Chapter 12
Emergent Oscillatory Properties in Modelling Ion Transport of Guard Cells

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Abstract Transport across eukaryotic cell membranes, including those of plant cells, is so complex that it defies intuitive understanding. Quantitative systems modelling is essential in these circumstances, to test our understanding of the system through the experimental scrutiny of predictions. OnGuard is the first, fully integrated and quantitative modelling package developed for the study of cellular homoeostasis in guard cells and its relevance to stomatal aperture dynamics. It offers a unique tool for exploring the properties arising from interactions between plasma membrane and tonoplast transporters with the processes involved in the control of pH, ionic and neutral osmolite concentrations, membrane potentials and ion buffering in cytoplasm and vacuole. OnGuard has already yielded detail sufficient to guide phenotypic and mutational studies, opening the way towards 'reverse-engineering' of stomatal guard cells with the aim of improving water-use efficiency. We focus here on the fundamental insights OnGuard models offer for understanding the physiological phenomena of oscillations in membrane voltage, cytosolic-free Ca^{2+} concentration, and their roles in stomatal movements. OnGuard models faithfully reproduce differences in stomatal closure with oscillation

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frequency, much as observed in vivo and including an optimal cycle period. Analysis shows that these oscillations arise from the balance of transport activities at the plasma membrane and tonoplast to generate a range of resonant frequencies. The lowest frequencies are of sufficient duration to permit substantial changes in cytosolic-free Ca^{2+} concentration. Thus, we demonstrate the oscillations as an emergent property of the system of transport at the two dominant membranes of the guard cell.

12.1 Introduction

Our intuitive understanding of cellular homoeostasis is hampered by the complexity of the interactions between all the relevant transport, metabolic and buffering components active in the different cellular compartments. Handling this level of complexity demands an integrative computational framework from which the dynamic behaviour of the cell in physiological and experimental conditions may be derived from a full and quantitative accounting of each of the contributing process. A substantial body of data now exists enabling integrated modelling of cellular homoeostasis in many eukaryotic cells. The physico-chemical principles that govern and connect this information have been developed and successfully implemented in a variety of cell systems in recent decades (Lew et al. 1979; Lew and Bookchin 1986; Mauritz et al. 2009; Hills et al. 2012).

The critical components in all these models are the primary ion and solute transporters of the cell membranes, encoded by unique sets of kinetic and regulatory descriptors. Every transport process that carries charge across a membrane will affect—and will be affected by—the voltage across that membrane. In some cases, this relationship is a consequence of mass action and the movement of the charged ions it carries; in others, it reflects direct effects of voltage and ion concentrations on regulatory sites on the channel. In either case, we are forced to consider voltage and solute concentration as both substrates and products of transport. Furthermore, the voltage—and often the ion concentration(s)—is shared between all of the charge-carrying transporters at the membrane, and the dependencies on voltage of the individual transporters are frequently nonlinear (Blatt et al. 1987; Blatt and Slayman 1987; Sanders 1990; Weiss 1996). Models of cellular homoeostasis in plants have been applied to validate a role for K^+ transport in phloem loading of sucrose as an 'energy reserve' (Gajdanowicz et al. 2011) and to describe oscillatory characteristics in H^+ and K^+ fluxes of roots (Shabala et al. 2006). However, the real test of any systems model is its capacity for predicting unexpected behaviours that are experimentally testable.

The Homoeostasis, Transport and Signalling (HoTSig) libraries (Hills et al. 2012) address this issue. HoTSig is built on an open, expandable structure that incorporates user-definable libraries for transporter kinetics, chemical buffering, macromolecular binding, metabolic reactions and macroscopic equations to couple

these processes to solute content, cell volume and turgor. Its open structure makes the platform adaptable to any single-cell system for which an accurate description of these macroscopic relations is known, and it offers the potential for applications to multicellular situations that are best scaled from the cellular to whole-tissue and organ structures. The OnGuard software (Chen et al. 2012; Hills et al. 2012) is the first implementation of the HoTSig libraries and is freely available at www.psrg.org.uk. OnGuard focuses on guard cell mechanics and on the control of stomatal aperture. It includes a graphical user interface for real-time monitoring of the individual transport and homoeostatic processes under simulation. A unique feature of OnGuard is its Reference State Wizard which serves as an aid to defining a starting point for experimental simulations with sensible outputs for all known variables.

OnGuard models of *Vicia* and Arabidopsis guard cells have reproduced many of the known properties of ion transport, solute content and stomatal aperture in response to well-defined experimental manipulations of these cells. They have also generated a number of unexpected and emergent outputs, notably demonstrating a homoeostatic 'communication' between the plasma membrane and tonoplast independent of ad hoc signal transduction networks, counterintuitive changes in cytosolic-free $[Ca^{2+}]$ ($[Ca^{2+}]_i$) and pH over the diurnal cycle, and an exchange of vacuolar Mal^{2-} with Cl^- subject to the availability of the anion, all of which found direct support in independent experimental data (Raschke and Schnabl 1978; MacRobbie 1991, 1995, 2000, 2006; Thiel et al. 1992; Blatt and Armstrong 1993; Willmer and Fricker 1996; Frohnmeyer et al. 1998; Dodd et al. 2005, 2006). Furthermore, OnGuard has been shown to possess true predictive power. Two examples suffice here. In studies with the Arabidopsis *slac1* Cl^- channel mutant, Wang et al. (2012) found that eliminating the Cl^- channel, which greatly slows stomatal closure, also suppresses inward-rectifying K^+ channel activity, enhances outward-rectifying K^+ channel activity, and slows stomatal opening. OnGuard analysis predicted these effects to arise from anion accumulation in the mutant which in turn affects the H^+ and Ca^{2+} loads on the cytosol, elevating cytosolic pH and $[Ca^{2+}]_i$, both factors that are well known to regulate the K^+ channels. These predictions were confirmed experimentally, and redressing the balance of cytosolic pH and $[Ca^{2+}]_i$ was shown to be sufficient to restore both the K^+ currents and wild-type stomatal opening kinetics. Thus, OnGuard modelling uncovered a homoeostatic network that was unexpected and which connects three unrelated ion channels in the guard cell. In a second study, Wang et al. (2014a) addressed the question of whether guard cell function might be manipulated through genetic means to improve stomatal dynamics. This study represents a crucial step towards the use of OnGuard modelling for in silico design and reverse-engineering of guard cell physiology, notably to improve water-use efficiency during photosynthesis (Lawson et al. 2012; Blatt et al. 2014; Lawson and Blatt 2014). These studies confirmed independent and concurrent experimental work (Wang et al. 2014b) that reported no substantial effects on water-use efficiency of overexpressing either the AHA2 H^+-ATPase or the dominant K^+ channels. Instead, the modelling predicted that enhanced stomatal dynamics and gains in water-use efficiency were most likely

to be realized through modification of the voltage- and K^+-dependent gating of the K^+ channels.

We focus now on one intriguing, and as yet unresolved question concerning the role of Ca^{2+} oscillations in stomatal behaviour. The $[Ca^{2+}]_i$ of guard cells at rest is about 50–200 nM, as it is in virtually all living cells, but can be induced to rise above 1 μM—and locally, most likely above 10 μM—following stimulation (Blatt 2000). Such elevations are often short-lived, lasting for periods of tens of seconds to a few minutes, and may be periodic (McAinsh et al. 1995; Webb et al. 1996; Grabov and Blatt 1998, 1999; Staxen et al. 1999; Allen et al. 2001). These transients in $[Ca^{2+}]_i$ have frequently been associated with stomatal closure. They are understood to accelerate closure by promoting controlled, often cyclic release of K^+, Cl^- and malate (Mal^{2-}) from the guard cell (Chen et al. 2012). Here, we examine one aspect of $[Ca^{2+}]_i$ oscillations, namely how different frequencies in the oscillation might arise, what the consequences are for osmotic solute flux, and whether specific frequencies are critical for stomatal closure.

