Tansley insight

Connecting vacuolar and plasma membrane transport networks

Paloma Cubero-Font and Alexis De Angeli

BPMP, Univ Montpellier, CNRS, INRAE, Montpellier SupAgro, Montpellier, 34060 France

Paloma Cubero-Font Orcid: 0000-0002-0231-9811
Alexis De Angeli Orcid: 0000-0003-3072-7932

Author for correspondence:
Alexis De Angeli
Tel: +33499613177
Email: alexis.deangeli@supagro.fr

Received: 10 July 2020
Accepted: 1 September 2020

Contents

Summary
I. Introduction
II. Vacuolar and plasma membrane transport in guard cells, a team work
III. Ion flux coordination under the control of ionic conditions in the cytosol

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/NPH.16983

This article is protected by copyright. All rights reserved
Summary
The coordinated control of ion transport across the two major membranes of differentiated plant cells, the plasma and the vacuolar membranes, is fundamental in cell physiology. The stomata responses to the fluctuating environmental conditions are an illustrative example. Indeed, they rely on the coordination of ion fluxes between the different cell compartments. The cytosolic environment, that is an interface between intracellular compartments, and the activity of the ion transporters localized in the different membranes influence one each other. Here we analyse the molecular mechanisms connecting and modulating the transport processes at both the plasma and the vacuolar membranes of guard cells.

Key words: flux coordination, intracellular network, cytosolic conditions, guard cells, ion channels, stomata, transporters, vacuole.

Boxes
Box 1 Driving forces in transport reactions
The electrochemical potential difference ($\Delta \mu_i$) for an ion $i$ between two sides of a membrane defines the direction and the limits of ion transport reactions across membranes. $\Delta \mu_i$ combines the chemical and the electrical potentials of ion $i$ and is defined by:

$$\Delta \mu_i = R \cdot T \cdot \ln \frac{[i]_{cyt}}{[i]_{out}} + z_i \cdot F \cdot (V_{cyt} - V_{out}) \quad \text{Eqn 1}$$

In the following equations we outline the driving force of the major types of transport reactions in cells: passive transport (ion channels), primary active transport (pumps) and secondary active transport (antiporters and symporters).

For ion channels transporting ion $i$, the electrochemical potential $\Delta \mu_i$ coincides with the driving force of the transport reaction ($\Delta \mu_{\text{channel}}$):

$$\Delta \mu_{\text{channel}} \biggem_{i_{out}} \biggem_{i_{cyt}} = \Delta \mu_i = R \cdot T \cdot \ln \frac{[i]_{cyt}}{[i]_{out}} + z_i \cdot F \cdot (V_{cyt} - V_{out}) \quad \text{Eqn 2}$$

For pumps that catalyse the transport of $n$ ion $i$ against $\Delta \mu_i$ coupling it to ATP hydrolysis, the driving force of the transport reaction ($\Delta \mu_{\text{pump}}$) is:

$$\Delta \mu_{\text{pump}} = n\Delta \mu_i + \Delta \mu_{\text{ATP}} =$$

$$= R \cdot T \cdot \ln \frac{ADP \cdot P_i}{ATP} \cdot \frac{[i]_{out}^n}{[i]_{cyt}^n} - n \cdot z_i \cdot F \cdot (V_{cyt} - V_{out}) \quad \text{Eqn 3}$$

For an antiporter that catalyse the exchange of $n$ ions $i$ and $m$ ions $j$, the driving force ($\Delta \mu_{\text{antiporter}}$) of the transport reaction is defined as:

$$\Delta \mu_{\text{antiporter}} = n\Delta \mu_i - m\Delta \mu_j =$$

$$= R \cdot T \cdot \ln \frac{[i]_{cyt}^n}{[i]_{out}^n} \cdot \frac{[j]_{out}^m}{[j]_{cyt}^m} + (n \cdot z_i - m \cdot z_j) \cdot F \cdot (V_{cyt} - V_{out}) \quad \text{Eqn 4}$$
For a symporters mediating the co-transport of \( n \) ions \( i \) and \( m \) ions \( j \), the driving force of the transport reaction \( (\Delta \mu_{\text{symporter}}) \) is defined as:

\[
\Delta \mu_{\text{symporter}} = n\Delta \mu_i + m\Delta \mu_j = R \cdot T \cdot \ln \left( \frac{[i]_{\text{cyt}}^n \cdot [j]_{\text{cyt}}^m}{[i]_{\text{out}}^n \cdot [j]_{\text{out}}^m} \right) + (n \cdot z_i + m \cdot z_j) \cdot F \cdot (V_{\text{cyt}} - V_{\text{out}})
\]

Eqn 5

In all equations \( R \) is the gas constant, \( T \) is the absolute temperature, \( z_{ij} \) is the charge of the ion \( i \) or \( j \), \( F \) is the Faraday’s constant, \( V_{\text{cyt}} - V_{\text{out}} \) is the membrane potential difference between the cytosol (\( \text{cyt} \)) and outside (\( \text{out} \)), \([i,j]_{\text{cyt}} \) is the cytosolic (\( \text{cyt} \)) concentration of the ion \( i \) or \( j \), \([i,j]_{\text{out}} \) is the concentration of the ion \( i \) or \( j \) outside (\( \text{out} \)), and \( n \) and \( m \) are the stoichiometric coefficients of ions \( i \) and \( j \), respectively. The outside (\( \text{out} \)) corresponds to the apoplast, to the vacuolar lumen or to the lumen of other compartments.

