Journal of Experimental Botany, Vol. 57, No. 1, pp. 171–184, 2006
doi:10.1093/jxb/erj022 Advance Access publication 5 December, 2005

Journal of Experimental Botany

RESEARCH PAPER

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

Sergey Shabala[1,*], Lana Shabala[1], Dietrich Gradmann[3], Zhonghua Chen[1], Ian Newman[2] and Stefano Mancuso[4]

[1] *School of Agricultural Science, University of Tasmania, Hobart, Australia*

[2] *School of Mathematics and Physics, University of Tasmania, Hobart, Australia*

[3] *Plant Biophysics Department, University of Göttingen, Germany*

[4] *Department of Horticulture, University of Florence, Italy*

Received 6 July 2005; Accepted 21 October 2005

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) 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 Ca^{2+} (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;

* To whom correspondence should be addressed. E-mail: Sergey.Shabala@utas.edu.au

Shabala and Newman, 1997*a*, 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, 1997*a*), with no oscillations found in roots growing slower than 2 μm 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 Vucinic, 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 $CaCl_2$, 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 2.0 l 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 O_2 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).

O_2 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 O_2 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% O_2, 1 ml l^{-1} CO_2, and the balance N_2. Bulk solution oxygen concentration in the measuring chamber was recorded polarographically using a Clark type electrode for pO_2 (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^+, Ca^{2+}, Cl^-, Na^+, NH_4^+). 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. Ca^{2+}; 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'

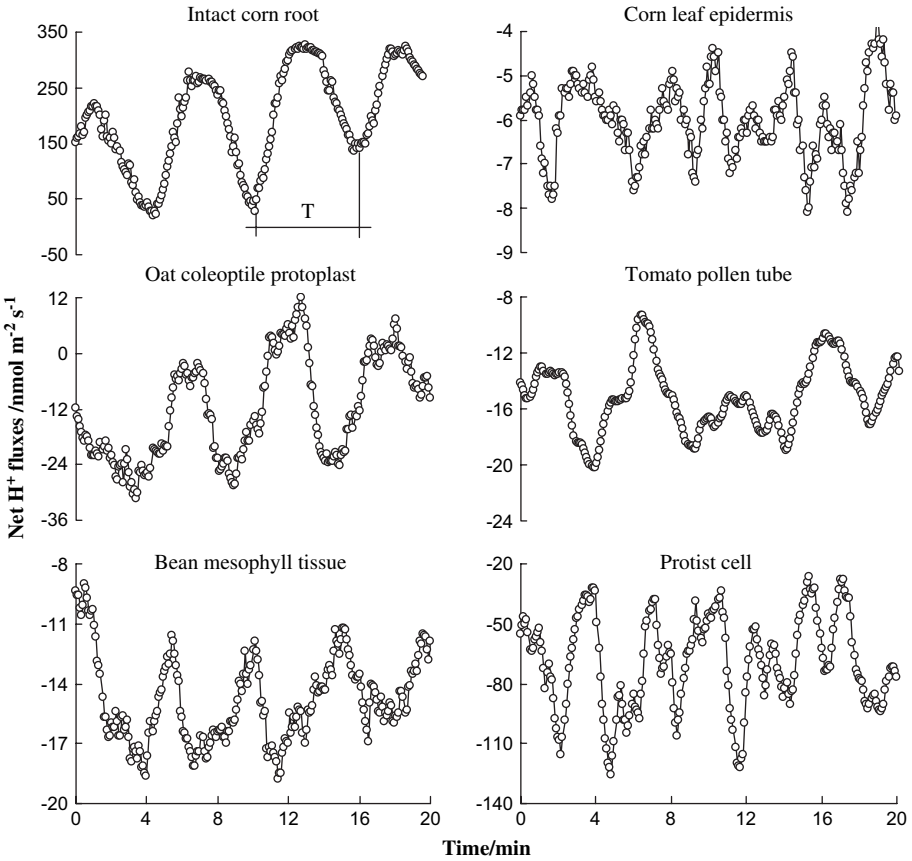

Fig. 1. Phenomenology of oscillations in membrane transport activity in plants. Examples of ultradian oscillations in net H^+ fluxes across the plasma membrane are shown for various systems: intact corn roots (cv. Aussie Gold; 3-d-old; root meristem region), oat coleoptile protoplasts (cv. Victory; 4-d-old), bean leaf mesophyll tissue (cv. Coles Dwarf; 8-d-old leaf), corn leaf epidermis (cv. SR073; 15-d-old plant); tomato pollen tube (cv. Chandler's English), and unicellular marine protist (*Thraustochytrium* sp.). Note that for all MIFE data, the sign convention is 'influx positive'.

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^- channel in the model); and a symporter for anion uptake ($2H^+$-Cl^- 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: Gazzarini *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

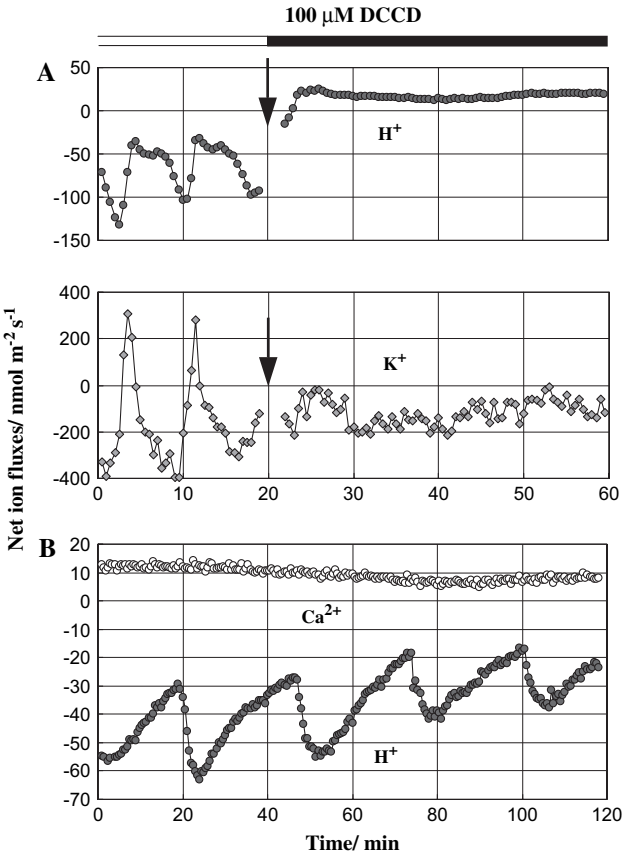

Fig. 2. Evidence for H^+ being a component of the oscillatory mechanism in root cells. One out of six typical examples is shown for each panel. (A) Suppression of ion flux oscillations by DCCD, a known H^+ pump inhibitor. Net H^+ and K^+ fluxes were measured from the middle of the elongation zone of 3-d-old corn roots (cv. SR073). Note that H^+ and K^+ flux oscillations are not in phase. (B) Ultradian H^+ flux oscillations (closed symbols) are measured from the mature zone of 3-d-old corn roots (cv. Terrific) in the virtual absence of Ca^{2+} flux oscillations (open symbols).