12.2 Sources of Ca^{2+} for $[Ca^{2+}]_i$ Elevation and Oscillations

A brief review of our experimental knowledge of Ca^{2+} and $[Ca^{2+}]_i$ signalling in guard cells is useful. The topic has been reviewed extensively (Blatt 2000; Hetherington and Brownlee 2004; Martinoia et al. 2007; Roelfsema and Hedrich 2010), and we make no attempt to be exhaustive in our coverage, but instead highlight elements most relevant to oscillatory behaviours. Three fundamental characteristics are prerequisite for the transience of $[Ca^{2+}]_i$ increases and their cyclic appearance: (1) Ca^{2+} flux into the cytosol must arise from at least two sources or pathways with different flux kinetics; (2) each of these pathways must incorporate mechanisms for self-limitation; and (3) each source must include mechanisms for Ca^{2+} recovery following a rise in $[Ca^{2+}]_i$. All three conditions must be met for any oscillatory behaviour in $[Ca^{2+}]_i$. Much controversy initially surrounded the origins of Ca^{2+} for $[Ca^{2+}]_i$ elevations, if only because Ca^{2+}-evoked signalling was frequently observed to be sensitive to Ca^{2+} depletion in the apoplast (De Silva et al. 1985). It is now generally accepted that guard cells draw on both Ca^{2+} from outside and on one or more endomembrane stores of Ca^{2+}. So the first of the three conditions is met. Similarly, the third condition is clearly satisfied by the distribution of energy-coupled Ca^{2+} transporters, both mediating Ca^{2+}/H^+ exchange—the so-called CAX antiporters—and the ACA Ca^{2+}-ATPases across all of the major membranes within the plant cell (Lopez-Marques et al. 2004; Pardo et al. 2006; Martinoia et al. 2007; Bonza and De Michelis 2011; Pittman 2011). Not only do these transporters provide the essential capacity for Ca^{2+} recovery but also the affinity to scavenge Ca^{2+} across a wide range of free concentrations [see Appendix 2 and Tables 1–6 of Hills et al. (2012)]. It is the second condition that has proven the more difficult to demonstrate based on the physiological and biophysical characteristics of known Ca^{2+} transport pathways in plants.

The major pathway for extracellular Ca^{2+}-entry is via Ca^{2+}-permeable channels at the plasma membrane—notably the highly Ca^{2+}-selective, hyperpolarization-activated Ca^{2+} channels of low conductance—and potentially via a number of other non-selective, Ca^{2+}- and cation-permeable channels (Very and Sentenac 2002), including several of the glutamate receptor-like channels (Qi et al. 2006; Cho et al. 2009). The hyperpolarization-activated Ca^{2+} channels mediate Ca^{2+} influx in a strongly voltage-dependent manner, especially at voltages negative of approximately −150 mV, and their activity (open probability) is suppressed as $[Ca^{2+}]_i$ rises to 1 μM and above (Grabov and Blatt 1997, 1998, 1999; Hamilton et al. 2000, 2001), thus satisfying the second condition for these channels to contribute to $[Ca^{2+}]_i$ transients and oscillations. Indeed, to date, the hyperpolarization-activated Ca^{2+} channels of guard cells are the only channels in plants known to exhibit such self-limitation.

Of interest, these Ca^{2+} channels are activated by ABA, which shifts the apparent threshold for activity to more positive voltages and, intriguingly, ABA acts on the channels even when isolated in membrane patches (Hamilton et al. 2000). These characteristics fit closely the observations that ABA affects the apparent voltage threshold for $[Ca^{2+}]_i$ elevations (Grabov and Blatt 1998). It may be that this shift in voltage sensitivity is connected to (de-)phosphorylation of the channels or proteins that associate with them. Certainly, Ca^{2+} influx is regulated by Ca^{2+}-dependent protein kinases, especially the Ca^{2+}-dependent protein kinases CPK3 and CPK6 (Mori et al. 2006). However, until the molecular identity of these Ca^{2+} channels is resolved, a clear knowledge of this regulatory process is hampered. This point not withstanding, it is clear that these channels are activated by reactive oxygen species (ROS), as their enhancement by ABA is impaired in the Arabidopsis *atrbohd* and *atrbohf* mutants which encode NADPH oxidases (Kwak et al. 2003). In a recent study, the ABA receptor quadruple mutant *pyr1/pyl1/pyl2/pyl4,* which shows a much reduced response to ABA (Park et al. 2009; Nishimura et al. 2010), was found to suppress $[Ca^{2+}]_i$ elevation in response to ABA. This impairment was linked to a loss in ABA-evoked ROS production, as adding the ROS hydrogen peroxide (H_2O_2) was sufficient to recover both enhanced activity of the Ca^{2+} channels and stomatal closure (Wang et al. 2013). Thus, at least one pathway for ABA to elevate $[Ca^{2+}]_i$ is probably via a ROS intermediate, although neither these studies, nor the earlier work with the *atrbohd* and *atrbohf* mutants, distinguish between the roles for ROS as a primary intermediate and as a 'permissive' factor.

Until recently, it was less obvious what the contributions are from Ca^{2+}-permeable but non-selective cation channels and their regulation. Two genes, *OSCA1* (Yuan et al. 2014) and its close homologue *AtCSC1* (Hou et al. 2014), are now known to encode such non-selective cation channels at the guard cell plasma membrane, and both show significant permeability to Ca^{2+}. The *OSCA1* mutant is impaired in Ca^{2+} signalling in response to hyperosmotic stress, suggesting that OSCA1 is likely to be important for $[Ca^{2+}]_i$ increases associated with mechanical or osmotically induced stress at the plasma membrane. However, the mutant was

found to respond normally to ROS (H_2O_2) treatments, and it showed a normal ABA response in stomatal closure. Furthermore, no evidence of self-limitation has come to light for OSCA1 as yet. These observations underscore the specificity of stimulus-specific Ca^{2+} signalling pathways in $[Ca^{2+}]_i$ elevation. They also suggest that these channels are unlikely to contribute to cyclic elevations in $[Ca^{2+}]_i$.

Calcium entry is important to trigger increases in $[Ca^{2+}]_i$, but best estimates (Chen et al. 2012) suggest that Ca^{2+} from outside accounts for some 5 % or less of the total Ca^{2+} entering the cytosol during $[Ca^{2+}]_i$ transients. The bulk of the cation flux comes from intracellular stores that are sequestered within endomembrane compartments. Like animals (Bezprozvanny et al. 1991; Hille 2001), guard cells clearly incorporate endomembrane channels for Ca^{2+} release. Furthermore, there is unequivocal evidence for Ca^{2+} release as $[Ca^{2+}]_i$ rises with Ca^{2+} influx across the plasma membrane (Grabov and Blatt 1998, 1999), a process often identified as Ca^{2+}-induced Ca^{2+} release (CICR). Channels that respond to cytosolic Ca^{2+} and ligands, including IP_3, cyclic ADP-ribose (cADPR), nitric oxide (NO) and inositol hexakisphosphate (IP_6), are known or have been implicated as Ca^{2+} flux pathways at the endoplasmic reticulum and tonoplast of plant cell types, including those of *Vicia* guard cells (Alexandre et al. 1990; Muir and Sanders 1996; Wu et al. 1997; Leckie et al. 1998; Grabov and Blatt 1999; Garcia-Mata et al. 2003; Lemtiri-Chlieh et al. 2003). Again, the prerequisite for self-limitation implies that the predominant pathways for Ca^{2+} release, like the animal counterparts, should show a Ca^{2+}-dependence that leads to flux suppression at elevated $[Ca^{2+}]_i$. It is of interest, therefore, that none of the pathways for Ca^{2+} release identified at a molecular level to date satisfy this second condition for $[Ca^{2+}]_i$ oscillations.

Consider, for example, the slow-vacuolar (SV) channels that have been identified with the *TPC1* gene product in Arabidopsis (Peiter et al. 2005). SV channels are well known as a Ca^{2+}- and K^+-permeable pathway (Peiter et al. 2005; dacz-Narloch et al. 2011) and have been repeatedly proposed as an important Ca^{2+} release pathway. Arguments for its potential regulation by dephosphorylation (Allen and Sanders 1995) not withstanding, however, SV channels in general are unsuited to the task of physiological Ca^{2+} release precisely because their Ca^{2+}-dependence is not linked to $[Ca^{2+}]_i$ nor is there evidence of suppression when $[Ca^{2+}]_i$ is elevated. Other endomembrane stores, including mitochondria and chloroplasts, may contribute as sources of Ca^{2+}, subject to their capacities both for Ca^{2+} sequestration and its release (Subbaiah et al. 1998; Coelho et al. 2002; Loro et al. 2012). However, their dominance in any process of CICR will be defined by the ability for elevated $[Ca^{2+}]_i$ to suppress the activities of the associated Ca^{2+} release pathways. Indeed, we predicted before (Chen et al. 2012) that one or more channels eventually will surface exhibiting these characteristics and consequently have used our knowledge of $[Ca^{2+}]_i$ dynamics to 'reverse-engineer' a hypothetical Ca^{2+} release channel in our models with the characteristics of the animal IP_3 and ryanodine receptor channels essential for self-limitiation.