**Box 2** Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AKT1 | Arabidopsis K\(^+\) Transporter 1 |
| ALMT | ALuminum Activated Malate Transporter |
| CBL | Calcineurin-B-Like protein |
| CIPK | CBL-Interacting Protein Kinase |
| GC | Guard Cell |
| GORK | Guard cell Outward Rectifying K\(^+\) channel |
| HAK5 | High Affinity K\(^+\) Transporter 5 |
| KAT1 | K\(^+\) channel in Arabidopsis Thaliana 1 |
| KIN7 | Kinase 7 |
| MAPK | Mitogen-Activated Protein Kinases |
| NHX | Na\(^+\)/H\(^+\) eXchanger |
| NRT1.1 | NitRate Transporter 1.1 |
| OSCA | Reduced hyperosmolality-induced [Ca\(^{2+}\)], increase |
| OST1/SnRK2.6 | Open STomata 1/ Snf1 Related protein Kinase type-2.6 |
I. Introduction

The vacuole occupies the major part of the cellular volume, up to 90% in differentiated plant cells (Krüger & Schumacher, 2018). It is an acidic compartment presenting dynamic morphology, composition and volume. The characteristics of the vacuole change during plant cell development and cellular responses to the environment (Martinoia et al., 2012), making the plasticity of the vacuole an essential property of this organelle. Given its size, in differentiated cells the vacuole is a key player in the building of the turgor pressure and is part of the intracellular signalling network (Martinoia et al., 2012; Peiter, 2011; Roelfsema & Hedrich, 2005). Vacuolar functions are intimately linked to the pool of transport systems residing in the vacuolar membrane (VM) and transporting a large diversity of molecules (nutrients, metals, metabolites, sugars, peptides) (Martinoia et al., 2012).

The vacuolar functions are important for the adaptation of plants to their environment. For example, during exposure to high salt or heavy metals several plants accumulate toxic elements in the vacuole to preserve metabolic active compartments (Martinoia, 2018). Interestingly, the VM is also involved in the translocation mechanisms of toxic species between plant organs (Baetz et al., 2016; Cosio et al., 2004; Ma et al., 2005; Thomine et al., 2003). In petunia flowers, specific proton pumps in the VM of petal cells generate extremely acidic vacuolar pHs influencing the colour of flowers (Faraco et al., 2014; Verweij et al., 2008). The VM is also fundamental for the activity of the specialized motor cells generating seismonastic leaf movements of Mimosa pudica pulvini (Fleurat-Lessard et al., 1997a,b; Hagihara & Toyota, 2020). Finally, the VM transporters...
participate in the control of stomata aperture in guard cells (Martinoia, 2018). In these cells, the morphological changes of the vacuole during stomata movements are linked to the transport activity of the VM (Andrés et al., 2014; Tanaka et al., 2007) (Fig. 1b).

The examples listed above demonstrate the importance of the specialised transport capacities of the vacuole in plant cell physiology. However, for proper cellular responses the VM needs to work in concert with the other cell membranes delimiting intracellular compartments. Thus, the processes occurring at the VM and other cellular membranes are interconnected and coordinated. Given its size, the VM is a central element of the intracellular transport network, and the vacuolar transport processes must be considered integrated in a network of fluxes mutually influencing each other.

II. Vacuolar and plasma membrane transport in guard cells, a team work

Guard cells (GC) perfectly illustrate the physiological interconnection of the vacuole with the other cellular membranes. Indeed, in guard cells massive fluxes of ions and sugars across the VM and the plasma membrane (PM) control intracellular turgor pressure in response to environmental stimuli (i.e. light, drought, CO₂, pathogens). The rapid modification of the turgor pressure relies on the movement of ions from the apoplast to the cytosol and then to the vacuole ( stomata opening), and from the vacuole to the cytosol and then to the apoplast ( stomata closure; Fig. 1) (Roelfsema & Hedrich, 2005). These movements of ions induce the swelling and shrinking of guard cells for opening and closing stomata, respectively (Fig. 1). But how many molecules of solutes cross the PM and VM during stomata opening/closure? To estimate this, we used data from *Vicia faba*. To drive stomata opening a net raise of 4-5 pmol of ionic solutes per guard cell, mainly K⁺, Cl⁻, NO₃⁻ or malate²⁻, is necessary (Allaway & Hsiao, 1973). This raise of solutes induces an increase of the total intracellular concentration between 0.8 and 1 M in open stomata. Considering an opening time of 60 minutes and a *V. faba* guard cell surface of 1899 µm² (Meckel et al., 2007), a net entry of up to ~7×10⁸ molecules·s⁻¹/GC across the PM and then across the VM can be calculated (Fig. 1a). When stomata close, ionic solutes leave the cell in about 20 minutes generating a net flux of ~2×10⁹ molecules·s⁻¹/GC firstly across the VM and then across the PM (Fig. 1a). If the VM and the PM were not coordinated and molecules could not enter the vacuole, the cytosolic concentration would increase of up to 150 mM of solutes·minute⁻¹. The latter is an extreme and hypothetical situation but it illustrates the importance of flux coordination between membranes.
Overall, these estimations provide the order of magnitude of the ion fluxes occurring in a guard cell.