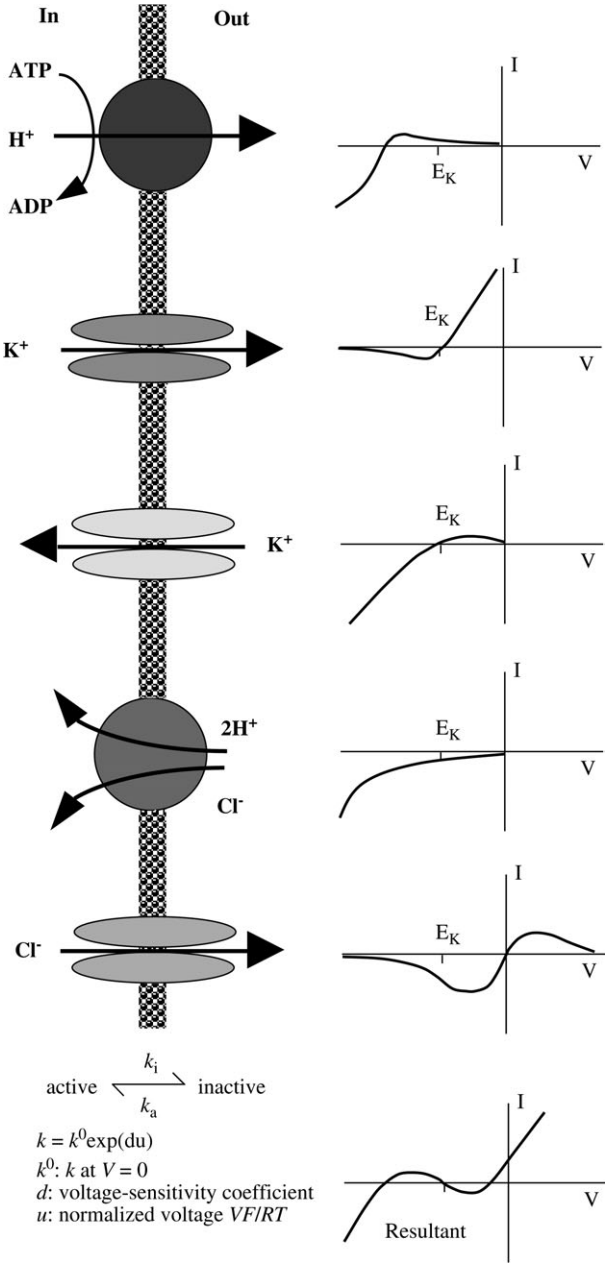

Fig. 3. A five-component model for plasma membrane transporters (based on Gradmann, 2001). Five major electroenzymes (K^+ inward- and outward rectifying channels; H^+ pump; anion channel and $2H^+$-Cl^- symporters) are electrochemically coupled, to produce a diversity of oscillations in net ion fluxes across the plasma membrane. The qualitative current–voltage (*I–V*) relationship for each individual electroenzyme is shown on the right, and the resultant *I–V* curve of the entire ensemble of transporters is shown at the very bottom. Ion flux oscillations are predicted to occur within the region with a negative slope.

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

Table 1. *Parameters of a five-component model used in this study*

	Parameter	d^a
Pump		
ΔG_{ATP}/F (mV)	−450	
I_{PU}, maximum H current through pump (A m^{-2} mM^{-1})	10 000	
k_{PUa}, rate constant for pump activation (s^{-1})	0.003	−0.5
k_{PUi}, rate constant for pump inactivation (s^{-1})	0.015	0.5
K^+ outward rectifier (KOR)		
G_{KO}, maximum GHK conductance (S m^{-2} mM^{-1})	1	
k_{KOa}, rate constant for KOR activation (s^{-1})	0.03	0.5
k_{KOi}, rate constant for KOR inactivation (s^{-1})	0.03	−0.5
K^+ inward rectifier (KIR)		
$G_{KI,}$ maximum GHK conductance (S m^{-2} mM^{-1})	2	
k_{KIa}, rate constant for KIR activation (s^{-1})	0.03	−0.5
k_{KIi}, rate constant for KIR inactivation (s^{-1})	3	0.5
$2H^+$-Cl^- symporter (SY)		
I_{SY}, maximum current through SY (A m^{-2} mM^{-1})	400 000	
k_{SYa}, rate constant for SY activation (s^{-1})	0.4	−0.5
k_{SYi}, rate constant for SY inactivation (s^{-1})	0.6	0.5
Cl^- channel (gating scheme: i1-a-i2)		
G_{Cl}, maximum GHK conductance (S m^{-2} mM^{-1})	0.2	
k_{Cli1a}, rate constant for activation from i1 (s^{-1})	5	0.5
k_{Clai1}, rate constant for inactivation to i1 (s^{-1})	0.07	−0.5
k_{Cli2a}, rate constant for activation from i2 (s^{-1})	0.007	−0.5
k_{Clai2}, rate constant for inactivation to i2 (s^{-1})	0.15	0.5

[a] Voltage-sensitivity coefficients of the rate constants as defined in Fig. 3.