12.3 $[Ca^{2+}]_i$ Control of Osmotic Solute Flux

Although a $[Ca^{2+}]_i$ increase has not always been observed with stomatal closure [cf. Gilroy et al. (1991); Armstrong et al. (1995); Romano et al. (2000); also discussion by Lemtiri-Chlieh and MacRobbie (1994)], the consensus is that its elevation is important to accelerate closure in the face of biotic and abiotic stress. For transport across the plasma membrane, the primary effects of $[Ca^{2+}]_i$ elevation are to suppress current through the inward-rectifying K^+ channels and the H^+-ATPase, and to enhance the activity of anion channels mediating Cl^- and Mal^{2-} efflux (Schroeder and Hagiwara 1989; Blatt et al. 1990, 2007; Kinoshita et al. 1995; Blatt 2000; Chen et al. 2010; Roelfsema and Hedrich 2010). At the tonoplast, increasing $[Ca^{2+}]_i$ suppresses H^+ transport via the K^+-activated pyrophosphatase (Darley et al. 1998) and the FV-type K^+ channels, and it activates CAX Ca^{2+}/H^+ exchange (Pittman 2011), the TPK1 K^+ channel (Gobert et al. 2007), the K^+- and Ca^{2+}-permeable TPC1 channel and vacuolar anion channels (Pei et al. 1996, 1999; Beyhl et al. 2009; Rienmuller et al. 2010). Of course, raising $[Ca^{2+}]_i$ also enhances Ca^{2+}-ATPases at both membranes to drive Ca^{2+} out of the cytosol (Geisler et al. 2000; Sze et al. 2000; Bonza and De Michelis 2011; Pittman 2011). Thus, the overall effects of raising $[Ca^{2+}]_i$ are to reduce direct, metabolically driven H^+ transport energizing both membranes and to increase their conductances for net export of K^+, Cl^- and Mal^{2-} from the vacuole, through the cytosol, and out across the plasma membrane. The exceptions of the inward-rectifying K^+ channels at the plasma membrane and the FV channels at the tonoplast also fit with this pattern, as both normally contribute to K^+ uptake rather than its release from the guard cells. In short, elevated $[Ca^{2+}]_i$ adjusts the balance of much of transport across both membranes to enhance the capacity for solute efflux from the guard cell and, at the same time, it reduces the energization of both membranes that might otherwise counter this efflux. There remain a smaller number of transporters that are demonstrably $[Ca^{2+}]_i$-insensitive or are anticipated to be so, notably the outward-rectifying K^+ channels and H^+-coupled transporters at the plasma membrane and the V-type H^+-ATPase and CLC Cl^-/H^+ antiporters at the tonoplast. These transporters are either regulated by the parallel signalling pathway of cytosolic pH or under direct substrate control of the H^+ concentration. They are also controlled directly by membrane voltage. An exhaustive list of the relevant kinetic properties and $[Ca^{2+}]_i$ sensitivities for all of these transporters will be found in Appendix 2 and Supplemental Tables 1–6 of Hills et al. (2012).

12.4 The Importance of Voltage Control

Membrane voltage, especially across the plasma membrane, is a major factor determining $[Ca^{2+}]_i$ at rest, during transient elevations and its recovery. Clearly, voltage exerts its control on Ca^{2+} entry by moderating the open probability of the hyperpolarization-activated Ca^{2+} channels and, hence, the Ca^{2+} influx they will

facilitate. Hamilton et al. (2000, 2001) reported an apparent gating charge—that is, voltage sensitivity—near unity, implying a twofold increase in Ca^{2+} permeability with each −18 mV shift in voltage. The result is to impose a substantial kinetic constraint on the rate of $[Ca^{2+}]_i$ rise in vivo that is related directly to the prevailing voltage and to the chemical driving force for Ca^{2+} entry (Grabov and Blatt 1998). Voltage will also exert control on Ca^{2+} efflux through the ACA Ca^{2+}-ATPases at this membrane, but antiparallel to its effect on the Ca^{2+} channels. These pumps almost certainly couple charge (Ca^{2+}) flux with ATP hydrolysis in a 2:1 ratio (Geisler et al. 2000; Sze et al. 2000; Bonza and De Michelis 2011), implying a reversal voltage near −200 mV at resting $[Ca^{2+}]_i$ with 1 mM Ca^{2+} outside and a significant kinetic enhancement as the membrane depolarizes from −150 mV towards voltages near 0 mV (Hills et al. 2012). Thus, voltage transitions across the physiological range from −200 to −50 mV can be expected to bias the plasma membrane alternately for Ca^{2+} influx and $[Ca^{2+}]_i$ elevation on the one hand and for Ca^{2+} efflux and $[Ca^{2+}]_i$ recovery on the other. Indeed, this is precisely what has been observed in the intact guard cell (Grabov and Blatt 1998; Hamilton et al. 2000; Garcia-Mata et al. 2003), and the effect is demonstrably coupled through endomembrane Ca^{2+} release (Grabov and Blatt 1999).

Equally, voltage exerts a primary control on osmotic solute flux across the guard cell plasma membrane, both as a driving force for transport and as a regulatory factor affecting the gating of several ion channels that contribute to these fluxes. K^+ and Cl^- (anion) channels at plasma membrane. These transporters dominate trans-membrane solute flux in many circumstances (Willmer and Fricker 1996; Blatt 2000; Blatt et al. 2007; Pandey et al. 2007; Melotto et al. 2008; Ward et al. 2009; Lawson and Blatt 2014) and are complemented by H^+-coupled transport of K^+ (Blatt 1988) and, undoubtedly, of Cl^- as well as malate (Mal^{2-}) (Barbier-Brygoo et al. 2011; Chen et al. 2012; Hills et al. 2012). They facilitate K^+ and anion uptake for stomatal opening and their efflux to the apoplast for stomatal closing. What determines the bias between these solute fluxes is largely dependent on energization of the membrane and H^+-ATPase activity. Indeed, guard cells, such as many plant cells studied to date, show two states of the membrane voltage. One state is characterized by voltages close to the K^+ equilibrium voltage (E_K), with a conductance that is dominated by currents through the GORK K^+ channel in Arabidopsis (Hosy et al. 2003; Eisenach et al. 2014) and similar outward-rectifying K^+ channels ($I_{K,out}$) (Blatt and Armstrong 1993; Blatt and Thiel 1994; Blatt and Gradmann 1997) and by the anion current (I_{Cl}) that in Arabidopsis is characterized by SLAC1 and its homologous (Negi et al. 2008; Vahisalu et al. 2008; Geiger et al. 2009; Wang et al. 2012). This state is typical of the guard cell during solute loss for stomatal closure. The second state is characterized by voltages well-negative of E_K with a conductance that derives from the activities of the AHA H^+-ATPases (Lohse and Hedrich 1992; Thiel et al. 1992; Gradmann et al. 1993), primarily AHA1 and AHA2 (Merlot et al. 2007; Blatt et al. 2014), and by inward-rectifying K^+ channel current ($I_{K,in}$) including that through the channels KAT1 and KAT2 (Nakamura et al. 1995; Pilot et al. 2001). This second state reflects a bias for K^+ and Cl^- (anion) accumulation and is typical of the guard cell during stomatal opening. So, much as it affects Ca^{2+} flux and $[Ca^{2+}]_i$

dynamics, transitions in plasma membrane voltage across the same physiological range generally define whether the guard cell is actively accumulating solute or losing it. Again, such oscillations in voltage are known and have been associated with stimuli that trigger the transition from open to closed stomata (Blatt and Armstrong 1993; Gradmann et al. 1993; Blatt and Thiel 1994).