Therefore, to avoid aberrant ionic concentrations in the cytosol and to generate coherent responses, the transport systems residing in both the VM and the PM of guard cells are co-regulated (Fig. 1). The coordination of the ion fluxes through the PM and the VM was suggested in seminal work in the 90s (summarized in MacRobbie, 2000). But which are the mechanisms allowing coordination? How fluxes of ions are coordinated between the PM and the VM? The transport properties of cellular membranes can be modified by changing the activity of transporters or by acting on the pool of transporters residing in a membrane. To coordinate the fluxes, a first level is the regulation of the solute concentrations in the cytosol; a second level is through signalling pathways targeting the transport systems residing in the different cellular membranes. These two levels will be discussed in the following sections with a special focus on the model plant Arabidopsis thaliana.

III. Ion flux coordination under the control of ionic conditions in the cytosol
1. Cytosolic ion concentration, a straightforward way to coordinate fluxes between membranes

The subcellular organisation of guard cells makes the cytosol a thin layer between two large membranes, the PM and VM. Since in open stomata the vacuole occupies nearly 90% of the whole cellular volume (Fig. 1b) the majority of the solutes crossing the PM will also cross the VM during opening. Therefore, the cytosolic compartment, which accounts for only a fraction of the cell, undergoes this flux of solutes (Fig. 1b). Thus, during stomatal movements ionic concentrations in the cytosol are likely to vary, and in silico modelling of guard cells shows such changes (Blatt et al., 2014; Chen et al., 2012; Wang et al., 2012). Recent in vivo data demonstrate that intracellular transport systems residing in the VM act on cytosolic ion homeostasis in guard cells, influencing pH and [NO$_3^-$] (Demes et al., 2020). Changes of the cytosolic concentrations of each ionic species $i$ modifies its electrochemical potential difference ($\Delta\mu_i$) between the two sides of the cellular membranes facing the cytosol. Such modifications can influence the different types of transport reactions across the different membranes involving ion $i$ (Box.1). Thus, $\Delta\mu_i$, together with the kinetic properties of the ion transporters, impacts on ion fluxes across cellular membranes, and changes of cytosolic ion concentrations modify the movement of ions across the membranes facing the cytosol (Horaruang et al., 2020; Wang et al., 2017). Therefore, changes of the ionic
conditions in the cytosol participate in the coordination of ion fluxes between cellular membranes (Fig. 1b).

2. Simultaneous regulation of VM and PM transport systems by cytosolic ions

Some ions and metabolites emerge as elements co-regulating solute transport across intracellular membranes (Fig. 2; Box 2). A first ‘classic’ candidate is Ca$^{2+}$. Although we still miss the genetic identity of Ca$^{2+}$ transport systems in plants, the occurrence of cytosolic Ca$^{2+}$ variations induced by environmental stimuli is well established (Jezek & Blatt, 2017; Konrad et al., 2018). Recently, a family of channels, the OSCA, was found to be PM ion channels involved in Ca$^{2+}$ signalling (Thor et al., 2020; Yuan et al., 2014). Interestingly, the OSCA channels are permeable to Ca$^{2+}$ but also to a similar extent to Na$^+$ (Thor et al., 2020). The VM harbours different ion transport systems sensitive to cytosolic Ca$^{2+}$. TPC1 is a vacuolar channel permeable to K$^+$, and to a lower extent to Ca$^{2+}$, which is activated by cytosolic Ca$^{2+}$ (Fig. 2) (Hedrich & Marten, 2011). One function of TPC1 seems to be linked to Ca$^{2+}$ signalling (Vincent et al., 2017), and recent data show that TPC1 regulates the VM excitability and in this way it could modulate Ca$^{2+}$ signalling (Jaślan et al., 2019). Additionally, a vacuolar anion channel permeable to malate$^{2-}$, ALMT6, was identified to be activated by Ca$^{2+}$ (Fig. 2) (Meyer et al., 2011). In the PM, ion channels like SLAC1, SLAH3, GORK and AKT1, the K$^+$/H$^+$ symporter HAK5, and the NRT1.1 transporter are regulated by Ca$^{2+}$ through interaction with the Ca$^{2+}$-dependent CBL-CIPK kinase complexes (Fig. 2) (reviewed in Kim et al., 2010; Saito & Uozumi, 2019; Tang et al., 2020a). Regarding the VM, CIPK9/7 with CBL2/3 were proposed to regulate the activity of the VM K$^+$ transporters NHX1 and NHX2 (Fig. 2) (Song et al., 2018). Recently, it has been shown that modules involving CBL2/3 and CIPK3/9/23/26 activate the VM K$^+$ channel TPK1, leading to K$^+$ efflux to the cytosol (Fig. 2) (Tang et al., 2020b). In the future a critical step to decipher the role of Ca$^{2+}$ will be to identify the molecular actors mediating its fluxes in plant cells.

