Communication between the plasma membrane and tonoplast is an emergent property of ion transport

Running head: Emergent membrane transport interactions

Dear Editor,

A very substantial body of data now exists for ion transport at both the plasma membrane and the tonoplast of several plant cell models, notably for guard cells, root hairs and epidermal cells of several species to name a few (Grierson et al., 2014; Jezek and Blatt, 2017; Wang et al., 2019). This knowledge has its foundation in detailed electrophysiological and flux studies that have provided quantitative biophysical and kinetic information. Our understanding has expanded through molecular biology to include the genetic identities of many transporters that operate at both membranes. Among these studies, it is common to deconstruct the mechanics and genetics of transport and to characterize each transporter in isolation. In many cases, this work has included the cloning and heterologous expression of specific transport gene products, their analysis under voltage clamp and by radiotracer flux measurements.

Where such studies often struggle is in phenotypic analysis in vivo to associate a genetic lesion with a function for the transport gene of interest. We suggest that, in focusing on single gene products, the deconstructionist approach can lose sight of the unique feature of transport, namely its physiological integration and apparent communication with other transport processes in situ, what is often referred to as 'emergent' properties arising from transport interactions. This communication is particularly evident when the transporter in question moves charge across the membrane. Although less often appreciated, similar considerations apply to transport across serial membranes when these operate on common pools of

solutes within an enclosed and finite compartment. Both situations are common to plants. Communication is especially important between serial membranes, for example between the plasma membrane and tonoplast, when the consequences of manipulating transport at one membrane affects the cytosolic contents and, hence, transport across the other membrane. In these circumstances quantitative mathematical modeling is frequently essential to gain true insight into the communication between membranes and their transport processes.

Within a single membrane there exist connections between different transporters that ensure their fundamental interdependency. Notably, voltage exerts a dominant control on charge-carrying transport, acting both as a driving force for transport and as a product of charge flux across the membrane (Jezek and Blatt, 2017). Because physical laws require that net charge flux across a membrane is zero in the steady state, transport of each ionic species is necessarily joined to the transport of all other ions that affect voltage across the same membrane. Only by imposing the circuit of a voltage clamp is this interconnection between transporters bypassed. In short, manipulations affecting charge flux through any one transporter will necessarily impact on all other charge-carrying transporters in the same membrane, often with unforeseen consequences. As one example, the interdependency between H^+-coupled K^+ transport and the primary H^+-ATPase (Blatt and Slayman, 1987; Gibrat et al., 1990; Maathuis and Sanders, 1994) was ultimately a key factor in explaining why more than a decade of research failed to uncover a hypothetical H^+/K^+ exchange ATPase in plants (Leonard and Hotchkiss, 1976), proposed to operate in a manner analogous to the mammalian Na^+/K^+-ATPase.

Similar considerations apply to communication between the plant plasma membrane and tonoplast. Because these serial membranes operate through a common and enclosed compartment - the cytosol - the transport activites of both are connected through this single pool of solutes. Analogies may be drawn here to transport interactions across the apoplast between cells, for example as highlighted in the plant vasculature (Gajdanowicz et al., 2011) and fungal symbiosis (Dreyer et al., 2019). Some caution in drawing such analogies is advisable however, as the nature of apoplast, if semi-open, may moderate interactions between membranes and thus precludes a direct comparison with the enclosed cytosolic compartment within a cell.

We focus here on communication between the serial membranes of the plasma membrane and tonoplast and how alterations in transport at one are a predictable consequence of manipulations affecting transport across the other membrane. The examples are for guard cells and the ensuing stomatal phenotypes, but apply equally to transport in other plant cell types. We use two studies (Wang et al., 2012; Wang et al., 2017) to address the mechanics of how Cl^- flux mediated by the SLAC1 channel at the plasma membrane affects transport across the tonoplast. The physiology of the SLAC1 Cl^- channel in guard cells ensures that it mediates Cl^- efflux for stomatal closure and, as expected, the *slac1* null mutation suppresses stomatal closure and greatly slows stomatal kinetics. Surprisingly, the mutant also greatly slows the kinetics of stomatal opening (Vahisalu et al., 2008; Wang et al., 2012; Wang et al., 2017), an effect that we now know arises because the mutation indirectly suppresses the activity of the K^+ channels that mediate K^+ uptake (Wang et al., 2012; Wang et al., 2017).

