduli of the complex amplitudes were returned from the DFT spectra. These moduli were later plotted against the period (T) of the harmonic components for the discrete frequencies v=0, 1/T, 2/T,⋯, (n–1)/T.

Results

Phenomenology

Oscillations in net ion fluxes were observed in a wide range of plant species and tissues, including root (epidermis and stele) and leaf (epidermis and mesophyll) tissues, single cells (pollen tubes, guard cells, unicellular organisms, macerated or cultured cells), and protoplasts derived from various plant tissues. Some representative traces are shown in Fig. 1 using H⁺ flux as an example. As a rule, H⁺ flux oscillations were accompanied by oscillations in fluxes of other ions measured (K⁺, Ca²⁺, Cl⁻, Na⁺, NH₄⁺). This is illustrated by a typical example of oscillations in H⁺ and K⁺ fluxes from the elongation zone of 3-d-old corn roots (Fig. 2A). On some occasions, H⁺ flux oscillations were measured in the virtual absence of oscillations in fluxes of another ion (e.g. Ca²⁺; Fig. 2B). The periods of ion flux oscillations depended on the specific tissue measured and plant age, as well as on environmental conditions (temperature, medium ionic composition, pH etc). This is further illustrated in the Model Predictions section below. Most oscillations ranged from 30 s to 15 min (Fig. 1), although slower oscillations, with periods 1–2 h, were also measured (Shabala et al., 1997; Shabala and Knowles, 2002). In most cases, a noticeable phase shift occurred between oscillations in fluxes of different ions (as illustrated in Fig. 2A for K⁺ and H⁺ ions).

The model

For practical purposes, the ionic relations of a plant cell can be adequately described by five major ion transporters operating in parallel (Gradmann, 2001). The term ‘major’
in this context means that their operation can have a direct and significant effect on the membrane voltage. The transporters include (Fig. 3): an electrogenic pump (typically an H+ ATPase) which indirectly energizes uptake and release of anions and cations; voltage-gated K+ inward (KIR)- and outward (KOR)-rectifying channels, mediating the uptake and release of cations; a uniporter channel for anion release (Cl⁻/C255 channel in the model); and a symporter for anion uptake (2H⁺-Cl⁻/C255 in the model).

The function of each ion transporter comprises the algebraic product of the kinetics of the active enzyme (the ‘transport function’, for example, conventional Michaelis–Menten kinetics) and its activity (the ‘gating function’). The latter is a probability of the enzyme being in an active state. This probability may be affected by the presence/absence of ligands (‘ligand gating’) or the electrical voltage across the membrane (‘voltage-gating’). For many transporters, these functions, including their temporal behaviour, have been extensively investigated (as channels: Gazzarrini et al., 2002; as a symporter: Boyd et al., 2003; as pumps: Blatt et al., 1990). As a result, the quantitative description of relationships between these transporters became possible (Gradmann, 2001). An appropriate model (called EPPM for Electrical Properties of Plant Membranes; Pascal file available on request) was developed based on Gradmann (2001). This model allows the voltage and current traces for each of these transporters (and, thus, net fluxes of each ion) to be quantified and plotted against time. The cornerstone of this model is voltage-coupling between transporters. When one of them changes its activity, membrane voltage will also change. That will cause the activities of all the voltage-gated transporters to change also, each with some particular rate (depending on the time constants). This, in turn, will cause another voltage change in time, and so on. As a result, oscillations in membrane transport activity can occur.

Model predictions and experimental validation
Stoichiometry and time constants: Using the basic set of parameters, described in Table 1, non-damped oscillations in net H⁺ and K⁺ fluxes were modeled. Several predictions were drawn from the model: (i) the model adequately describes ion flux oscillations within the minute range of periods; (ii) the period of oscillation is strongly determined by the rate constants for gating the H⁺ pump. The smaller
the rate constants, the slower are the oscillations; (iii) a significant phase shift occurs between oscillations in net H+ and K+ fluxes; this phase shift may be as large as 180° (maximum value in the flux of one ion corresponds to the minimum value of the flux of the other ion); and (iv) inhibition of the proton pump activity causes oscillations to cease in all ion fluxes.