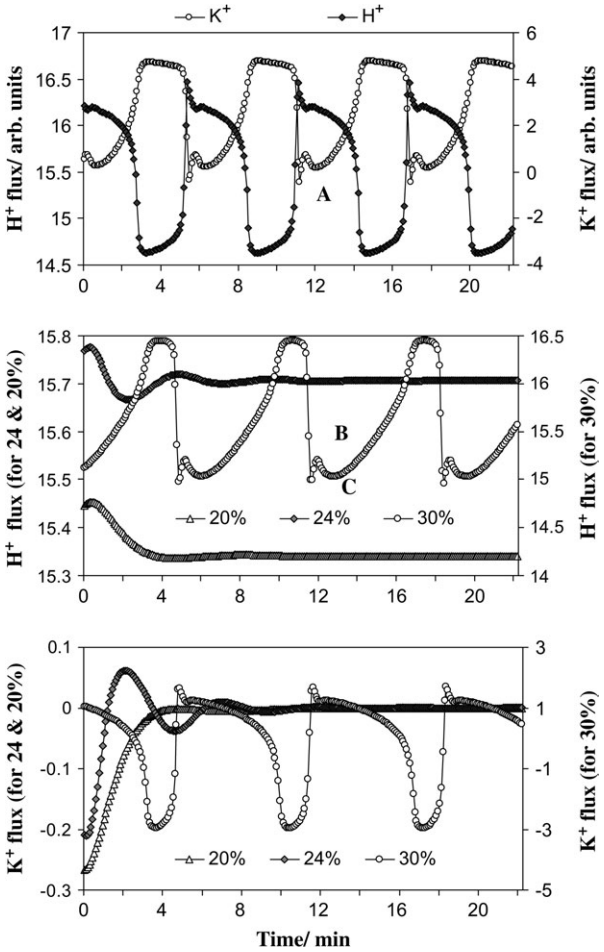

Fig. 4. Stoichiometry between plasma membrane H^+ and K^+ flux oscillations predicted by the model. (A) Non-damped oscillations resulting from the basic model settings as specified in Table 1. (B) Effect of reduced H^+ pump current on net H^+ flux oscillatory patterns. Numbers for each curve show the percentage of the maximum pump current (compared with the basic parameter set). (C) Effect of the reduced H^+ pump current on oscillatory patterns in net K^+ flux. The flux sign convention is the same as for the MIFE experimental data.

only H^+ fluxes, but fluxes of other ions were also strongly damped (Fig. 4C).

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 μM 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 Ca^{2+}. 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_{PUa}=0.003 and k_{PUi}=0.015 s^{-1}, i.e. time constants ($1/k$) ~300 and ~60 s, respectively (Table 1). Such slow 'gating' of metabolic processes is common (Chay, 1981). Time constants for the activation/inactivation of K^+ channels were in the seconds range (Table 1). The plasma membranes of plant cells are usually dominated by two classes of K^+-permeable channels: (i) slowly activating channels with a strong voltage dependence and (ii) instantaneously activating weakly rectifying channels (Zhang *et al.*, 2002). The latter are activated within ms, while the former have time constants of several seconds (Findlay *et al.*, 1994; Zhang *et al.*, 2002; Carpaneto, 2003).

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

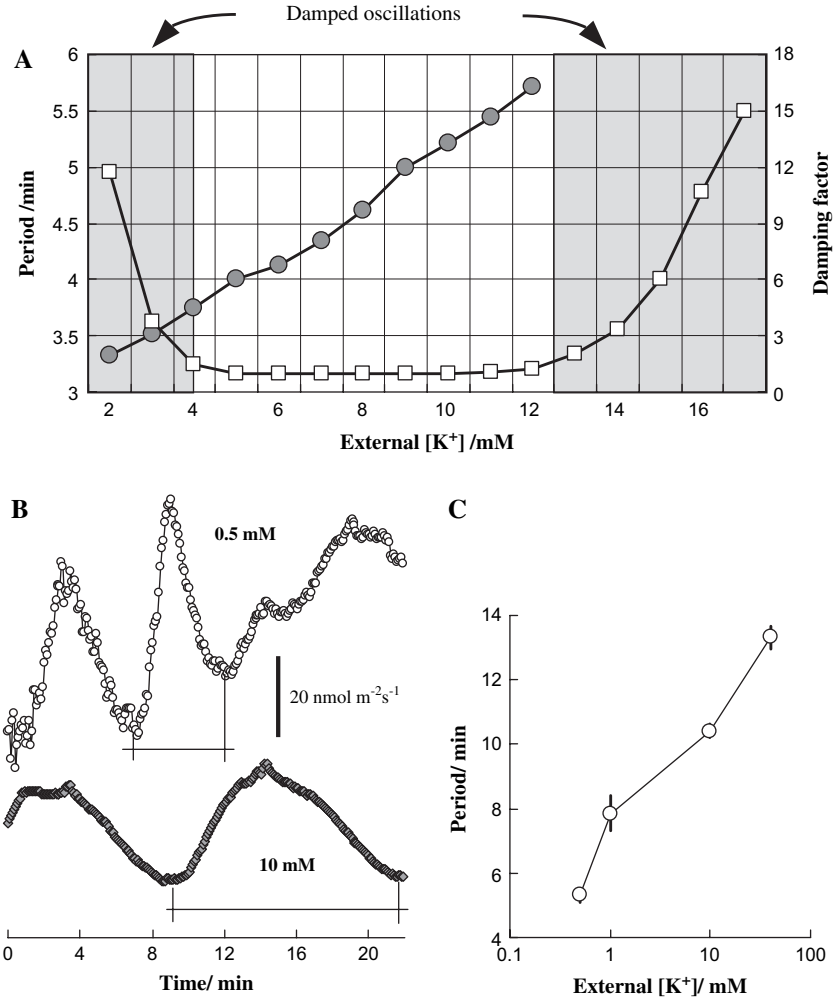

Fig. 5. Effect of external [K^+] on membrane-transport oscillations in plant cells. (A) Model predictions for period and damping of net K^+ and H^+ flux oscillation as a function of external [K^+]. (B, C) Experimental evidence for the dependence of H^+ flux oscillations on external [K^+], measured from the elongation zone of 4-d-old corn root (cv. Terrific). Typical fragments of H^+ flux oscillations are shown in (B) for 0.5 mM and 10 mM external [K^+], while their concentration-dependence is shown in (C). Means ±SE (n=4–9).