Nucleotides, like ATP, also modify the activity of ion transporters in both the PM and the VM (Fig. 2). ATP is the source of energy of the H$^+$-ATPase pumps as P- and V-type H$^+$ pumps in the PM and the VM, respectively. Additionally, ATP negatively regulates the activity of the VM anion/H$^+$ exchanger CLCa (De Angeli et al., 2009) and of the VM anion channel ALMT9 (Fig. 2) (Zhang et al., 2014). In the PM, ATP blocks Rapid-type anion currents (Frachisse et al., 1999) that, in guard cells, they are mediated by ALMT12 (Fig. 2) (Meyer et al., 2010). A relevant aspect
of the role of nucleotides like ATP, ADP or AMP is their potential to coordinate ion fluxes with the energetic status of the cell.

Recently, malate emerge as a regulator of ion transport systems in both the VM and the PM. Cytosolic malate concentration depends on the starch degradation/synthesis cycle, on the metabolic consumption, on the vacuolar stocks and on its transport from the apoplast (Santelia & Lawson, 2016). In the last years it was found that the vacuolar anion channels ALMT4 (Eisenach et al., 2017) and ALMT9 (De Angeli et al., 2013) are directly activated by cytosolic malate (Fig. 2). In the PM SLAC1 (Wang & Blatt, 2011; Wang et al., 2018) and ALMT12 (Meyer et al., 2010) channels are also regulated by malate (Fig. 2). These data open to the possibility that cytosolic malate plays a role in the coordination of ion fluxes between both membranes during the opening and closure of stomata.

IV. Kinases and phosphatases targeting vacuolar and plasma membrane transporters

PM ion channels and transporters are target of kinases and phosphatases (Kim et al., 2010; Saito & Uozumi, 2019). Less is known on the VM side. Nonetheless, signalling pathways targeting vacuolar ion transporters and channels are emerging (Carpaneto et al., 2017; Eisenach et al., 2017; Wege et al., 2014). The signalling pathway responsible of ABA-induced stomata closure is an illustrative example (Fig. 2). In the last decade, the identification of the PYR/PYL/RCAR ABA receptors was a considerable advance (Ma et al., 2009; Park et al., 2009). PYR/PYL/RCAR interaction with ABA is the starting point of the signalling cascade inducing stomata closure (Kim et al., 2010). Interestingly PYR/PYL/RCAR receptors reside in the cytosol as soluble proteins (Fig. 2) (Ma et al., 2009). The cytosolic localisation of PYR/PYL/RCAR receptors is conceptually intriguing. It suggests that the PM and the VM events taking place during ABA response are not hierarchically organised in time and space. In other words, the signalling cascade starts in the cytosol with ABA binding to PYR/PYL/RCAR and is then targeting transport systems in the PM and the VM coordinating the release of ions from the vacuole to the apoplast. The ABA-PYR/PYL/RCAR complex inhibits a PP2C phosphatase, and this leads to the activation of the cytosolic kinase OST1/SnRK2.6 (Fig. 2) (Lee et al., 2013; Park et al., 2009). This kinase is central in ABA response targeting and activating, among others, the PM anion channels SLAC1 (Geiger et al., 2009; Lee et al., 2009) and ALMT12 (Imes et al., 2013), the aquaporin PIP2;1 (Grondin et al., 2015) as well as the VM anion/H⁺ exchanger CLCa (Fig. 2) (Wege et al., 2014). The activation of these ion transport systems residing in the VM and PM is required for the release of
ions and water to close stomata (Fig. 1b). In addition, OST1 inhibits inward K$^+$ currents mediated by KAT1 through protein interaction (Fig. 2) (Acharya et al., 2013). Besides OST1/SnRK2.6, other kinase proteins were found to regulate the activity of PM and VM ion transport systems. Recently, the vacuolar K$^+$ channel TPK1 was shown to be the target of the kinase KIN7 (Isner et al., 2018). This kinase phosphorylates TPK1 and seems to participate in ABA and CO$_2$ signalling (Fig. 2) (Gobert et al., 2007; Isner et al., 2018). Interestingly, KIN7 is localised in both the PM and the VM (Isner et al., 2018). Although its role in the PM is unknown, the dual localisation suggests that it could regulate transport systems in both membranes. Finally, MAPK were also found to target ion channels (Fig. 2) (Lee et al., 2016). A phosphorylation site targeted by MAPK was identified in the vacuolar ion channel ALMT4 (Eisenach et al., 2017). It was shown that the phosphorylation of ALMT4 inhibits its ion transport activity and that it is involved in ABA-induced stomata closure (Fig. 2) (Eisenach et al., 2017). Apart from SLAC1 (Fig. 2) (Prodhan et al., 2018), no target of MAPKs in the PM have been identified so far.