In short, plasma membrane voltage is a common denominator that connects $[Ca^{2+}]_i$ and the osmotic solute fluxes that drive stomatal movements within an elegant counterpoint. At its negative extreme, voltage favours K^+ and anion uptake but also triggers Ca^{2+} entry to elevate $[Ca^{2+}]_i$; at its positive extreme, voltage activates the channels mediating K^+ and anion efflux as well as engaging Ca^{2+}-ATPases to restore $[Ca^{2+}]_i$ to its resting level. Blatt (2000) originally described this relationship as a 'signalling cassette' in four steps which can now be updated with new kinetic detail pertaining to the H^+-ATPase (Merlot et al. 2007) and to the anion channels (Chen et al. 2010): (1) with resting $[Ca^{2+}]_i$ low, negative membrane voltage triggers Ca^{2+} influx across the plasma membrane, stimulating intracellular Ca^{2+} release to elevate $[Ca^{2+}]_i$; (2) the rise in $[Ca^{2+}]_i$ inactivates $I_{K,in}$, the Ca^{2+} channels, and it inactivates the H^+-ATPase and activates I_{Cl} to promote membrane depolarization; (3) depolarization favours K^+ and Cl^- efflux and, with the Ca^{2+} influx suppressed, permits the Ca^{2+}-ATPases and CAX transporters to resequester Ca^{2+} and restore $[Ca^{2+}]_i$; and finally, (4) with a falling $[Ca^{2+}]_i$, I_{Cl} declines, $I_{K,in}$ and the H^+-ATPases recover sufficiently to repolarize the membrane and enable K^+ and H^+-coupled anion uptake. At the core of this process is the intrinsic feedback of membrane transporters operating across a common membrane, and so sharing the common electrical driver of membrane voltage; it also incorporates a second feedback loop of $[Ca^{2+}]_i$-dependent controls on several of the major transporters that dominate osmotic solute flux and on the H^+-ATPase, not just on the Ca^{2+} channels at the plasma membrane and those predicted to associate with endomembrane stores. The overall consequence is that voltage and $[Ca^{2+}]_i$ oscillate in a syncopated rhythm with K^+ and anion flux, solute content and stomatal aperture.

12.5 An Oscillatory Optimum for Stomatal Response

However intuitively the preceding description may appear, it hides much subtlety in the oscillatory behaviour of guard cells. In the simplest sense, we might anticipate, for example, that the time-averaged ratio of residence in the hyper- and depolarized states should determine whether the guard cell shows a net uptake or loss in solute. In other words, averaged over a period of 30 min, three cycles of $[Ca^{2+}]_i$ elevation—and membrane depolarization—totalling a period of 24 min should promote solute loss and stomatal closure; similar cycles totalling a period of 15 min of $[Ca^{2+}]_i$ elevation and membrane depolarization should have no net effect on solute content or aperture; and, with a total of 6 min $[Ca^{2+}]_i$ elevation and depolarization, the solute content and aperture should increase. To an extent, this pattern has been found to hold true. Allen et al. (2001) imposed oscillations in $[Ca^{2+}]_i$ and found that

increasing the period of its elevation promoted stomatal closure. Intriguingly, however, the most effective cycle period was of 10 min with $[Ca^{2+}]_i$ elevations of 5 min interspersed with 5-min recovery periods. Shorter recovery periods were less effective, whether they were interspersed with the same or with longer periods in $[Ca^{2+}]_i$ elevation.

Interpreting these observations is complicated by the use of external buffers that varied both the Ca^+ and K^+ concentrations. Not only would elevated K^+ outside have affected membrane voltage, but it would also have greatly increased K^+ availability, the associated K^+ conductance (Blatt 1987; Schroeder 1988; Blatt 1992) and the rates of its uptake (Willmer and Fricker 1996). Even so, that there should exist an optimal $[Ca^{2+}]_i$ oscillation frequency for stomatal closure is not intuitively obvious, and it underscores two points. First, coupling between $[Ca^{2+}]_i$, voltage and osmotic solute flux is not linear. Indeed, this nonlinearity is amply demonstrated in the voltage dependence of the component currents, as we have noted above, but its consequences are less obvious. Second, the response of solute flux to $[Ca^{2+}]_i$ and voltage depends on the recent history of both, in other words solute flux almost certainly relaxes with time following transitions in $[Ca^{2+}]_i$ and voltage. This second point almost certainly relates to emergent properties that arise from the interactions between the different transporters, and this interpretation makes sense in context of related studies of the *det3* mutant (Allen et al. 2000). The *det3* mutation, which suppresses the vacuolar H^+-ATPase, showed prolonged $[Ca^{2+}]_i$ increases without oscillation and reduced stomatal closure in the face of high Ca^{2+} outside. Thus, both observations beg the question of what we can learn from quantitative systems modelling of these oscillations.

12.6 Modelling Oscillations and Stomatal Responsiveness

Detailed information is available on the construction of the *Vicia* and *Arabidopsis* models (Chen et al. 2012; Hills et al. 2012; Wang et al. 2012), including a complete listing of the transporters and their characteristics incorporated in these models [see Appendix 2 and Supplemental Tables 1–6 in Hills et al. (2012)]. Also available is a video introduction to OnGuard (www.psrg.org.uk) and a step-by-step guide to its basic application (Blatt et al. 2014). We used the Arabidopsis model with the published settings to analyse the $[Ca^{2+}]_i$ and voltage oscillations. In the model, the kinetics of primary ATP-dependent transport is coupled to fluence rate (Chen et al. 2012). Voltage and $[Ca^{2+}]_i$ oscillations arise near the end of the daylight period, shortly before and during stomatal closure, as primary transport activity declines and the accumulated energy in the various ionic gradients is released. Much of the diurnal cycle is shown in Fig. 12.1 and the times late in the daylight period are expanded in Fig. 12.2. The overviews show oscillations in voltage as they arise, and they illustrate the gradual shift over a period of 3 h as these rapid and shallow cycles first develop, then contract through accelerating oscillatory cycles, culminating with a series of long membrane depolarizations, each of several minutes' duration.

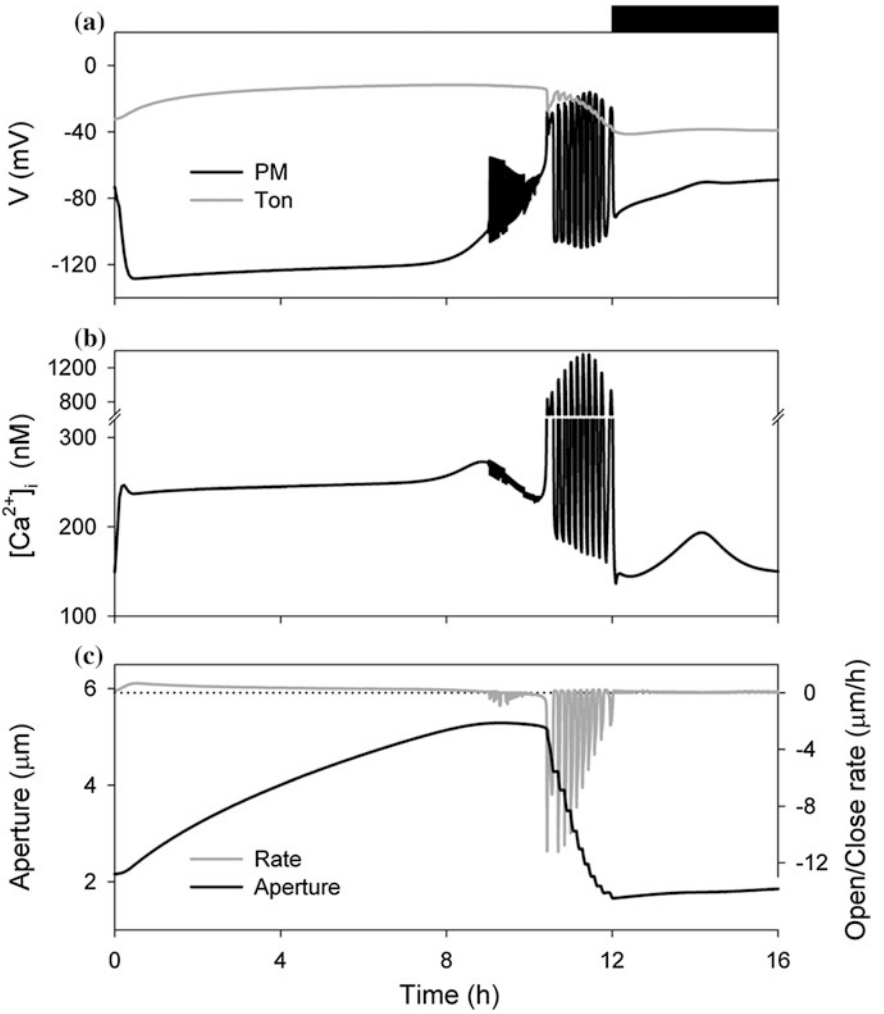

Fig. 12.1 Macrosocpic ouputs from the OnGuard model. Outputs resolved over a standard diurnal cycle (12 h light:12 h dark; *dark period* indicated by bar above) with 10 mM KCl, 1 mM $CaCl_2$ and pH 6.5 outside (Chen et al. 2012; Wang et al. 2012). Only the first 16 h of the diurnal cycle is shown. The full set of model parameters and initialising variables are listed in Wang et al. (2012) and are available with the OnGuard software at www.psrg.org.uk. Shown are **a** plasma membrane and tonoplast voltage, **b** cytosolic-free $[Ca^{2+}]$ ($[Ca^{2+}]_i$) and **c** stomatal aperture and the rate of opening/closing in stomatal aperture (=ΔAperture/Δt). Note that, positive rates here indicate opening