K^+ concentration. The higher the 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 to10 mM increased the period of ion flux oscillations from ~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 Ca^{2+} 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

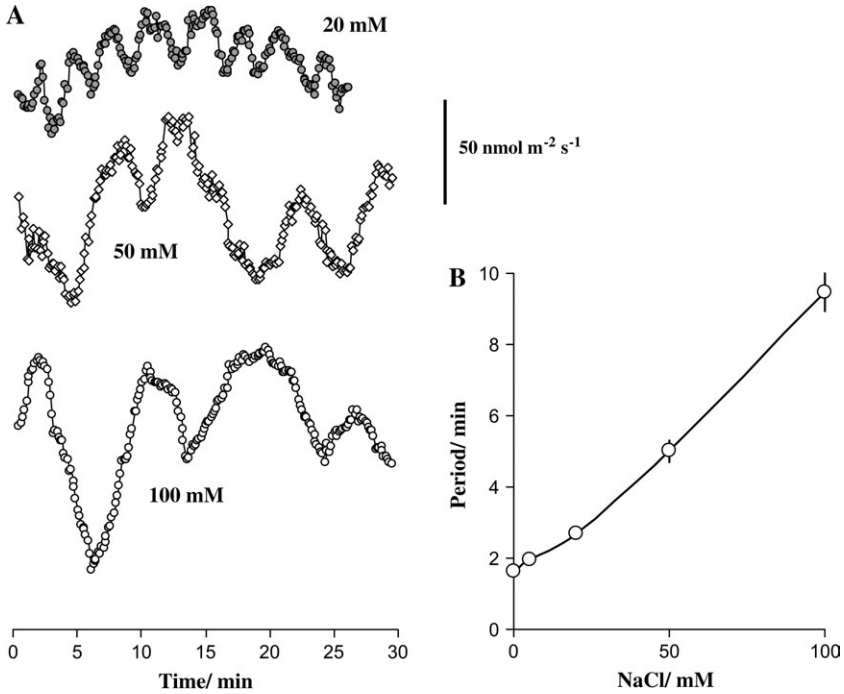

Fig. 6. Effect of salinity on ion flux oscillations in 3-d-old barley (cv. Franklin) roots. Plants were treated in various NaCl concentrations (ranging from 0 to 100 mM) for 1 h prior to measurements. (A) Typical examples of net H^+ flux oscillations for three salt levels. (B) Dependence of period of H^+ flux oscillations on NaCl concentrations in the bath solutions. Means ±SE (n=30–40).

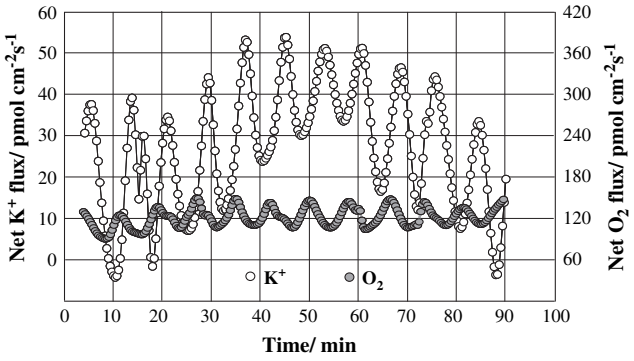

Fig. 7. Stoichiometry between net K^+ and O_2 flux oscillations in 4-d-old corn roots (cv. Gritz). One (out of 15) representative example is shown. Fluxes were measured in the transition zone of the root apex.

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 O_2 fluxes, with the same period ~8 min and constant phase difference (Fig. 7). When O_2 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 [O_2] 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 O_2 oscillations (and, hence, oscillations in net ion fluxes: see Fig. 7 for phase relations between O_2 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 2^2=4-fold bigger, while the volume increases 2^3=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.

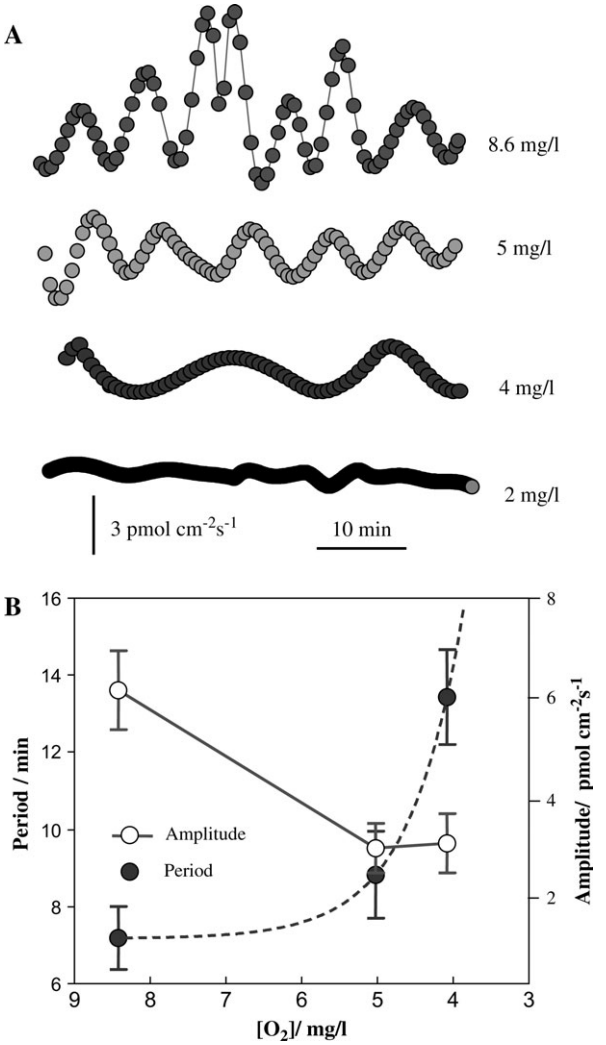

Fig. 8. Net O_2 flux oscillations in the transition zone of corn root (cv. Gritz) as a function of oxygen availability. (A) Fragments of oscillations at four levels of $[O_2]$ in the root medium. (B) Dependence of O_2 flux oscillations on $[O_2]$ availability. Means ±SE (n=32).

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).