V. Conclusions and perspectives

The vacuole presents a high functional plasticity and is involved in a multitude of cellular processes. The transporters fluxing molecules across the VM determine the specialized functions of the vacuole in plant cells. In the last decade, the knowledge on the molecular identity of the vacuolar transport systems has considerably expanded. Currently, a consistent number of proteins forming transports systems in the VM is known, highlighting the role of the vacuolar fluxes in plants (Eisenach & De Angeli, 2017; Martinoia et al., 2012; Zhang et al., 2017). Based on this knowledge, a major step will be to decipher how vacuolar transport is integrated within the cell. Only some flux studies on Commelina communis L. using $^{86}$Rb$^+$ as a tracer investigated simultaneously ion fluxes across the PM and the VM (summarized in MacRobbie, 2000). Otherwise, the transport processes occurring in the VM and in other membranes, such as the PM, have been considered separately. Compared with the PM, only a restricted number of regulatory mechanisms targeting the VM transport systems is known. An integrative perspective on the intracellular transport reactions comes from in silico modelling, but only few data are available in vivo. Recently, in vivo data demonstrated the impact of ion transport on cytosolic conditions and connected it with stomatal aperture (Demes et al., 2020). Therefore, a future challenge will be to visualise and decipher, in living cells, the connection between transport systems residing in different membranes during cellular responses.
Acknowledgements

ADA was supported by a CNRS ATIP-Avenir grant 2018. PC-F was supported by a Postdoctoral Grant from Fundación Alfonso Martin Escudero. We thank J. Jáslan for critical reading and comments.

References

Acharya BR, Jeon BW, Zhang W, Assmann SM. 2013. Open Stomata 1 (OST1) is limiting in abscisic acid responses of Arabidopsis guard cells. *New Phytologist* **200**: 1049–1063.

Allaway WG, Hsiao TC. 1973. Preparation of rolled epidermis of *Vicia faba* L. so that stomata are the only viable cells: analysis of guard cell potassium by flame photometry. *Australian Journal of Biological Sciences* **26**: 309–318.

Andrés Z, Pérez-Hormaeche J, Leidi EO, Schlucking K, Steinhorst L, McLachlan DH, Schumacher K, Hetherington AM, Kudla J, Cubero B et al. 2014. Control of vacuolar dynamics and regulation of stomatal aperture by tonoplast potassium uptake. *Proceedings of the National Academy of Sciences* **111**: E1806–E1814.

Baetz U, Eisenach C, Tohge T, Martinoia E, De Angeli A. 2016. Vacuolar chloride fluxes impact ion content and distribution during early salinity stress. *Plant Physiology* **172**: 1167–1181.

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. *Journal of Plant Physiology* **171**: 770–778.

Carpaneto A, Boccaccio A, Lagostena L, Di Zanni E, Scholz-Starke J. 2017. The signaling lipid phosphatidylinositol-3,5-bisphosphate targets plant CLC-a anion/H⁺ exchange activity. *EMBO Reports* **18**: 1100–1107.

Chen ZH, Hills A, Bätz 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 Physiology* **159**: 1235–1251.

Cosio C, Martinoia E, Keller C. 2004. Hyperaccumulation of cadmium and zinc in *Thlaspi caerulescens* and *Arabidopsis halleri* at the leaf cellular level. *Plant Physiology* **134**: 716–725.

De Angeli A, Moran O, Wege S, Filleur S, Ephritikhine G, Thomine S, Barbier-Brygoo H, Gambale F. 2009. ATP binding to the C terminus of the *Arabidopsis thaliana* nitrate/proton antiporter, AtCLCa, regulates nitrate transport into plant vacuoles. *Journal of Biological
De Angeli A, Zhang J, Meyer S, Martinoia E. 2013. AtALMT9 is a malate-activated vacuolar chloride channel required for stomatal opening in Arabidopsis. Nature Communications 4: 1804.

Demès E, Besse L, Cubero-Font P, Satiat-Jeunemaitre B, Thomine S, De Angeli A. 2020. Dynamic measurement of cytosolic pH and [NO$_3^-$] uncovers the role of the vacuolar transporter AtCLCa in cytosolic pH homeostasis. Proceedings of the National Academy of Sciences, doi: 10.1073/pnas.2007580117.

Eisenach C, Baetz U, Huck N V, Zhang J, De Angeli A, Beckers G, Martinoia E. 2017. ABA-induced stomatal closure involves ALMT4, a phosphorylation-dependent vacuolar anion channel of Arabidopsis. The Plant Cell 29: 2552–2569.

Eisenach C, De Angeli A. 2017. Ion transport at the vacuole during stomatal movements. Plant Physiology 174: 520–530.

Faraco M, Spelt C, Bliék M, Verweij W, Hoshino A, Espen L, Prinsi B, Jaarsma R, Tarhan E, de Boer AH et al. 2014. Hyperacidification of vacuoles by the combined action of two different P-ATPases in the tonoplast determines flower color. Cell Reports 6: 32–43.