How does the *slac1* mutation influence transport at the tonoplast? Direct access to the vacuolar membrane in vivo is not practicable. However, it is possible to assess the consequences for solute contents and to examine the underlying mechanisms that can explain these phenomena through simulation. We used the OnGuard platform (Chen et al., 2012; Hills et al., 2012; Wang et al., 2012) to explore the connections between these membranes. OnGuard2 (freely available at www.psrg.org.uk) incorporates all of the quantitative detail for transport and the relevant metabolic activities in guard cells to reproduce the charcteristics of solute flux, stomatal aperture and conductance known in the literature, and it has yielded a number of unexpected predictions, many now validated experimentally. For comparison with guard cells of wild-type Arabidopsis in OnGuard2, simulations of the *slac1* mutant were generated by setting to zero the ohmic (voltage-independent) Cl^- conductance and the dominant fraction of the voltage-gated Cl^- conductance which, combined, normally represent the characteristics of SLAC1 (Wang et al., 2017). Negi et al (2008) reported that the *slac1* mutant accumulates osmotically-active solutes, not only K^+ and Cl^- but also substantial amounts of organic anions. The simulations carried out by Wang et al (2012; 2017) similarly yielded accumulations of K^+, Cl^- and organic anions, the latter subsumed as malate in the OnGuard platform.

It is not surprising that eliminating a major pathway for anion efflux should

result in its accumulation in the cytosol and vacuole through its build-up in the cytosol and trans-inhibition of efflux across the tonoplast. However, a comparison of the fluxes through each of the major tonoplast transporters is instructive. These data are available in the Supplemental Figures of Wang et al (2012). The simulations predict an early increase in daytime K^+ flux through the tonoplast TPK1 and FV K^+ channels (see Figure S7 of Wang et al (2012)), thereby accounting for the overall accumulation of this cation in the guard cell as reported previously (Negi et al., 2008); they also shows a daytime reversal of Cl^- flux through the tonoplast Cl^- channels (VCl). Additional to these outputs, a detailed analysis here throws up a number of other predictions highlighting the apparent communication between plasma membrane and tonoplast. It predicts, counterintuitively, roughly 3-fold increases in net Ca^{2+} transport by the tonoplast Ca^{2+}-ATPases (VCa^{2+}-ATPase) and the Ca^{2+} channels (VCa_{in}), as is clearly evident in the Supplemental Figure S6 of Wang et al (2012) and a substantial increase in the activity of both sets of transporters (Figure 1). These changes arise from the enhanced Ca^{2+} influx with plasma membrane hyperpolarization in the *slac1* mutant and stimulation of endomembrane Ca^{2+} release and recycling (Wang et al., 2012). The juxtaposition of these two Ca^{2+} fluxes accounts for the overall lower total Ca^{2+} levels in the vacuole as well as the elevated cytosolic-free [Ca^{2+}] ($[Ca^{2+}]_i$) reported both in simulation and as validated through experimental measurements (Wang et al., 2012; Wang et al., 2017).

OnGuard2 yields a number of other predictions which, although still to be tested experimentally, gain credibility from the accuracy of simulations to date in predicting experimental observations. Notable among these, in simulation the effect of the *slac1* mutant is to increase the activity of the tonoplast FV, TPK and VCl channels as would be resolved under voltage clamp (Figure 1). The effect on all three currents is substantially greater than might be expected for the overall K^+ and Cl^- fluxes [Supplemental Figure S7 of Wang et al (2012)] and is seemingly counterintuitive. However, the effects on these transporters is a natural consequence of changes in $[Ca^{2+}]_i$ and cytosolic pH: all three channels are $[Ca^{2+}]_i$-sensitive and the K^+ channels are also subject to the elevated cytosolic pH that is characteristic of the *slac1* mutant (Allen and Sanders, 1996; Wang et al., 2012; Wang et al., 2017). In short, the simulation predicts, in the *slac1* mutant, an enhanced *capacity* for K^+ and

Cl^- flux, even if this capacity is kinetically restricted by charge balance and the free-running voltage across the tonoplast.