Each of the oscillations in voltage is accompanied by comparably scaled cycles in $[Ca^{2+}]_i$. Voltage initially traverses a range of approximately 50 mV in cycles with periods near 0.3 min, these cycles decaying to nothing in ever shorter cycles over the next hour. Likewise, $[Ca^{2+}]_i$ cycles largely in phase throughout this time and its oscillations, too, relax to nothing in parallel. These oscillations and, in part those of

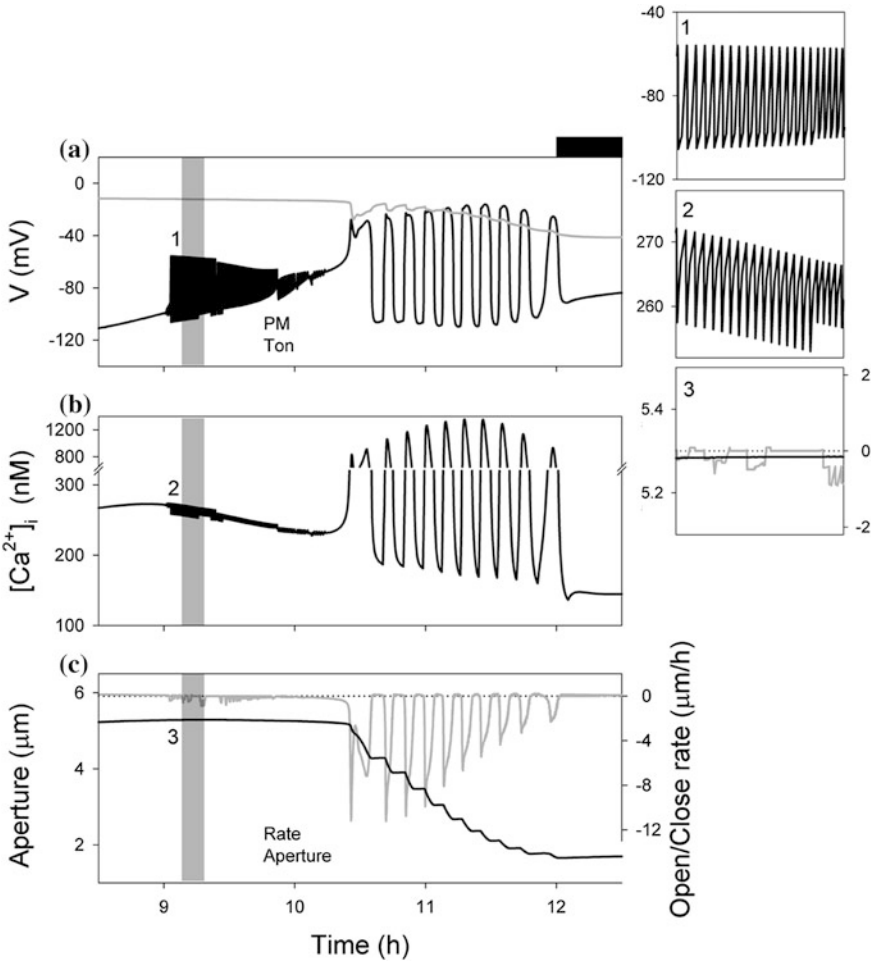

Fig. 12.2 Expanded view of the macrosocpic ouputs from the OnGuard model. Outputs as in Fig. 12.1 for the diurnal period of 8.5–12.5 h (*dark period* indicated by bar above). Shown are **a** plasma membrane and tonoplast voltage, **b** cytosolic-free $[Ca^{2+}]$ ($[Ca^{2+}]_i$) and **c** stomatal aperture and the rate of opening/closing in stomatal aperture (=ΔAperture/Δt). Note that, positive rates here indicate opening. *Insets* (*right*) correspond to the period highlighted by the grey bars (9.1–9.3 h) and show the rapid cycles in voltage, $[Ca^{2+}]_i$, and the corresponding effects on the rates of opening/closing in expanded view (cross-referenced by numbers)

voltage, are clipped to give a saw-tooth pattern throughout. Similarly, the long voltage oscillations are closely matched by long cycles of $[Ca^{2+}]_i$ elevation and recovery. However, unlike the shorter cycles, the transitions in voltage appear to settle at each extreme. The accompanying $[Ca^{2+}]_i$ changes relax and, on elevation, rise and then decay as Ca^{2+} release is followed by its resequestration and export from the cytosol (Chen et al. 2012). Thus, these extended time periods are long enough to permit the rise and fall in $[Ca^{2+}]_i$ to 'catch up' with the voltage cycle.

Coupled with the long cycles in voltage and $[Ca^{2+}]_i$ are cyclic variations in the H^+-ATPase, anion and outward- and inward-rectifying K^+ currents (Chen et al. 2012). From the standpoint of total membrane current, these currents are evident in a bi-stable relationship with voltage—an N-shaped current–voltage curve with two positive inflection points on the voltage axis—and its displacement alternately above and below the voltage axis (Jack et al. 1983; Blatt and Thiel 1994). Indeed, all of the oscillatory behaviour is coupled to N-shapes in the current–voltage curve and corresponding alternations between solute uptake and efflux (Chen et al. 2012), but the effects on aperture are most obvious when the cycles are of sufficient duration. In this case, the oscillations are accompanied by substantial decreases in stomatal aperture separated by periods of little change in aperture. The effect is of stepwise decay from roughly 5.5 to 1.8 μm in aperture. What is of interest is the difference in rates of stomatal closure as the cycles in voltage and $[Ca^{2+}]_i$ expand. To illustrate this point, Fig. 12.3 summarizes the mean rates of stomatal closure as a function of oscillation period. The closure rates here are corrected for the decline in thermodynamic driving forces for K^+ and anion flux. Closure clearly follows a biphasic relation with oscillation period, showing a maximum rate near a period of 8.9 min and falling off steeply to either side of this value. Analysis (C. Minguet, Y. Wang and M.R. Blatt in preparation) shows that this maximum associates with a mean time fraction around 0.5–0.55 in the depolarized phase of the cycle—that is, closure is most rapid with a near-symmetrical cycle of elevated $[Ca^{2+}]_i$ (depolarized voltage) and resting $[Ca^{2+}]_i$ (hyperpolarized voltage)—and that the peak in closure rate corresponds directly with the maximum in the dynamic, $[Ca^{2+}]_i$-dependent suppression of the H^+-ATPase. By contrast, the same analysis shows no substantial association with the underlying activities of the Ca^{2+} channels, nor on that of the K^+ and anion channels at the plasma membrane.

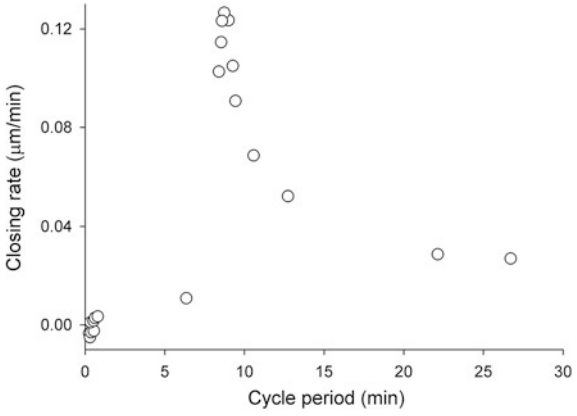

Fig. 12.3 Aperture closing rate as a function of the oscillation period in voltage and cytosolic-free Ca^{2+} concentration ($[Ca^{2+}]_i$). Mean aperture closing rates during each of the long oscillatory cycles and a selection of the initial rapid cycles are taken from the data of Figs. 12.1 and 12.2. Note the sharp maximum in closing rate at a period of 8.9 min (0.0019 Hz)

How, then, can we encapsulate these observations to define a role for $[Ca^{2+}]_i$ and voltage oscillations in the guard cell? Clearly, the simulations provide a close match to the behaviours observed in vivo and, thereby, support the initial perceptions of an interplay between voltage, the Ca^{2+} channels and endomembrane Ca^{2+} release in driving the $[Ca^{2+}]_i$ cycle (Blatt 2000). The OnGuard models give quantitative substance to these previous experimental findings of voltage and $[Ca^{2+}]_i$ oscillations (Grabov and Blatt 1998; Allen et al. 2001). They support the perception that the oscillations accelerate the rate of stomatal closure and that the most effective bandwidths fall within a narrow range of frequencies near 0.0019 Hz (= 8.9 min period). It is remarkable that the OnGuard models faithfully reproduce experimental data suggesting an optimal cycle period for accelerated stomatal closure. What is all the more important, therefore, is that the OnGuard analysis offers a new and truly mechanistic basis from which to understand the origins of this optimum cycle frequency. The analysis does not support the perception that this frequency is in any way unique, and this viewpoint is underscored by the range of oscillatory frequencies arising from the OnGuard models. All of these oscillations find their origins in the underlying N-shaped relationships between current and voltage which, in turn, arise from the component currents of the various charge-carrying transporters (Chen et al. 2012). Perhaps, more useful, then, is to recognize that these cycles of voltage and $[Ca^{2+}]_i$ simply reflect the several resonance frequencies that emerge from the balance of intrinsic transport activities of the guard cell. It happens that the slowest of these frequencies is sufficiently long-lived to permit substantial elevations in $[Ca^{2+}]_i$ and its recovery, and these oscillations in turn facilitate K^+, Cl^- and Mal^{2-} efflux from the guard cells.