Some results of the modelling are shown in Fig. 4. Both K+ and H+ fluxes oscillate with ~6 min periods (Fig. 4A). Flux oscillations are opposite in phase, with maximum uptake of K+ coinciding with lowest values for net H+ flux. A 10-fold increase in the rate of H+ pump activation (k_{PUa}) and inactivation (k_{PUi}) shortened the period of oscillations by ~35% (data not shown). Modelled oscillations were extremely sensitive to changes in H+ pump characteristics within some narrow parameter ‘window’. For example, at 30% H+ pump current (decrease from 10 000 to 3000 A m^{-2} mM^{-1}), only a minor effect on the characteristics of H+ flux oscillations was observed (Fig. 4B), specifically a slight decrease in the amplitude and ~30% increase in the period compared with the non-inhibited pump (Fig. 4A). No damping was observed. However, at 24% H+ pump current, a strong flux damping was observed (Fig. 4B), while a further 4% inhibition (20% H+ pump current) caused a complete cessation of oscillation (Fig. 4B). Importantly, not
The ‘gating’ of metabolic processes is common (Chay, 1981). As far as is known, there is no direct inhibitory effect of DCCD on K+ channels. In mathematical terms, the application of DCCD is equivalent to inhibiting the H+ pump current and, thus, makes the results in Fig. 2A comparable with those in Fig. 4B and C. Consistent with the model (Fig. 4A), net H+ and K+ flux oscillations were not in phase (Fig. 2A). Under some experimental conditions, net H+ flux oscillations occurred without obvious rhythmicity in the fluxes of other ions, as illustrated in Fig. 2B for Ca2+. All this strongly implicates the H+ pump as a key ‘pacemaker’ in oscillatory membrane behaviour.

It is interesting to notice that the ~6 min oscillations shown in Fig. 4A were obtained for the set of parameters, having \( k_{\text{PUi}} = 0.003 \) and \( k_{\text{PUi}} = 0.015 \) s\(^{-1}\), i.e. time constants \((1/k) \approx 300\) and \( \approx 60\) s, respectively (Table 1). Such slow ‘gating’ of metabolic processes is common (Chay, 1981).

### Only H+ Fluxes, but Fluxes of Other Ions Were Also Strongly Damped

All the above model predictions were supported by experimental observations. Oscillations in the minute range of periods were ubiquitous and observed in various plant systems (Fig. 1). Oscillations in the H+ pump seem to play a key role in driving the system, as the application of 100 \( \mu \)M DCCD (dicyclohexylcarbodiimide; an H+-ATPase uncoupler) caused cessation not only of H+ flux oscillations, but also of oscillations in other ions measured (e.g. K+; Fig. 2A). As far as is known, there is no direct inhibitory effect of DCCD on K+ channels. In mathematical terms, the application of DCCD is equivalent to inhibiting the H+ pump current and, thus, makes the results in Fig. 2A comparable with those in Fig. 4B and C. Consistent with the model (Fig. 4A), net H+ and K+ flux oscillations were not in phase (Fig. 2A). Under some experimental conditions, net H+ flux oscillations occurred without obvious rhythmicity in the fluxes of other ions, as illustrated in Fig. 2B for Ca2+. All this strongly implicates the H+ pump as a key ‘pacemaker’ in oscillatory membrane behaviour.

### Frequency Modulation

One of the important model predictions is that oscillations in membrane transport activity occur only in a certain range of external K+ concentrations. Outside this range, even small changes in external K+ lead to progressive damping and aperiodic behaviour, as shown in Fig. 5A. The model also predicts frequency modulation in net ion flux oscillations in response to changing external conditions.
The higher external K+ concentration, the slower are net ion flux oscillations (Fig. 5A). The above predictions were validated in direct experiments on corn roots. As shown in Fig. 5B, increasing external [K+] from 0.5 mM to 10 mM increased the period of ion flux oscillations from 4.5 min to 10.5 min, with a clear dose–response dependence (Fig. 5C). Also, oscillations were slightly damped at the lowest [K+] tested (0.5 mM; Fig. 5B). All these observations are consistent with the model prediction (Fig. 5A).

The observed dependence of the oscillatory period upon external [K+] is consistent with the idea of the frequency encoding of environmental information, advocated in the literature for animal cells (Rapp et al., 1981; Rapp, 1987) and plant guard cells (McAinsh and Hetherington, 1998; Evans and Hetherington, 2001). It was expected that not only [K+] but also other environmental parameters might have some impact on oscillatory patterns in membrane transport activity. This is further illustrated in Figs 6 and 7.

Figure 6A shows modulation of net ion flux oscillations measured from barley roots grown at various salinity levels. Only net H+ fluxes are shown, although K+ and Ca2+ fluxes showed qualitatively similar behaviour (data not shown, but see Shabala and Knowles 2002). Increasing external NaCl concentration caused a significant (P=0.01) increase in the period of ion flux oscillations, with an almost linear relationship in the 20–100 mM NaCl range (Fig. 6B). It is important to mention that, being a relatively salt-tolerant species, barley roots were still growing while exposed to 100 mM NaCl. No oscillations were present in roots treated by higher NaCl concentrations which led to the complete arrest of root growth (data not shown).