Fleurat-Lessard P, Bouche-Pillon S, Leloup C, Bonnemain JL. 1997a. Distribution and activity of the plasma membrane H$^+$-ATPase in Mimosa pudica L. in relation to ionic fluxes and leaf movements. Plant Physiology 113: 747–754.

Fleurat-Lessard P, Frangne N, Maeshima M, Ratajczak R, Bonnemain JL, Martinoia E. 1997b. Increased expression of vacuolar aquaporin and H$^+$-ATPase related to motor cell function in Mimosa pudica L. Plant Physiology 114: 827–834.

Frachisse JM, Thomine S, Colcombet J, Guern J, Barbier-Brygoo H. 1999. Sulfate is both a substrate and an activator of the voltage-dependent anion channel of Arabidopsis hypocotyl cells. Plant Physiology 121: 253–62.

Geiger D, Scherzer S, Mumm P, Stange A, Marten I, Bauer H, Ache P, Matschi S, Liese A, Al-Rasheid KAS et al. 2009. Activity of guard cell anion channel SLAC1 is controlled by drought stress signaling kinase-phosphatase pair. Proceedings of the National Academy of Sciences 106: 21425–21430.

Gobert A, Isayenkov S, Voelker C, Czempinski K, Maathuis FJ. 2007. The two-pore channel TPK1 gene encodes the vacuolar K$^+$ conductance and plays a role in K$^+$ homeostasis. Proceedings of the National Academy of Sciences 104: 10726–10731.

Grondin A, Rodrígues O, Verdoucq L, Merlot S, Leonhardt N, Maurel C. 2015. Aquaporins...
contribute to ABA-triggered stomatal closure through OST1-mediated phosphorylation. *The Plant Cell* **27**: 1945–54.

Hagihara T, Toyota M. 2020. Mechanical Signaling in the Sensitive Plant *Mimosa pudica* L.*. *Plants* **9**: 587.

Hedrich R, Marten I. 2011. TPC1-SV channels gain shape. *Molecular Plant* **4**: 428–41.

Imes D, Mumm P, Böhm J, Al-Rasheid KAS, Marten I, Geiger D, Hedrich R. 2013. Open stomata 1 (OST1) kinase controls R-type anion channel QUAC1 in Arabidopsis guard cells. *The Plant Journal* **74**: 372–382.

Horaruang W, Hills A, Blatt MR. 2020. Communication between the plasma membrane and tonoplast is an emergent property of ion transport. *Plant Physiology* **182**: 1833–1835.

Isner JC, Begum A, Nuehse T, Hetherington AM, Maathuis FJM. 2018. KIN7 kinase regulates the vacuolar TPK1 K+ channel during stomatal closure. *Current Biology* **28**: 466–472.

Jaślan D, Dreyer I, Lu J, O’Malley R, Dindas J, Marten I, Hedrich R. 2019. Voltage-dependent gating of SV channel TPC1 confers vacuole excitability. *Nature Communications* **10**: 1–9.

Jezek M, Blatt MR. 2017. The membrane transport system of the guard cell and its integration for stomatal dynamics. *Plant Physiology* **174**: 487–519.

Kim T-H, Böhmer M, Hu H, Nishimura N, Schroeder JI. 2010. Guard cell signal transduction network: advances in understanding abscisic acid, CO2, and Ca2+ signaling. *Annual Review of Plant Biology* **61**: 561–591.

Konrad K, Maierhofer T, Hedrich R. 2018. Spatio-temporal aspects of Ca2+ signalling: Lessons from guard cells and pollen tubes. *Journal of Experimental Botany* **69**: 4195–4214.

Krüger F, Schumacher K. 2018. Pumping up the volume – vacuole biogenesis in *Arabidopsis thaliana*. *Seminars in Cell and Developmental Biology* **80**: 106–112.

Lee SC, Lan W, Buchanan BB, Luan S. 2009. A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. *Proceedings of the National Academy of Sciences* **106**: 21419–21424.

Lee SC, Lim CW, Lan W, He K, Luan S. 2013. ABA signaling in guard cells entails a dynamic protein–protein interaction relay from the PYL-RCAR family receptors to ion channels. *Molecular Plant* **6**: 528–538.

Lee Y, Kim YJ, Kim M-H, Kwak JM. 2016. MAPK cascades in guard cell signal transduction. *Frontiers in Plant Science* **11**: 80.
Ma JF, Ueno D, Zhao FJ, McGrath SP. 2005. Subcellular localisation of Cd and Zn in the leaves of a Cd-hyperaccumulating ecotype of Thlaspi caerulescens. *Planta* **220**: 731–736.

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E. 2009. Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* **324**: 1064–1068.

MacRobbie EAC. 2000. ABA activates multiple Ca\(^{2+}\) fluxes in stomatal guard cells, triggering vacuolar K\(^{+}\) (Rb\(^{+}\)) release. *Proceedings of the National Academy of Sciences* **97**: 12361–12368.

Martinoia E. 2018. Vacuolar transporters – Companions on a longtime journey. *Plant Physiology*. **176**: 1384–14007.