12.7 Conclusion and Outlook

From the discussion and simulations presented here, two general conclusions can be drawn. The first—that transporters operating at one membrane will interact—is implicit to their situation, operating in parallel across a common membrane. We highlighted this point at the start of this chapter. It arises from the shared membrane voltage, as well as the common pools of ions on either side of the membrane, and it generates interactions, both direct and indirect, often between unrelated transport processes. The second observation similarly arises from the commonality of the pools of ionic species and the controls exerted by $[Ca^{2+}]_i$ and voltage. Both act on several key solute transporters at both the plasma membrane and the tonoplast, including the Ca^{2+} channels and ATPases that transport Ca^{2+} itself. The interactions of these interlinked regulatory loops, one mediated through the common voltage across the plasma membrane and the second through $[Ca^{2+}]_i$, give rise to resonance behaviours that emerge only when the system is treated as a whole. In short, we can conclude that there is nothing intrinsic to these resonance frequencies other than these interactions between these regulatory loops and their component transport processes.

What, then, can we learn that might be applied in experimentation? Clearly, one message is that 'the consequence of manipulating a single transporter at a membrane is rarely (if ever) restricted to this one process or solely to the distributions of the transported species' (Blatt et al. 2014). Inevitably, the challenge is to recognize the 'off-target' effects of experimental manipulations. These are almost always beyond intuitive grasp, and so their identification is best addressed through quantitative mathematical modelling. For work with the premier model of the guard cell, researchers and students have access to OnGuard and the models we have constructed. It is our expectation that these models will find applications in exploring many other fundamental problems that arise in studies of these cells.

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References

Alexandre J, Lassalles JP, Kado RT (1990) Opening of Ca^{2+} channels in isolated red beet root vacuole membrane by inositol 1,4,5-trisphosphate. Nature 343:567–570

Allen GJ, Sanders D (1995) Calcineurin, a type 2B protein phosphatase, modulates the Ca^{2+} permeable slow vacuolar ion channel of stomatal guard cells. Plant Cell 7:1473–1483

Allen GJ, Chu SP, Schumacher K, Shimazaki CT, Vafeados D, Kemper A, Hawke SD, Tallman G, Tsien RY, Harper JF, Chory J, Schroeder JI (2000) Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in *Arabidopsis* det3 mutant. Science 289:2338–2342

Allen GJ, Chu SP, Harrington CL, Schumacher K, Hoffman T, Tang YY, Grill E, Schroeder JI (2001) A defined range of guard cell calcium oscillation parameters encodes stomatal movements. Nature 411:1053–1057

Armstrong F, Leung J, Grabov A, Brearley J, Giraudat J, Blatt MR (1995) Sensitivity to abscisic acid of guard cell K+ channels is suppressed by abi1-1, a mutant *Arabidopsis* gene encoding a putative protein phosphatase. Proc Natl Acad Sci USA 92:9520–9524

Barbier-Brygoo H, De Angeli A, Filleur S, Frachisse JM, Gambale F, Thomine S, Wege S (2011) Anion channels/transporters in plants: from molecular bases to regulatory networks. Annu Rev Plant Biol 62:25–51

Beyhl D, Hortensteiner S, Martinoia E, Farmer EE, Fromm J, Marten I, Hedrich R (2009) The fou2 mutation in the major vacuolar cation channel TPC1 confers tolerance to inhibitory luminal calcium. Plant Journal 58:715–723

Bezprozvanny I, Watras J, Ehrlich BE (1991) Bell-shaped calcium-response curves of Ins(1,4,5)P 3 —and calcium-gated channels from endoplasmic reticulum of cerebellum. Nature 351:751–754

Blatt MR (1987) Electrical characteristics of stomatal guard cells: the ionic basis of the membrane potential and the consequence of potassium chloride leakage from microelectrodes. Planta 170:272–287

Blatt MR (1988) Mechanisms of fusicoccin action: a dominant role for secondary transport in a higher-plant cell. Planta 174:187–200

Blatt MR (1992) K^+ channels of stomatal guard cells: characteristics of the inward rectifier and its control by pH. J Gen Physiol 99:615–644

Blatt MR (2000) Cellular signaling and volume control in stomatal movements in plants. Annu Rev Cell Dev Biol 16:221–241

Blatt MR, Armstrong F (1993) K^+ channels of stomatal guard cells: abscisic acid-evoked control of the outward rectifier mediated by cytoplasmic pH. Planta 191:330–341

Blatt MR, Gradmann D (1997) K^+ -sensitive gating of the K^+ outward rectifier in Vicia guard cells. J Membr Biol 158:241–256

Blatt MR, Slayman CL (1987) Role of "active" potassium transport in the regulation of cytoplasmic pH by nonanimal cells. Proc Natl Acad Sci USA 84:2737–2741

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

Blatt MR, Rodriguez-Navarro A, Slayman CL (1987) Potassium-proton symport in Neurospora: kinetic control by pH and membrane potential. J Membr Biol 98:169–189

Blatt MR, Thiel G, Trentham DR (1990) Reversible inactivation of K^+ channels of Vicia stomatal guard cells following the photolysis of caged inositol 1,4,5- trisphosphate. Nature 346:766–769

Blatt MR, Garcia-Mata C, Sokolovski S (2007) Membrane transport and Ca^{2+} oscillations in guard cells. In: Mancuso S, Shabala S (eds) Rhythms in plants. Springer, Berlin, pp 115–134

Blatt MR, Wang Y, Leonhardt N, Hills A (2014) Exploring emergent properties in cellular homeostasis using OnGuard to model K^+ and other ion transport in guard cells. J Plant Physiol 171:770–778

Bonza MC, De Michelis MI (2011) The plant Ca^{2+} -ATPase repertoire: biochemical features and physiological functions. Plant Biology 13:421–430

Chen ZH, Hills A, Lim CK, Blatt MR (2010) Dynamic regulation of guard cell anion channels by cytosolic free Ca^{2+} concentration and protein phosphorylation. Plant J 61:816–825

Chen ZH, Hills A, Baetz U, Amtmann A, Lew VL, Blatt MR (2012) Systems dynamic modeling of the stomatal guard cell predicts emergent behaviors in transport, signaling, and volume control. Plant Physiol 159:1235–1251

Cho D, Kim SA, Murata Y, Lee S, Jae SK, Nam HG, Kwak JM (2009) De-regulated expression of the plant glutamate receptor homolog AtGLR3.1 impairs long-term Ca^{2+} -programmed stomatal closure. Plant J 58:437–449

Coelho SM, Taylor AR, Ryan KP, Sousa-Pinto I, Brown MT, Brownlee C (2002) Spatiotemporal patterning of reactive oxygen production and Ca 2 + wave propagation in Fucus rhizoid cells. Plant Cell 14:2369–2381

dacz-Narloch B, Beyhl D, Larisch C, Lopez-Sanjurjo EJ, Reski R, Kuchitsu K, Muller TD, Becker D, Schonknecht G, & Hedrich R (2011). A novel calcium binding site in the slow vacuolar cation channel TPC1 senses luminal calcium levels. Plant Cell 23 2696–2707

Darley CP, Skiera LA, Northrop F, Sanders D, Davies JM (1998) Tonoplast inorganic pyrophosphatase in Vicia faba guard cells. Planta 206:272–277

De Silva DLR, Hetherington AM, Mansfield TA (1985) Synergism between calcium ions and abscisic acid in preventing stomatal opening. New Phytol 100:473–482