As the model used in this study does not use [Na+] as one of the variables, direct comparison of these results with the model predictions remains to be done. Several possibilities
are likely. One of them is that Na⁺ may enter the cell through K⁺-permeable channels (Maathuis and Amtmann, 1999) and, thus, contribute to ‘K⁺ current’ in the model. Another possibility is that Na⁺ simply disrupts the coupling between transporters, due to membrane depolarization (Shabala et al., 2003). It is probable that both these components occur at the same time.

Finally, the effect of oxygen availability on membrane transport oscillations was studied from the transient elongation zone of corn roots. Non-damped oscillations were measured in net K⁺ and O₂ fluxes, with the same period ~8 min and constant phase difference (Fig. 7). When O₂ availability to roots was reduced from 8.6 mg l⁻¹ (fully aerated solution) to 4 mg l⁻¹, a significant (P=0.05) decrease in the magnitude of oscillations was observed (Fig. 8), accompanied by an increase in period. No regular oscillations were present at hypoxic conditions of 2 mg l⁻¹ of [O₂] in solution.

Temperature dependence: The model also predicts that ion flux oscillations are temperature sensitive (Fig. 9). The higher the ambient temperature, the faster are the oscillations. At very low temperatures, a strong damping is predicted (Fig. 9B).

Experimental validation was done on corn roots (Fig. 10). A step-wise reduction in the ambient temperature from 18°C to 10°C caused a progressive increase in the period of O₂ oscillations (and, hence, oscillations in net ion fluxes: see Fig. 7 for phase relations between O₂ and K⁺ flux) from ~7 min at 18°C to ~24 min at 10°C (Fig. 10). Further reduction in ambient temperature caused strong damping, and oscillations were not observed (data not shown).

Cell geometry: Another prediction from the model is that smaller cells oscillate faster. A simplistic approach to relate frequency and size is based on the surface/volume ratio. If the diameter is doubled, the surface will be 2²=4-fold bigger, while the volume increases 2³=8-fold. So the absolute transport rates (mol s⁻¹) will increase 4-fold, but the concentration changes (mol vol⁻¹ s⁻¹) will be 4/8=0.5.
This will double the duration of concentration-related processes.

The above prediction was validated by measuring net ion fluxes from the unicellular marine protist *Thraustochytrium* sp. It has been shown previously that *Thraustochytrium* cells exhibit pronounced oscillations during growth (Shabala et al., 2001). A typical example of such oscillations is shown in Fig. 11A. Several oscillatory components are evident (Fig. 11B). In addition to the major one (>4 min period), Fourier analysis revealed several more resonant frequencies, with a period <1 min (Fig. 11B). The analysis of a large population of oscillating cells has revealed a strong relationship between period of these fast (<1 min) oscillations and cell diameter. Consistent with the model prediction, the larger the cell, the slower were ion flux oscillations (significant at *P* <0.01 between all pairs; Fig. 11C).

**Discussion**

*Oscillations in membrane transport as a feature inherent in every non-linear feedback system*

Theoretically, to generate oscillatory behaviour requires only two or three independent dynamic variables and non-linear terms in the set of equations that describe their
interaction (Baker and Gollub, 1990; Feijo et al., 2001).

Talking specifically about membrane-transport oscillations, relevant dynamic variables are concentration and the activities of ion transporters, while voltage and ligand gating provide the necessary feedback (Hansen, 1978; Chay, 1981; Felle, 1988; Elzenga and Prins, 1989; Beffagna and Romani, 1991; Miedema and Prins, 1991; Kocks and Ross, 1995; Miedema et al., 2000). It is not surprising, therefore, that membranes often exhibit oscillatory behaviour.

Why then are oscillations in membrane transport activity not seen every time? Mathematical analysis of the model shows that non-damped oscillations will be present only when the resultant $I$–$V$ curve (Fig. 3) has a region with a negative slope. Outside this range, oscillations are either damped or non-existing. Due to space limitations, these issues are not discussed here. For more theoretical details, refer to Gradmann (2001) and appropriate references within. With over 20 variables used in the model to describe kinetics of the five major electroenzymes, it is obvious that the above condition is not likely to be met at all times. As shown in Fig. 4B and C, even a slight variation in $H^+$ pump current (from 3000 to 2400 A m$^{-2}$ mM$^{-1}$) resulted in strong damping. When $H^+$ pump current was 2000 A m$^{-2}$ mM$^{-1}$, no oscillations were present (Fig. 4B, C).