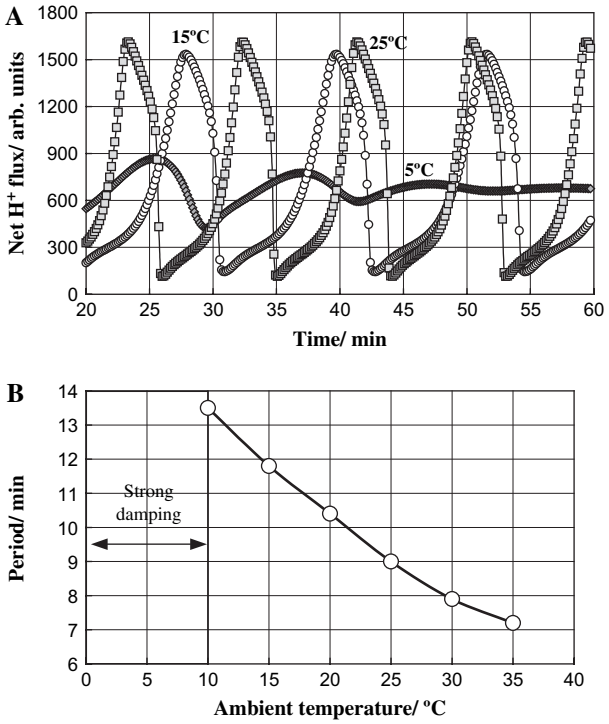

Fig. 9. Model predictions for the temperature dependence of membrane oscillators. (A) H^+ flux oscillations at three ambient temperatures (25, 15 and 5 °C) predicted by the model. Please note that oscillations at 5 °C are damped. (B) Temperature-response curve for H^+ flux oscillations modelled above.

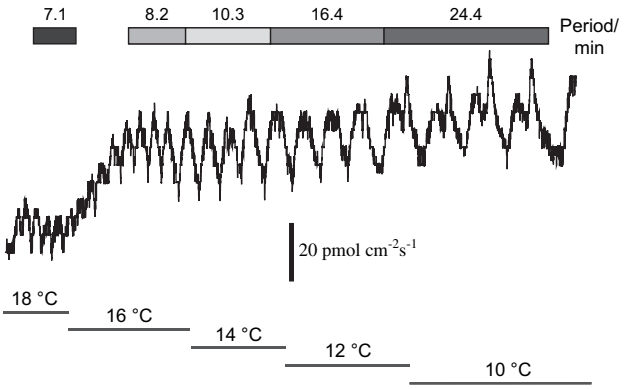

Fig. 10. Effect of temperature on membrane-transport oscillations in corn (cv. Gritz) roots. A progressive increase in the period of net O_2 flux oscillations is observed in response to a step-wise reduction of the ambient temperature. One (out of 15) representative example is shown. Numbers above the bar indicate average period of O_2 oscillations for each of the temperatures.

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

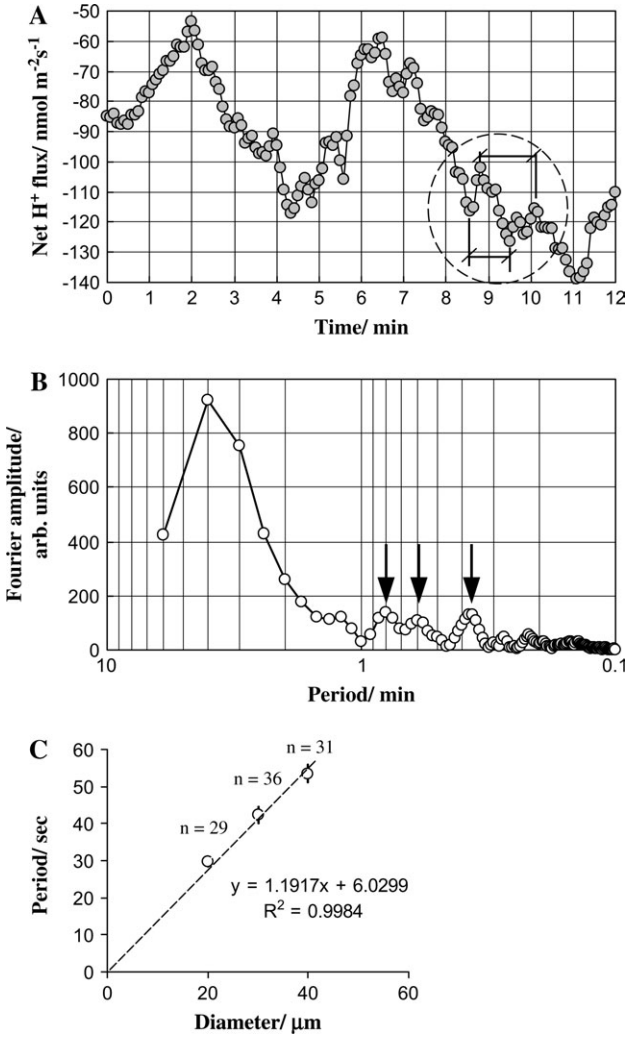

Fig. 11. Effect of cell diameter on period of oscillations in membrane-transport activity for *Thraustochytrium* sp. cells. (A) Fragment of oscillations in net H^+ fluxes from the *Thraustochytrium* cell, depicting two oscillatory components, with periods of ~5 and ~1 min, respectively. (B) Amplitude Fourier spectrum of oscillations shown in (A). Additional resonant peaks are marked by arrows. (C) Period of H^+ flux oscillations ('1 min' component circled in (A) as a function of the cell diameter. Means ±SE (*n* values are shown on a graph).

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. Antkowiak 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 (Kourie, 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 circumnutation patterns and oscillating ion flux profiles at the root surface (Shabala and Newman, 1997*a*, *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 O_2 consumption. Such a model is favoured by the strong relationship existing between the amplitude of the oxygen flux oscillations and the O_2 availability for the cell (Fig. 8). Moreover, the decrement of the amplitude of O_2 oscillation at lowered levels of O_2 availability was strongly correlated with the hypoxia-tolerance of different species of *Vitis* (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.

Acknowledgement

This work was supported by ARC Discovery grant to S Shabala.

References

Allen GJ, Chu SP, Schumacher K, *et al.* 2000. Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in *Arabidopsis* det3 mutant. *Science* **289,** 2338–2342.