Dodd AN, Love J, Webb AAR (2005) The plant clock shows its metal: circadian regulation of cytosolic free Ca^{2+}. Trends Plant Sci 10:15–21

Dodd AN, Jakobsen MK, Baker AJ, Telzerow A, Hou SW, Laplaze L, Barrot L, Poethig RS, Haseloff J, Webb AAR (2006) Time of day modulates low-temperature Ca^{2+} signals in *Arabidopsis*. Plant J 48:962–973

Eisenach C, Papanatsiou M, Hillert EK, Blatt MR (2014) Clustering of the K^+ channel GORK of *Arabidopsis* parallels its gating by extracellular K^+. Plant J 78:203–214

Frohnmeyer H, Grabov A, Blatt MR (1998) A role for the vacuole in auxin-mediated control of cytosolic pH by Vicia mesophyll and guard cells. Plant J 13:109–116

Gajdanowicz P, Michard E, Sandmann M, Rocha M, Correa LGG, Ramirez-Aguilar SJ, Gomez-Porras JL, Gonzalez W, Thibaud JB, van Dongen JT, Dreyer I (2011) Potassium gradients serve as a mobile energy source in plant vascular tissues. PNAS 108:864–869

Garcia-Mata C, Gay R, Sokolovski S, Hills A, Lamattina L, Blatt MR (2003) Nitric oxide regulates K^+ and Cl—channels in guard cells through a subset of abscisic acid-evoked signaling pathways. Proc Natl Acad Sci USA 100:11116–11121

Geiger D, Scherzer S, Mumm P, Stange A, Marten I, Bauer H, Ache P, Matschi S, Liese A, Al-Rasheid KAS, Romeis T, Hedrich R (2009) Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase pair. Proc Natl Acad Sci USA 106:21425–21430

Geisler M, Axelsen KB, Harper JF, Palmgren MG (2000) Molecular aspects of higher plant P-type Ca^{2+} -ATPases. Biochim Et Biophys Acta-Biomembr 1465:52–78

Gilroy S, Fricker MD, Read ND, Trewavas AJ (1991) Role of calcium in signal transduction of Commelina guard cells. Plant Cell 3:333–344

Gobert A, Isayenkov S, Voelker C, Czempinski K, Maathuis FJM (2007) The two-pore channel TPK1 gene encodes the vacuolar K^+ conductance and plays a role in K^+ homeostasis. Proc Natl Acad Sci USA 104:10726–10731

Grabov A, Blatt MR (1997) Parallel control of the inward-rectifier K^+ channel by cytosolic-free Ca^{2+} and pH in Vicia guard cells. Planta 201:84–95

Grabov A, Blatt MR (1998) Membrane voltage initiates Ca^{2+} waves and potentiates Ca^{2+} increases with abscisic acid in stomatal guard cells. Proc Natl Acad Sci USA 95:4778–4783

Grabov A, Blatt MR (1999) A steep dependence of inward-rectifying potassium channels on cytosolic free calcium concentration increase evoked by hyperpolarization in guard cells. Plant Physiol 119:277–287

Gradmann D, Blatt MR, Thiel G (1993) Electrocoupling of ion transporters in plants. J Membr Biol 136:327–332

Hamilton DWA, Hills A, Kohler B, Blatt MR (2000) Ca^{2+} channels at the plasma membrane of stomatal guard cells are activated by hyperpolarization and abscisic acid. Proc Natl Acad Sci USA 97:4967–4972

Hamilton DWA, Hills A, Blatt MR (2001) Extracellular Ba 2 + and voltage interact to gate Ca^{2+} channels at the plasma membrane of stomatal guard cells. FEBS Lett 491:99–103

Hetherington AM, Brownlee C (2004) The generation of Ca^{2+} signals in plants. Annu Rev Plant Biol 55:401–427

Hille B (2001) Ionic channels of excitable membranes. Sinauer Press, Sunderland, Mass

Hills A, Chen ZH, Amtmann A, Blatt MR, Lew VL (2012) OnGuard, a computational platform for quantitative kinetic modeling of guard cell physiology. Plant Physiol 159:1026–1042

Hosy E, Vavasseur A, Mouline K, Dreyer I, Gaymard F, Poree F, Boucherez J, Lebaudy A, Bouchez D, Very AA, Simonneau T, Thibaud JB, Sentenac H (2003) The *Arabidopsis* outward K^+ channel GORK is involved in regulation of stomatal movements and plant transpiration. Proc Natl Acad Sci USA 100:5549–5554

Hou C, Tian W, Kleist T, He K, Garcia V, Bai F, Hao Y, Luan S, Li L (2014) DUF221 proteins are a family of osmosensitive calcium-permeable cation channels conserved across eukaryotes. Cell Res 24:632–635

Jack JJB, Noble D, Tsien RW (1983) Electric current flow in excitable cells. Clarendon Press, Oxford

Kinoshita T, Nishimura M, Shimazaki KI (1995) Cytosolic concentration of Ca^{2+} regulates the plasma membrane H^+ ATPase in guard cells of fava bean. Plant Cell 7:1333–1342

Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JDG, Schroeder JI (2003) NADPH oxidase AtrbohD and AtrbohF genes function in ROS- dependent ABA signaling in *Arabidopsis*. EMBO J 22:2623–2633

Lawson T, Blatt MR (2014) Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. Plant Physiol 164:1556–1570

Lawson T, Kramer DM, Raines CA (2012) Improving yield by exploiting mechanisms underlying natural variation of photosynthesis. Curr Opin Biotechnol 23:215–220

Leckie CP, McAinsh MR, Allen GJ, Sanders D, Hetherington AM (1998) Abscisic acid-induced stomatal closure mediated by cyclic ADP-ribose. Proc Natl Acad Sci Unit States Am 95:15837–15842

Lemtirichlieh F, MacRobbie EAC (1994) Role of calcium in the modulation of Vicia guard cell potassium channels by abscisic acid—a patch clamp study. J Membr Biol 137:99–107

Lemtiri-Chlieh F, MacRobbie EAC, Webb AAR, Manison NF, Brownlee C, Skepper JN, Chen J, Prestwich GD, Brearley CA (2003) Inositol hexakisphosphate mobilizes an endomembrane store of calcium in guard cells. Proc Natl Acad Sci Unit States Am 100:10091–10095

Lew VL, Bookchin RM (1986) Volume, pH, and ion-content regulation in human red-cells—analysis of transient-behavior with an integrated model. J Membr Biol 92:57–74

Lew VL, Ferreira HG, Moura T (1979) Behavior of transporting epithelial-cells.1. Computer-analysis of a basic model. Proc R Soc Lon Ser B-Biol 206:53–83

Lohse G, Hedrich R (1992) Characterization of the plasma-membrane H^+ -ATPase from Vicia faba guard cells. Planta 188:206–214

Lopez-Marques RL, Schiott M, Jakobsen MK, Palmgren MG (2004) Structure, function and regulation of primary H^+ and Ca^{2+} pumps. In: Blatt MR (ed) Membrane transport in plants. Blackwell, Oxford, pp 72–104

Loro G, Drago I, Pozzan T, Lo Schiavo F, Zottini M, Costa A (2012) Targeting of Cameleons to various subcellular compartments reveals a strict cytoplasmic/mitochondrial Ca^{2+} handling relationship in plant cells. Plant J 71:1–13

MacRobbie EAC (1991) Effects of ABA on ion transport and stomatal regulation. J Exp Bot 42:11

MacRobbie EAC (1995) Effects of ABA on 86 Rb^+ fluxes at plasmalemma and tonoplast of stomatal guard cells. Plant J 7:835–843

MacRobbie EAC (2000) ABA activates multiple Ca^{2+} fluxes in stomatal guard cells, triggering vacuolar K^+ (Rb^+) release. Proc Natl Acad Sci Unit States Am 97:12361–12368

MacRobbie EAC (2006) Osmotic effects on vacuolar ion release in guard cells. Proc Natl Acad Sci Unit States Am 103:1135–1140

Martinoia E, Maeshima M, Neuhaus HE (2007) Vacuolar transporters and their essential role in plant metabolism. J Exp Bot 58:83–102

Mauritz JMA, Esposito A, Ginsburg H, Kaminski CF, Tiffert T, Lew VL (2009) The homeostasis of plasmodium falciparum—infected red blood cells. Plos Comput Biol 5:e1000339

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

Melotto M, Underwood W, He SY (2008) Role of stomata in plant innate immunity and foliar bacterial diseases. Ann Rev Phytopathol 46:101–122