Physiologically, the $H^+$ pump current may be limited by any of the following factors: (i) availability of substrates; (ii) $H^+$-ATPase density in the plasma membrane; (iii) maximum turnover rate of the enzyme; (iv) small rate constant for activation; and (v) large rate constant for inactivation. Thus, the fact that oscillations in membrane transport activity are almost always observed in the root apex, but seldom in the mature zone (Shabala et al., 1997; Shabala, 2003) may be caused simply by the difference in one or several of the above factors. It should be mentioned that the highest $H^+$ efflux was always found in the middle of the elongation zone of roots of various species (Pilet et al., 1983; Shabala et al., 1997; Zieschang et al., 1993).

Pharmacological experiments using DCCD (Fig. 2) or vanadate (Shabala and Shabala, 2002) suggested that such $H^+$ fluxes are mediated by $H^+$ pump activity. Taken together, this supports the idea that the difference in temporal behaviour of membrane transport activity between elongation and mature root zones is determined largely by different functional expression of $H^+$-ATPase pumps. Of course, contributions of other transporters (e.g. $2H^+–Cl^-$ symporter) should also be taken into account. To answer this issue explicitly, all the basic variables used in this model should be quantified in a series of experiments comparing root cells from the elongation and mature zones.

Regardless of the underlying mechanism, the fact that oscillatory ion fluxes are mostly observed in the root apex (Shabala, 2003) points to a possible physiological role of oscillations in this region. The root apex has always been found to be more sensitive to various treatments, including hormones (Ludidi et al., 2004), cadmium (Pineros et al., 1998), aluminium stress (Ryan et al., 1993), and salinity (Chen et al., 2005). It is tempting to suggest that such oscillatory behaviour provides more efficient control and better stability needed for fine-tuning of physiological and metabolic processes in this dynamic region. One component of such a mechanism may be frequency and amplitude modulation to encode environmental information.

**Frequency encoding in biological systems**

The hypothesis of frequency encoding in biological systems was proposed about two decades ago (Rapp et al., 1981; Berridge et al., 1988). Frequency modulation of any signal offers many advantages compared with amplitude modulation, for example, lower sensitivity to...
noise (Rapp et al., 1981; Rapp, 1987; Putney, 1998). A classical example of such frequency encoding is Ca\(^{2+}\) oscillations (Stucki and Somogyi, 1994; Izu and Spangler, 1995). By relying on large, discrete digital events (such as Ca\(^{2+}\) spikes), cells can readily distinguish an ‘intentional’ Ca\(^{2+}\) signal from potentially spurious wanderings of the steady-state cytosolic Ca\(^{2+}\). A much broader range of signal strengths can potentially be distinguished with a digitally encoded system, because the baseline value is essentially zero (Stucki and Somogyi, 1994).

The above concept of frequency encoding of environmental information by oscillations in cytosolic free Ca\(^{2+}\) ([Ca\(^{2+}\)\(_{cyt}\)]) has been widely advocated for stomatal guard cells (McAinsh and Hetherington, 1998). An increase in [Ca\(^{2+}\)\(_{cyt}\)] has been observed in guard cells in response to ABA, auxin, CO\(_2\), oxidative stress, external Ca\(^{2+}\) and K\(^+\), high and low temperatures, salinity, red light, fungal elicitors, and mechanical stimuli (McAinsh et al., 1995; Leckie et al., 1998, and references within). Each of these responses had its own [Ca\(^{2+}\)\(_{cyt}\)] ‘signature’; one of its features is repetitiveness of [Ca\(^{2+}\)\(_{cyt}\)] spikes (McAinsh et al., 1997; McAinsh and Hetherington, 1998). Surprisingly, the concept of frequency encoding has not been extrapolated to a full extent to other types of plant cells and tissues. Meanwhile, model predictions (Figs 5, 9) strongly suggest that such encoding should be observed in any feedback controlled membrane-transport system possessing nonlinear dynamics. Some of these aspects are discussed in the following section.

**Frequency encoding and plant adaptive responses to their environment**

**Temperature:** According to this study’s model predictions, information about ambient temperature may be encoded by the frequency of ion flux oscillations (Fig. 9). The higher the ambient temperature, the faster are the oscillations. This was confirmed in direct experiments on corn roots (Fig. 10) and is consistent with the literature. Antkowski and Engelmann (1995) reported a pronounced temperature effect on the period of changes in external K\(^+\) in the apoplast of pulvini tissue in Desmodium. Macduff and Dhanoa (1996) reported temperature dependence of ultradian rhythms of K\(^+\) uptake by roots of white clover, with periods 7 h and 4 h for 13 °C and 25 °C, respectively.