Antkowiak B, Engelmann W. 1995. Oscillations of apoplasmic K^+ and H^+ activities in *Desmodium motorium* (Houtt.) Merril. pulvini in relation to the membrane potential of motor cells and leaflet movements. *Planta* **196,** 350–356.

Baker GL, Gollub JP. 1990. *Chaotic dynamics*. New York: Cambridge University Press.

Bauer CS, Plieth C, Bethmann B, Popescu O, Hansen U-P, Simonis W, Schonknecht G. 1998. Strontium-induced repetitive calcium spikes in a unicellular green alga. *Plant Physiology* **117,** 545–557.

Beffagna N, Romani G. 1991. Modulation of the plasmalemma proton pump activity by intracellular pH in *Elodea densa* leaves: correlation between acid load and H^+ pumping activity. *Plant Physiology and Biochemistry* **29,** 471–480.

Berridge MJ, Cobbold PH, Cuthbertson KSR. 1988. Spatial and temporal aspects of cell signalling. *Philosophical Transactions of the Royal Society of London Series B* **320,** 325–343.

Berridge MJ, Rapp PE. 1979. A comparative survey of the function, mechanism and control of cellular oscillators. *Journal of Experimental Biology* **81,** 217–279.

Blatt MR. 2000. Cellular signalling and volume control in stomatal movements in plants. *Annual Review of Cell Developmental Biology* **16,** 221–241.

Blatt MR, Beilby MJ, Tester M. 1990. Voltage dependence of the *Chara* proton pump revealed by current–voltage measurement during rapid metabolic blockade with cyanide. *Journal of Membrane Biology* **114,** 205–23.

Blatt MR, Thiel G. 1994. K^+ channels of stomatal guard-cells: bimodal control of the K^+ inward-rectifier evoked by auxin. *The Plant Journal* **5,** 55–68.

Boyd J, Gradmann D, Boyd CM. 2003. Transinhibition and voltage-gating in a fungal nitrate transporter. *Journal of Membrane Biology* **195,** 1–12.

Buer CS, Masle J, Wasteneys GO. 2000. Growth conditions modulate root-wave phenotypes in *Arabidopsis*. *Plant and Cell Physiology* **41,** 1164–1170.

Buschmann P, Gradmann D. 1997. Minimal model for oscillations of membrane voltage in plant cells. *Journal of Theoretical Biology* **188,** 323–332.

Carpaneto A. 2003. Nickel inhibits the slowly activating channels of radish vacuoles. *European Biophysical Journal* **32,** 60–66.

Chay TR. 1981. A model for biological oscillations. *Proceedings of the National Academy of Sciences, USA* **78,** 2204–2207.

Chen Z, Newman I, Zhou M, Mendham N, Zhang G, Shabala S. 2005. Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. *Plant, Cell and Environment* **28,** 1230–1246.

Ehrhardt DW, Wais R, Long SR. 1996. Calcium spiking in plant root hairs responding to rhizobium nodulation signals. *Cell* **85,** 673–681.

Elzenga JTM, Prins HBA. 1989. Light-induced polar pH changes in leaves of *Elodea canadensis*. I. Effect of carbon concentration and light intensity. *Plant Physiology* **91,** 62–67.

Engelmann W, Antkowiak B. 1998. Ultradian rhythms in *Desmodium*. *Chronobiology International* **15,** 293–307.

Erdei L, Szegletes Z, Barabas KN, Pestenacz A, Fulop K, Kalmar L, Kovacs A, Toth B, Der A. 1998. Environmental stress and the biological clock in plants: changes of rhythmic behavior of carbohydrates, antioxidant enzymes and stomatal resistance by salinity. *Journal of Plant Physiology* **152,** 265–271.

Evans NH, Hetherington AM. 2001. Plant physiology: the ups and downs of guard cell signalling. *Current Biology* **11,** R92–R94.

Feijo JA, Sainhas J, Holdaway-Clarke T, Cordeiro MS, Kunkel JG, Hepler PK. 2001. Cellular oscillations and the regulation of growth: the pollen tube paradigm. *Bioassays* **23,** 86–94.

Felle H. 1988. Short-term pH regulation in plants. *Physiologia Plantarum* **74,** 583–591.

Findlay GP, Tyerman SD, Garrill A, Skerrett M. 1994. Pump and K^+ inward rectifiers in the plasmalemma of wheat root protoplasts. *Journal of Membrane Biology* **139,** 103–116.

Fisahn J, Hansen UP, Gradmann D. 1986. Determination of charge, stoichiometry and reaction constants from IV curve studies on a K^+ transporter in *Nitella*. *Journal of Membrane Biology* **94,** 245–252.

Gazzarini S, Van Etten JL, DiFrancesco D, Thiel G, Moroni A. 2002. Voltage-dependence of virus-encoded miniature K^+ channel Kcv. *Journal of Membrane Biology* **187,** 15–25.

Grabov A, Blatt MR. 1998. Co-ordination of signalling elements in guard cell ion channel control. *Journal of Experimental Botany* **49,** 351–360.

Gradmann D. 2001. Models for oscillations in plants. *Australian Journal of Plant Physiology* **28,** 577–590.

Gradmann D, Blatt MR, Thiel G. 1993. Electrocoupling of ion transporters in plants. *Journal of Membrane Biology* **136,** 327–332.

Gradmann D, Boyd CM. 1995. Membrane voltage of marine-phytoplankton, measured in the diatom *Coscinodiscus radiatus*. *Marine Biology* **123,** 645–650.

Gradmann D, Buschmann P. 1997. Oscillatory interactions between voltage gated electroenzymes. *Journal of Experimental Botany* **48,** 399–404.

Gradmann D, Slayman CL. 1975. Oscillations of an electrogenic pump in the plasma membrane of Neurospora. *Journal of Membrane Biology* **23,** 181–212.

Hansen U-P. 1978. Do light-induced changes in the membrane potential of *Nitella* reflect the feed-back regulation of a cytoplasmic parameter? *Journal of Membrane Biology* **41,** 197–224.