Merlot S, Leonhardt N, Fenzi F, Valon C, Costa M, Piette L, Vavasseur A, Genty B, Boivin K, Mueller A, Giraudat M, Leung J (2007) Constitutive activation of a plasma membrane H^+ -ATPase prevents abscisic acid-mediated stomatal closure. EMBO J 26:3216–3226

Mori IC, Murata Y, Yang YZ, Munemasa S, Wang YF, Andreoli S, Tiriac H, Alonso JM, Harper JF, Ecker JR, Kwak JM, Schroeder JI (2006) CDPKs CPK6 and CPK3 function in ABA regulation of guard cell S-type anion- and Ca^{2+} -permeable channels and stomatal closure. Plos Biol 4:1749–1762

Muir SR, Sanders D (1996) Pharmacology of Ca^{2+} release from red beet microsomes suggests the presence of ryanodine receptor homologs in higher-plants. FEBS Lett 395:39–42

Nakamura RL, McKendree WL, Hirsch RE, Sedbrook JC, Gaber RF, Sussman MR (1995) Expression of an *Arabidopsis* potassium channel gene in guard cells. Plant Physiol 109:371–374

Negi J, Matsuda O, Nagasawa T, Oba Y, Takahashi H, Kawai-Yamada M, Uchimiya H, Hashimoto M, Iba K (2008) CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. Nature 452:483–486

Nishimura N, Sarkeshik A, Nito K, Park SY, Wang A, Carvalho PC, Lee S, Caddell DF, Cutler SR, Chory J, Yates JR, Schroeder JI (2010) PYR/PYL/RCAR family members are major in-vivo ABI1 protein phosphatase 2C-interacting proteins in *Arabidopsis*. Plant J 61:290–299

Pandey S, Zhang W, Assmann SM (2007) Roles of ion channels and transporters in guard cell signal transduction. FEBS Lett 581:2325–2336

Pardo JM, Cubero B, Leidi EO, Quintero FJ (2006) Alkali cation exchangers: roles in cellular homeostasis and stress tolerance. J Exp Bot 57:1181–1199

Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TFF, Alfred SE, Bonetta D, Finkelstein R, Provart NJ, Desveaux D, Rodriguez PL,

McCourt P, Zhu JK, Schroeder JI, Volkman BF, Cutler SR (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science 324:1068–1071

Pei ZM, Ward JM, Harper JF, Schroeder JI (1996) A novel chloride channel in Vicia faba guard cell vacuoles activated by the serine/threonine kinase, CDPK. EMBO J 15:6564–6574

Pei ZM, Ward JM, Schroeder JI (1999) Magnesium sensitizes slow vacuolar channels to physiological cytosolic calcium and inhibits fast vacuolar channels in fava bean guard cell vacuoles. Plant Physiol 121:977–986

Peiter E, Maathuis FJM, Mills LN, Knight H, Pelloux M, Hetherington AM, Sanders D (2005) The vacuolar Ca^{2+} -activated channel TPC1 regulates germination and stomatal movement. Nature 434:404–408

Pilot G, Lacombe B, Gaymard F, Cherel I, Boucherez J, Thibaud JB, Sentenac H (2001) Guard cell inward K^+ channel activity in Arabidopsis involves expression of the twin channel subunits KAT1 and KAT2. J Biol Chem 276:3215–3221

Pittman JK (2011) Vacuolar Ca^{2+} uptake. Cell Calcium 50:139–146

Qi Z, Stephens NR, Spalding EP (2006) Calcium entry mediated by GLR3.3, an *Arabidopsis* glutamate receptor with a broad agonist profile. Plant Physiol 142:963–971

Raschke K, Schnabl H (1978) Availability of chloride affects balance between potassium chloride and potassium malate in guard cells of Vicia faba L. Plant Physiol 62:84–87

Rienmuller F, Beyhl D, Lautner S, Fromm J, Al-Rasheid KAS, Ache P, Farmer EE, Marten I, Hedrich R (2010) Guard cell-specific calcium sensitivity of high density and activity SV/TPC1 channels. Plant Cell Physiol 51:1548–1554

Roelfsema MR, Hedrich R (2010) Making sense out of Ca^{2+} signals: their role in regulating stomatal movements. Plant Cell Environ 33:305–321

Romano LA, Jacob T, Gilroy S, Assmann SM (2000) Increases in cytosolic Ca^{2+} are not required for abscisic acid-inhibition of inward K^+ currents in guard cells of Vicia faba L. Planta 211:209–217

Sanders D (1990) Kinetic modeling of plant and fungal membrane transport systems. Ann Rev Plant Physiol Plant Mol Biol 41:77–108

Schroeder JI (1988) K^+ transport properties of K^+ channels in the plasma membrane of Vicia faba guard cells. J Gen Physiol 92:667–683

Schroeder JI, Hagiwara S (1989) Cytosolic calcium regulates ion channels in the plasma membrane of Vicia faba guard cells. Nature 338:427–430

Shabala S, Shabala L, Gradmann D, Chen ZH, Newman I, Mancuso S (2006) Oscillations in plant membrane transport: model predictions, experimental validation, and physiological implications. J Exp Bot 57:171–184

Staxen I, Pical C, Montgomery LT, Gray JE, Hetherington AM, McAinsh MR (1999) Abscisic acid induces oscillations in guard-cell cytosolic free calcium that involve phosphoinositide-specific phospholipase C. Proc Natl Acad Sci Unit States Am 96:1779–1784

Subbaiah CC, Bush DS, Sachs MM (1998) Mitochondrial contribution to the anoxic Ca^{2+} signal in maize suspension-cultured cells. Plant Physiol 118:759–771

Sze H, Liang F, Hwang I, Curran AC, Harper JF (2000) Diversity and regulation of plant Ca^{2+} pumps: Insights from expression in yeast. Ann Rev Plant Physiol Plant Mol Biol 51:433–462

Thiel G, MacRobbie EAC, Blatt MR (1992) Membrane transport in stomatal guard cells: the importance of voltage control. J Membr Biol 126:1–18

Vahisalu T, Kollist H, Wang YF, Nishimura N, Chan WY, Valerio G, Lamminmaki A, Brosche M, Moldau H, Desikan R, Schroeder JI, Kangasjarvi J (2008) SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling. Nature 452:487–491

Very AA, Sentenac H (2002) Cation channels in the Arabidopsis plasma membrane. Trends Plant Sci 7:168–175

Wang Y, Papanatsiou M, Eisenach C, Karnik R, Williams M, Hills A, Lew VL, Blatt MR (2012) Systems dynamic modelling of a guard cell Cl—channel mutant uncovers an emergent homeostatic network regulating stomatal transpiration. Plant Physiol 160:1956–1972

Wang Y, Chen ZH, Zhang B, Hills A, Blatt MR (2013) PYR/PYL/RCAR abscisic acid receptors regulate K^+ and Cl^- channels through reactive oxygen species-mediated activation of Ca^{2+} channels at the plasma membrane of intact *Arabidopsis* guard cells. Plant Physiol 163:566–577

Wang Y, Hills A, Blatt MR (2014a) Systems analysis of guard cell membrane transport for enhanced stomatal dynamics and water use efficiency. Plant Physiol 164:1593–1599

Wang Y, Noguchi K, Ono N, Inoue SI, Terashima I, Kinoshita T (2014b) Overexpression of plasma membrane H^+ -ATPase in guard cells promotes light-induced stomatal opening and enhances plant growth. PNAS 111:533–538

Ward JM, Maser P, Schroeder JI (2009) Plant ion channels: gene families, physiology, and functional genomics analyses. Ann Rev Physiol 71:59–82

Webb AAR, McAinsh MR, Mansfield TA, Hetherington AM (1996) Carbon dioxide induces increases in guard cell cytosolic free calcium. Plant J 9:297–304

Weiss TF (1996) Cellular biophysics. MIT Press, Cambridge

Willmer C, Fricker MD (1996) Stomata .Chapman and Hall, London, pp 1–375

Wu Y, Kuzma J, Marechal E, Graeff R, Lee HC, Foster R, Chua NH (1997) Abscisic acid signaling through cyclic ADP-Ribose in plants. Science 278:2126–2130

Yuan F, Yang H, Xue Y, Kong D, Ye R, Li C, Zhang J, Theprungsirikul L, Shrift T, Krichilsky B, Johnson DM, Swift GB, He Y, Siedow JN, Pei Z-M (2014) OSCA1 mediates osmotic-stress-evoked Ca^{2+} increases vital for osmosensing in *Arabidopsis*. Nature 514:367