Martinoia E, Meyer S, De Angeli A, Nagy R. 2012. Vacuolar transporters in their physiological context. *Annual Review of Plant Biology* **63**: 183–213.

Meckel T, Gall L, Semrau S, Homann U, Thiel G. 2007. Guard cells elongate: relationship of volume and surface area during stomatal movement. *Biophysical Journal* **92**: 1072–1080.

Meyer S, Mumm P, Imes D, Endler A, Weder B, Al-Rasheid KAS, Geiger D, Marten I, Martinoia E, Hedrich R. 2010. AtALMT12 represents an R-type anion channel required for stomatal movement in Arabidopsis guard cells. *Plant Journal* **63**: 1054–1062.

Meyer S, Scholz-Starke J, De Angeli A, Kovermann P, Burla B, Gambale F, Martinoia E. 2011. Malate transport by the vacuolar AtALMT6 channel in guard cells is subject to multiple regulation. *Plant Journal* **67**: 247–57.

Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TFF et al. 2009. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* **324**: 1068–1071.

Peiter E. 2011. The plant vacuole: Emitter and receiver of calcium signals. *Cell Calcium* **50**: 120–128.

Prodhan MY, Munemasa S, Nahar MN-E-N, Nakamura Y, Murata Y. 2018. Guard cell salicylic acid signaling is integrated into abscisic acid signaling via the Ca\(^{2+}\)/CPK-dependent pathway. *Plant Physiology* **178**: 441–450.

Roelfsema MRG, Hedrich R. 2005. In the light of stomatal opening: new insights into ‘the Watergate’. *New Phytologist* **167**: 665–691.

Saito S, Uozumi N. 2019. Guard cell membrane anion transport systems and their regulatory components: an elaborate mechanism controlling stress-induced stomatal closure. *Plants* **8**: 9.

Santelia D, Lawson T. 2016. Rethinking Guard Cell Metabolism. *Plant Physiology* **172**: 1371–1392.
Song S-J, Feng Q-N, Li C, Li E, Liu Q, Kang H, Zhang W, Zhang Y, Li S. 2018. A tonoplast-associated calcium-signaling module dampens ABA signaling during stomatal movement. *Plant Physiology* 177: 1666–1678.

Tanaka Y, Kutsuna N, Kanazawa Y, Kondo N, Hasezawa S, Sano T. 2007. Intra-vacuolar reserves of membranes during stomatal closure: the possible role of guard cell vacuoles estimated by 3-D reconstruction. *Plant and Cell Physiology* 48: 1159–1169.

Tang R-J, Wang C, Li K, Luan S. 2020a. The CBL–CIPK calcium signaling network: Unified paradigm from 20 years of discoveries. *Trends in Plant Science* 25: 604–616.

Tang R-J, Zhao F-G, Yang Y, Wang C, Li K, Kleist TJ, Lemaux PG, Luan S. 2020b. A calcium signalling network activates vacuolar K⁺ remobilization to enable plant adaptation to low-K environments. *Nature Plants* 6: 384–393.

Thomine S, Lelièvre F, Debarbieux E, Schroeder JI, Barbier-Brygoo H. 2003. AtNRAMP3, a multispecific vacuolar metal transporter involved in plant responses to iron deficiency. *Plant Journal* 34: 685–695.

Thor K, Jiang S, Michard E, George J, Scherzr S, Huang S, Dindas J, Derbyshire P, Leitão N, DeFalco TA et al. 2020. The calcium-permeable channel OSCA1.3 regulates plant stomatal immunity. *Nature*: doi.org/10.1038/s41586-020-2702-1.

Verweij W, Spelt C, Di Sansebastiano G Pietro, Vermeer J, Reale L, Ferranti F, Koes R, Quattrocchio F. 2008. An H⁺ P-ATPase on the tonoplast determines vacuolar pH and flower colour. *Nature Cell Biology* 10: 1456.

Vincent TR, Avramova M, Canham J, Higgins P, Bilkey N, Mugford ST, Pitino M, Toyota M, Gilroy S, Miller TJ et al. 2017. Interplay of plasma membrane and vacuolar ion channels, together with BAK1, elicits rapid cytosolic calcium elevations in Arabidopsis during aphid feeding. *The Plant Cell* 29: 1460–1479.

Wang C, Zhang J, Wu J, Brodsky DE, Schroeder JI. 2018. Cytosolic malate and oxaloacetate activate S-type anion channels in *Arabidopsis* guard cells. *New Phytologist* 220: 178–186.

Wang Y, Blatt MR. 2011. Anion channel sensitivity to cytosolic organic acids implicates a central role for oxaloacetate in integrating ion flux with metabolism in stomatal guard cells. *Biochemical Journal* 439: 161–170.