Hayashi Y, Nishiyama H, Tanoi K, Ohya T, Nihei N, Tanioka K, Nakanishi TM. 2004. An aluminium influence on root circumnutation in dark revealed by a new super-HARP (high-gain avalanche rushing amorphous photoconductor) camera. *Plant and Cell Physiology* **45,** 351–356.

Hecks B, Hejnowicz Z, Sievers A. 1992. Spontaneous oscillations of extracellular electric potentials measured on *Lepidium sativum* L. roots. *Plant, Cell and Environment* **15,** 115–121.

Hetherington AM, Gray JE, Leckie CP, McAinsh MR, Ng C, Pical C, Priestley AJ, Staxen I, Webb AAR. 1998. The control of specificity in guard cell signal transduction. *Philosophical Transactions of the Royal Society London, Series B* **353,** 1489–1494.

Holdaway-Clarke TL, Feijo JA, Hacket GR, Kunkel JG, Hepler PK. 1997. Pollen tube growth and the intracellular cytosolic calcium gradient oscillate in phase while extracellular calcium influx is delayed. *The Plant Cell* **9,** 1999–2010.

Holdaway-Clarke TL, Hepler PK. 2003. Control of pollen tube growth: role of ion gradients and fluxes. *New Phytologist* **159,** 539–563.

Homble F. 1996. Membrane transport and oscillations in plants. In: Driessche TV, ed. *Membranes and circadian rhythms*. Berlin: Springer, 125–138.

Hynne R, Dano S, Sorensen PG. 2001. Full-scale model of glycolysis in *Saccharomyces cerevisiae*. *Biophysical Chemistry* **94,** 121–163.

Izu LT, Spangler RA. 1993. Mathematical analysis of a model for calcium oscillations based on calcium-induced calcium release and diffusion. *FASEB Journal* **7,** A239–A239.

Jenkinson IS, Scott BIH. 1961. Bioelectric oscillations of bean roots: further evidence for a feedback oscillator. I. Extracellular response to oscillations in osmotic pressure and auxin. *Australian Journal of Biological Science* **14,** 231–247.

Jeschke WD, Pate JS. 1991. Cation and chloride partitioning through xylem and phloem within the whole plant of *Ricinus communis* L. under conditions of salt stress. *Journal of Experimental Botany* **42,** 1105–1116.

Kocks P, Ross J. 1995. Kinetic-model for (damped) oscillations of transthylakoid pH in plants. *Journal of Physical Chemistry* **99,** 16490–16497.

Kourie JI. 1996. Oscillation of membrane currents during the action potential in *Chara corallina*: modification and significance for repolarisation of the membrane potential and salt sensitivity. *Australian Journal of Plant Physiology* **23,** 361–369.

Leckie CP, McAinsh MR, Montgomery L, Priestley AJ, Staxen I, Webb AAR, Hetherington AM. 1998. Second messengers in guard cells. *Journal of Experimental Botany* **49,** 339–349.

LeFebvre J, Lefever R, Gillet C. 1970. Oscillations auto-entretenues des potentials de membrane de *Nitella*. *Bulletin Societe Royal Botanique Belgique* **103,** 157.

Lloyd D, Lemar KM, Salgado EJ, Gould TM, Murray DB. 2003. Respiratory oscillation in yeast: mitochondrial reactive oxygen species, apoptosis and time; a hypothesis. *FEMS Yeast Research* **3,** 333–339.

Ludidi N, Morse M, Sayed M, Wherrett T, Shabala S, Gehring C. 2004. A recombinant plant natriuretic peptide causes rapid and spatially differentiated K^+, Na^+ and H^+ flux changes in *Arabidopsis thaliana* roots. *Plant and Cell Physiology* **45,** 1093–1098.

Maathuis FJM, Amtmann A. 1999. K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. *Annals of Botany* **84,** 123–133.

Macduff JH, Dhanoa MS. 1996. Diurnal and ultradian rhythms in K^+ uptake by *Trifolium repens* under natural light patterns: evidence for segmentation at different root temperatures. *Physiologia Plantarum* **98,** 298–308.

Mancuso S, Boselli M. 2002. Characterisation of the oxygen fluxes in the division, elongation and mature zone of *Vitis* roots: influence of oxygen availability. *Planta* **214,** 767–774.

Mancuso S, Papeschi G, Marras AM. 2000. A polarographic, oxygen-selective, vibrating-microelectrode system for the spatial and temporal characterisation of transmembrane oxygen fluxes in plants. *Planta* **211,** 384–389.

Markevich NI, Sel'kov EE. 1986. Mathematical model of resonance amplification on external effects on the membranes. *Biofizika* **31,** 662–666.

Marschner H. 1995. *Mineral nutrition of higher plants*, 2nd edn. London: Academic Press.

McAinsh MR, Brownlee C, Hetherington AM. 1997. Calcium ions as second messengers in guard cell signal transduction. *Physiologia Plantarum* **100,** 16–29.

McAinsh M, Hetherington A. 1998. Encoding specificity in Ca^{2+} signalling systems. *Trends in Plant Science* **3,** 32–36.

McAinsh MR, Webb AAR, Taylor JE, Hetherington AM. 1995. Stimulus-induced oscillations in guard-cell cytosolic-free calcium. *The Plant Cell* **7,** 1207–1219.

Miedema H, Balderas E, Pantoja O. 2000. Current oscillations under voltage-clamp conditions: an interplay of series resistance and negative slope conductance. *Journal of Membrane Biology* **173,** 31–37.

Miedema H, Prins HBA. 1991. pH-dependent proton permeability of the plasma membrane is a regulating mechanism of polar transport through the submerged leaves of *Potamogeton lucens*. *Canadian Journal of Botany* **69,** 1116–1122.

Newman IA. 1963. Electric potentials and auxin translocation in *Avena*. *Australian Journal of Biological Science* **16,** 629–646.

Njus D, Sulzman FM, Hastings JW. 1974. Membrane model for the circadian clock. *Nature* **248,** 116–119.

Pilet P-E, Versel JM, Mayor G. 1983. Growth distribution and surface pH pattern along maize roots. *Planta* **158,** 398–402.

Pineros MA, Shaff JE, Kochian V. 1998. Development, characterization, and application of a cadmium-selective microelectrode for the measurement of cadmium fluxes in roots of *Thlaspi* species and wheat. *Plant Physiology* **116,** 1393–1401.