Each of these predictions, and other outputs of OnGuard2, highlight the emergent properties of transport communication within and, especially, between the membranes of the guard cells. This communication is natural consequence of a system of non-linear biological processes that share substrates and products across each membrane and within cellular compartments. It arises from the membrane voltage that is shared between all charge-carrying transporters on any one membrane, as well as the common pool of ionic substrates shared between the membranes and enclosed by them. Between the plasma membrane and tonoplast, it arises from the common pool of cytosolic solutes that contribute to transport across both membranes.

We stress that there is nothing unusual to this network of interactive communication or the component transport processes. However, the intrinsic non-linearities in flux behavior of each transporter ensures that the consequences of experimental manipulations are beyond intuitive understanding. Thus, in vivo the consequence of manipulating a single transporter at a membrane is rarely (if ever) restricted to this one process, the distributions of the transported species alone or, in plants, solely to the target membrane. Distinguishing between the primary effects of a mutation and 'off target' effects clearly benefits in these circumstances from quantitative mathematical modelling.

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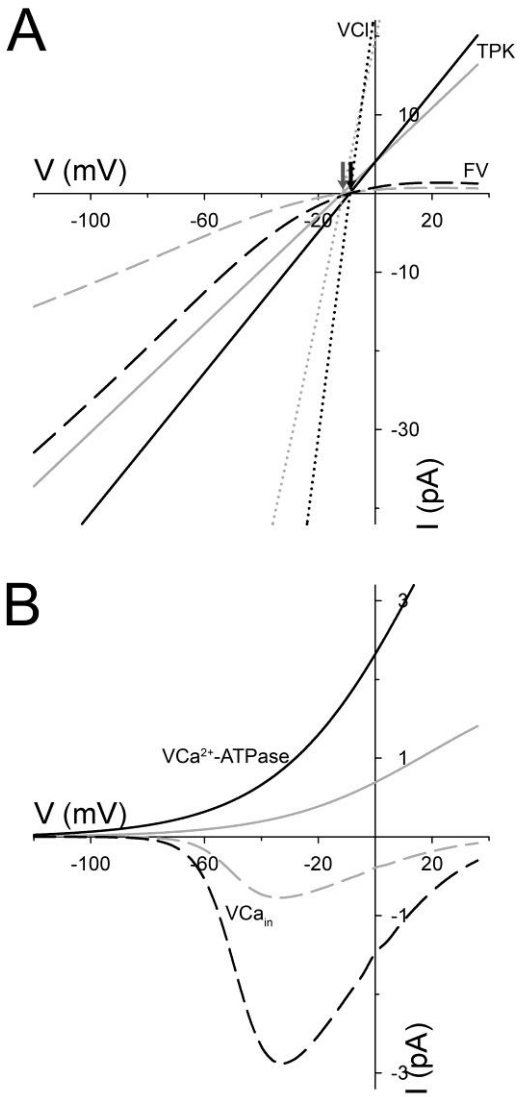

Figure 1. Current-voltage (IV) curves predicted for the major tonoplast K^+ and Cl^- channels and for the tonoplast Ca^{2+} ATPases and channels in guard cells of wild-type (grey lines) and the *slac1* mutant (black lines) Arabidopsis. Data were extracted from OnGuard2 simulations, as described, at 8 h into the daylight period, this timepoint corresponding to the maximum diurnal stomatal conductance in each case. The curves are plotted separately here for clarity.
(A) IV curves predicted for the TPK1 (solid lines) and FV (dashed lines) K^+ channels and for the VCl Cl^- channel (dotted lines). The free-running tonoplast voltages are indicated by the arrows (wild-type, grey; *slac1*, black). Note the substantial increase in the conductances (slopes) for the current of each channel type in the *slac1* mutant. (B) IV curves predicted for the VCa_{in} Ca^{2+} channels (dashed lines) and for the VCa^{2+}-ATPase (solid lines). The identity of the VCa_{in} remains unknown and, in the OnGuard platform, the VCa^{2+}-ATPase subsumes the characteristics of all endomembrane Ca^{2+}-ATPases (Chen et al., 2012; Hills et al., 2012; Wang et al., 2012; Wang et al., 2017). Again, the analysis yields a substantial increase in the conductances and amplitudes of both currents in the *slac1* mutant.

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