Wang Y, Hills A, Vialet-Chabrand S, Papanatsiou M, Griffiths H, Rogers S, Griffiths H, Rogers S, Lawson T, Lew VL et al. 2017. Unexpected connections between humidity and ion transport discovered using a model to bridge guard cell-to-leaf scales. *The Plant Cell* 29: 2921–
Wang Y, Papanatsiou M, Eisenach C, Karnik R, Williams M, Hills A, Lew VL, Blatt MR. 2012. Systems dynamic modeling of a guard cell Cl$^{-}$ channel mutant uncovers an emergent homeostatic network regulating stomatal transpiration. *Plant Physiology* **160**: 1956–1967.

Wege S, De Angeli A, Droillard M-J, Kroniewicz L, Merlot S, Cornu D, Gambale F, Martinoia E, Barbier-Brygoo H, Thomine S, et al. 2014. Phosphorylation of the vacuolar anion exchanger AtCLCa is required for the stomatal response to abscisic acid. *Science Signaling* **7**: ra65.

Yuan F, Yang H, Xue Y, Kong D, Ye R, Li C, Zhang J, Theprungsirikul L, Shrift T, Krichilsky B et al. 2014. OSCA1 mediates osmotic-stress-evoked Ca$^{2+}$ increases vital for osmosensing in Arabidopsis. *Nature* **514**: 367–371.

Zhang H, Zhao F-G, Tang R-J, Yu Y, Song J, Wang Y, Li L, Luan S. 2017. Two tonoplast MATE proteins function as turgor-regulating chloride channels in *Arabidopsis*. *Proceedings of the National Academy of Sciences* **114**: E2036–E2045.

Zhang J, Martinoia E, De Angeli A. 2014. Cytosolic nucleotides block and regulate the arabidopsis vacuolar anion channel AtALMT9. *Journal of Biological Chemistry* **289**: 25581–25589.

**Figure Legends**

**Fig. 1** The coordination of the fluxes of solutes across the vacuolar and plasma membranes is required to control stomata movements. (a) Opening (blue) and closure (red) of the stomata are based on rapid changes of the intracellular osmotic pressure (Π) leading to water entry/release into/from the guard cell (GC). In *Vicia faba* GC, during stomata opening ionic solutes raise of ~ 4 pmol, increases the intracellular concentration of solutes of ~ 0.8 M. $J_T$ is the total flux of solutes between the apoplast and the GC, that is majorly composed of anions ($J_{A^{-}}$) and of cations ($J_{C^{+}}$) fluxes. During closure, the total flux of ionic solutes ($J_T$) from the GC to the apoplast is ~2×10$^9$ molecules·s$^{-1}$/GC. (b) Osmotically active solutes move from the apoplast to the vacuole (opening, blue) and from the vacuole to the apoplast (closure, red). In GCs, the major osmotically active solutes are cations (C$^+$) like K$^+$, anions (A$^-$) like Cl$^{-}$, NO$_3^-$ and malate$^{2-}$, and sugars. To reach the vacuole/apoplast, solutes need to cross both the plasma (PM) and vacuolar (VM) membranes (*insets*). The cytosol faces both the PM and the VM, thus the cytosolic concentration of ions
influences ion fluxes across both the VM and the PM. Notably, during stomata opening and closure the vacuole undergoes morphological changes and modifications of its relative volume.

**Fig. 2** Identified mechanisms co-regulating ion transport systems in the vacuolar membrane (VM) and plasma membrane (PM). Cytosolic Ca\(^{2+}\) raise induces the activation of different CBL/CIPK kinase complexes that activates the PM anion channels SLAC1, SLAH3, the potassium (K\(^+\)) channels AKT1 and GORK, and the K\(^+\)/H\(^+\) symporter HAK5. In the VM, CBL/CIPK target the K\(^+\) exchangers NHX1 and NHX2 and the K\(^+\) channel TPK1. Cytosolic Ca\(^{2+}\) can also directly interact and activate vacuolar channels like TPC1 and ALMT6. The ABA signalling induces phosphorylation by OST1 of the PM channels SLAC1, KAT1 and ALMT12, the PIP2;1 aquaporin, and of the VM anion/proton exchangers CLCa. ABA signalling also acts on the vacuolar exchanger CLCc by an unknown pathway, and on the K\(^+\) channel TPK1 through KIN7 kinase. MAPK kinases activate SLAC1 in the PM and inhibit ALMT4 in the VM. Several cytosolic molecules induce the activation/inhibition of ion transporters like ALMT9, CLCa, and H\(^+\) pumps. Malate activates the anion channels ALMT4 and ALMT9 in the VM, and ALMT12 and SLAC1 in the PM. ATP is the substrate for the pumping activity of the H\(^+\) ATPases and is a negative regulator of anion channels in the PM and VM.
Figure 1

Tansley Insight 33874

(a) $\Delta [\text{solutes}] = 0.8 \text{ M}$

$J = J_A^- + J_C^+ 
\approx 7 \times 10^8 \text{ molecules s}^{-1}/\text{GC}$

Opening
(60 min)

Closure
(20 min)

(b) $J = J_A^- + J_C^+ 
\approx 2 \times 10^9 \text{ molecules s}^{-1}/\text{GC}$

This article is protected by copyright. All rights reserved
This article is protected by copyright. All rights reserved