The model also predicts that, at very low temperatures, oscillations will be strongly damped, and nutrient uptake will be aperiodic (Fig. 9B). The physiological significance of this phenomenon has yet to be revealed. The oscillations may be important to regulate long-distance nutrient transport in plants. It is known that a significant amount of nutrients, delivered from the root to the shoot, is recycled back to roots (Marschner, 1995). In some species, the amount of K\(^+\) returning to roots in this process is as high as 80% (Jeschke and Pate, 1991). Thus, an oscillating pattern of root nutrient acquisition may allow rapid regulation to maintain the required level of recycled nutrients in root vascular tissues. This rapidity becomes unnecessary when plants are chilled, and shoot nutrient demands are greatly reduced.

**Salinity and osmotic stresses:** There is only fragmentary evidence reporting amplitude modulation of oscillating ion currents in plant cells under saline conditions (Kouzie, 1996). In this work, the first evidence is provided for the frequency encoding of such oscillations under saline conditions (Fig. 6). The idea that sustained oscillations in ion transport, based on periodical switching between net uptake and net release of salt, may allow long-term osmotic adjustment was advocated previously (Gradmann et al., 1993; Buschmann and Gradmann, 1997) and supported by experimental observations (Shabala et al., 2000; Shabala and Lew, 2002). Also earlier, Gradmann and Boyd (1995) suggested that oscillatory ion transport mechanisms, operating in planktonic diatoms, were important for adjustment of buoyancy by appropriate uptake and release of ions. The full physiological significance of this phenomenon, as well as specific mechanisms of encoding and decoding, has yet to be revealed. In particular, it would be interesting to test whether isotonic NaCl and mannitol treatments cause the same changes in oscillatory patterns at cell membranes. Also, comparison of NaCl effect on oscillatory patterns of plant varieties, contrasting in their salt tolerance, might shed light on the adaptive significance of such oscillations.

**Chemical composition of the medium:** It was shown earlier that both amplitude and frequency of cytosolic Ca\(^{2+}\) oscillations in Eremosphaera depended strongly on the concentration of Sr\(^{2+}\) in the external medium (Bauer et al., 1998). McAinsh et al. (1995) reported a similar relationship for external Ca\(^{2+}\) concentration. In this work, both theoretical (Fig. 5A) and experimental (Fig. 5B) evidence is provided for the period (or frequency) of ion flux oscillations being strongly dependent on K\(^+\) availability. In this context, it is worth mentioning that waving patterns of Arabidopsis roots, grown on a solid medium surface, showed strong dependence on the nutrient availability (Buer et al., 2000). When plants were grown on nutrient-depleted media, roots had the shortest wavelengths, the greatest wave tangent angles, and the waving occurred more often. Previously, a very close correlation was shown between root circum-nutation patterns and oscillating ion flux profiles at the root surface (Shabala and Newman, 1997a, b). Another example is Al\(^{3+}\) toxicity. In rice roots, treatment with 5 μM Al\(^{3+}\) changed root nutational patterns, without reduction in the rate of root elongation (Hayashi et al., 2004). All these observations point strongly to the possibility of ‘encoding’ information about chemical composition of the root medium by oscillations in membrane-transport.

**Hypoxia/anoxia:** Under normoxic conditions, the oxygen influx in the elongation zone of the roots shows dynamic behavior, characterized by oscillations with period around...
8 min (Mancuso et al., 2000). However, until now no evidence was available about amplitude and frequency modulation in plant cells under hypoxic conditions. Interestingly, evidence of oxygen-mediated oscillations do exist in yeast (see Richard, 2003, for a review), where the oscillatory activity has been ascribed to energetic optimization, signalling or timekeeping functions (Hynne et al., 2001; Lloyd et al., 2003).

A mechanism for the oxygen oscillation in root apex cells could include oxidative phosphorylation and ATP utilizing processes. According to this scenario, mitochondrial electron transport chains, enslaved to a periodic requirement for ATP generation, would respond by periodic O₂ consumption. Such a model is favoured by the strong relationship existing between the amplitude of the oxygen flux oscillations and the O₂ availability for the cell (Fig. 8). Moreover, the decrement of the amplitude of O₂ oscillation at lowered levels of O₂ availability was strongly correlated with the hypoxia-tolerance of different species of Viitis (S Mancuso and AM Marras, unpublished data), suggesting a good predictive power of this parameter as a screening tool in plant breeding for waterlogging tolerance.

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Oscillations in membrane transport


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