Putney JW. 1998. Calcium signalling: up, down, up, down... What's the point? *Science* **279,** 191–192.

Rapp PE. 1987. Why are so many biological systems periodic? *Progress in Neurobiology* **29,** 261–273.

Rapp PE, Mees AI, Sparrow CT. 1981. Frequency encoded biochemical regulation is more accurate than amplitude dependent control. *Journal of Theoretical Biology* **90,** 531–544.

Richard P. 2003. The rhythm of yeast. *FEMS Microbiological Reviews* **791,** 1–11.

Ryan PR, DiTomaso JM, Kochian LV. 1993. Aluminium toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. *Journal of Experimental Botany* **44,** 437–446.

Satter RL, Galston AW. 1981. Mechanisms of control of leaf movements. *Annual Review of Plant Physiology* **32,** 83–110.

Scott BIH. 1957. Electric oscillations generated by plant roots and a possible feedback mechanism responsible for them. *Australian Journal of Biological Science* **10,** 164–179.

Shabala S. 2000. Ionic and osmotic components of salt stress specifically modulate net ion fluxes from bean leaf mesophyll. *Plant, Cell and Environment* **23,** 825–837.

Shabala S. 2003. Physiological implications of ultradian oscillations in plant roots. *Plant and Soil* **255,** 217–226.

Shabala S, Babourina O, Newman I. 2000. Ion-specific mechanisms of osmoregulation in bean mesophyll cells. *Journal of Experimental Botany* **51,** 1243–1253.

Shabala S, Knowles A. 2002. Rhythmic patterns of nutrient acquisition by wheat roots. *Functional Plant Biology* **29,** 595–605.

Shabala S, Lew RR. 2002. Turgor regulation in osmotically stressed *Arabidopsis* epidermal root cells. Direct support for the role of inorganic ion uptake as revealed by concurrent flux and cell turgor measurements. *Plant Physiology* **129,** 290–299.

Shabala S, Newman IA. 1997*a*. Proton and calcium flux oscillations in the elongation region correlate with root nutation. *Physiologia Plantarum* **100,** 917–926.

Shabala S, Newman IA. 1997*b*. Root nutation modelled by two ion flux-linked growth waves around the root. *Physiologia Plantarum* **101,** 770–776.

Shabala S, Newman IA. 1998. Osmotic sensitivity of Ca^{2+} and H^+ transporters in corn roots: effect on fluxes and their oscillations in the elongation region. *Journal of Membrane Biology* **161,** 45–54.

Shabala S, Newman I. 1999. Light-induced changes in hydrogen, calcium, potassium, and chloride ion fluxes and concentrations from the mesophyll and epidermal tissues of bean leaves. Understanding the ionic basis of light-induced bioelectrogenesis. *Plant Physiology* **119,** 1115–1124.

Shabala S, Newman IA, Morris J. 1997. Oscillations in H^+ and Ca^{2+} ion fluxes around the elongation region of corn roots and effects of external pH. *Plant Physiology* **113,** 111–118.

Shabala S, Newman I, Whittington J, Juswono U. 1998. Protoplast ion fluxes: their measurement and variation with time, position and osmoticum. *Planta* **204,** 146–152.

Shabala S, Shabala L. 2002. Kinetics of net H^+, Ca^{2+}, K^+, Na^+, NH_4^+, and Cl^- fluxes associated with post-chilling recovery of plasma membrane transporters in *Zea mays* leaf and root tissues. *Physiologia Plantarum* **114,** 47–56.

Shabala L, Shabala S, Ross T, McMeekin T. 2001. Membrane transport activity and ultradian ion flux oscillations associated with cell cycle of *Thraustochytrium* sp. *Australian Journal of Plant Physiology* **28,** 87–99.

Shabala S, Shabala L, Van Volkenburgh E. 2003. Effect of calcium on root development and root ion fluxes in salinized barley seedlings. *Functional Plant Biology* **30,** 507–514.

Stucki JW, Somogyi R. 1994. A dialog on Ca^{2+} oscillations: an attempt to understand the essentials of mechanisms leading to hormone-induced intracellular Ca^{2+} oscillations in various kinds of cell on a theoretical level. *Biochimica et Biophysica Acta* **1183,** 453–472.

Tegg RS, Melian L, Wilson CR, Shabala S. 2005. Plant cell growth and ion flux responses to the *Streptomycete* phytotoxin Thaxtomin A: calcium and hydrogen flux patterns revealed by the non-invasive MIFE technique. *Plant and Cell Physiology* **46,** 638–648.

Tyerman SD, Beilby M, Whittington J, Juswono U, Newman I, Shabala S. 2001. Oscillations in proton transport revealed from simultaneous measurements of net current and net proton fluxes from isolated root protoplasts: MIFE meets patch-clamp. *Australian Journal of Plant Physiology* **28,** 591–604.

Vucinic Z, Radenovic C, Damjanovic Z. 1978. Oscillations of the vacuolar potential in *Nitella*. *Physiologia Plantarum* **44,** 181–186.

Webb AAR. 2003. The physiology of circadian rhythms in plants. *New Phytologist* **160,** 281–303.

Zhang WH, Skerrett M, Walker NA, Patrick JW, Tyerman SD. 2002. Nonselective currents and channels in plasma membranes of protoplasts from coats of developing seeds of bean. *Plant Physiology* **128,** 388–99.

Zieschang HE, Kohler K, Sievers A. 1993. Changing proton concentrations at the surfaces of gravistimulated *Phleum* roots. *Planta* **190,** 546–554.

Zivanovic B, Vucinic Z. 1996. Photoperiodic induction of flowering in *Chenopodium rubrum* L. might be controlled by an oscillatory mechanism. *Journal of Plant Physiology* **149,** 707–713.

Zivanovic BD, Pang J, Shabala S. 2005. Light-induced transient ion flux responses from maize leaves and their association with leaf growth and photosynthesis. *Plant, Cell and Environment* **28,** 340–352.

Zonia L, Cordeiro S, Tupy J, Feijo JA. 2002. Oscillatory chloride efflux at the pollen tube apex has a role in growth and cell volume regulation and is targeted by inositol 3,4,5,6-tetrakisphosphate. *The Plant Cell* **14,** 2233–2249.