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Authors: Kwak, June M., Mäser, Pascal, and Schroeder, Julian I.

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The Clickable Guard Cell, Version II: Interactive Model of Guard Cell Signal Transduction Mechanisms and Pathways

June M. Kwak,[a,1] Pascal Mäser,[b] and Julian I. Schroeder,[c,1]

[a]Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland 20742
[b]Institute of Cell Biology, University of Berne, CH-3012 Bern, Switzerland
[c]Division of Biological Sciences, Cell and Developmental Biology Section and Center for Molecular Genetics, University of California, San Diego, La Jolla, California 92093-0116
[1]Address correspondence to jkwak@umd.edu and julian@biomail.ucsd.edu

ABSTRACT

Guard cells are located in the leaf epidermis and pairs of guard cells surround and form stomatal pores, which regulate CO_2 influx from the atmosphere into leaves for photosynthetic carbon fixation. Stomatal guard cells also regulate water loss of plants via transpiration to the atmosphere. Signal transduction mechanisms in guard cells integrate a multitude of different stimuli to modulate stomatal apertures. Stomata open in response to light. Stomata close in response to drought stress, elevated CO_2, ozone and low humidity. In response to drought, plants synthesize the hormone abscisic acid (ABA) that triggers closing of stomatal pores. Guard cells have become a highly developed model system for dissecting signal transduction mechanisms in plants and for elucidating how individual signaling mechanisms can interact within a network in a single cell. Many new findings have been made in the last few years. This chapter is an update of an electronic interactive chapter in the previous edition of *The Arabidopsis Book* (Mäser et al. 2003). Here we focus on mechanisms for which genes and mutations have been characterized, including signaling components for which there is substantial signaling, biochemical and genetic evidence. Ion channels have been shown to represent targets of early signal transduction mechanisms and provide functional signaling and quantitative analysis points to determine where and how mutations affect branches within the guard cell signaling network. Although a substantial number of genes and proteins that function in guard cell signaling have been identified in recent years, there are many more left to be identified and the protein-protein interactions within this network will be an important subject of future research. A fully interactive clickable electronic version of this publication can be accessed at the following web site: http://www-biology.ucsd.edu/labs/schroeder/clickablegc2/. The interactive clickable version includes the following features:

Figure 1. Model for the roles of ion channels in ABA signaling.
Figure 2. Blue light signaling pathways in guard cells.
Figure 3. ABA signaling pathways in guard cells.

Figure 1 is linked to explanations that appear upon mouse-over. Figures 2 and 3 are clickable and linked to info boxes, which in turn are linked to TAIR, to relevant abstracts in PubMed, and to updated background explanations from Schroeder et al (2001), used with permission of Annual Reviews of Plant Biology.

INTRODUCTION

Being rooted in one place, plants are required to respond to changes in environmental conditions and stresses. Hormone and light signal transduction pathways form complex networks that control plant responses to the environment. Although key mechanisms and genes have been identified in these signal transduction pathways, the protein-protein interaction networks and the functions of individual interactions largely remain be explored. In guard cells, a network of signal transduction mechanisms integrates water status, hormone responses, light, CO_2 and other environmental conditions to regulate stomatal apertures in leaves, for optimal plant growth and survival under diverse conditions. Stomatal guard cells have developed into a favorite model system for understanding how various signaling components can interact within a network in a single plant cell, creating graded bi-directional outputs (stomatal 'opening' or 'closing' responses). Moreover, cell signaling and genetic studies have shown that guard cell ion channels are targets of signal transduction. Thus detailed insights into the signaling machinery are gained through eletrophysiological recordings of ion channel currents (Schroeder and Hagiwara, 1989; Blatt and Armstrong, 1993; Ward and Schroeder, 1994; Li et al., 2000; Kwak et al., 2003; Mori et al., 2006). Detailed overviews of guard cell signal transduction can be found in several recent reviews (Kuhn and Schroeder, 2003; Pei and Kuchitsu, 2005; Roelfsema and Hedrich, 2005; Israelsson et al., 2006; Pandey et al., 2007; Shimazaki et al., 2007).

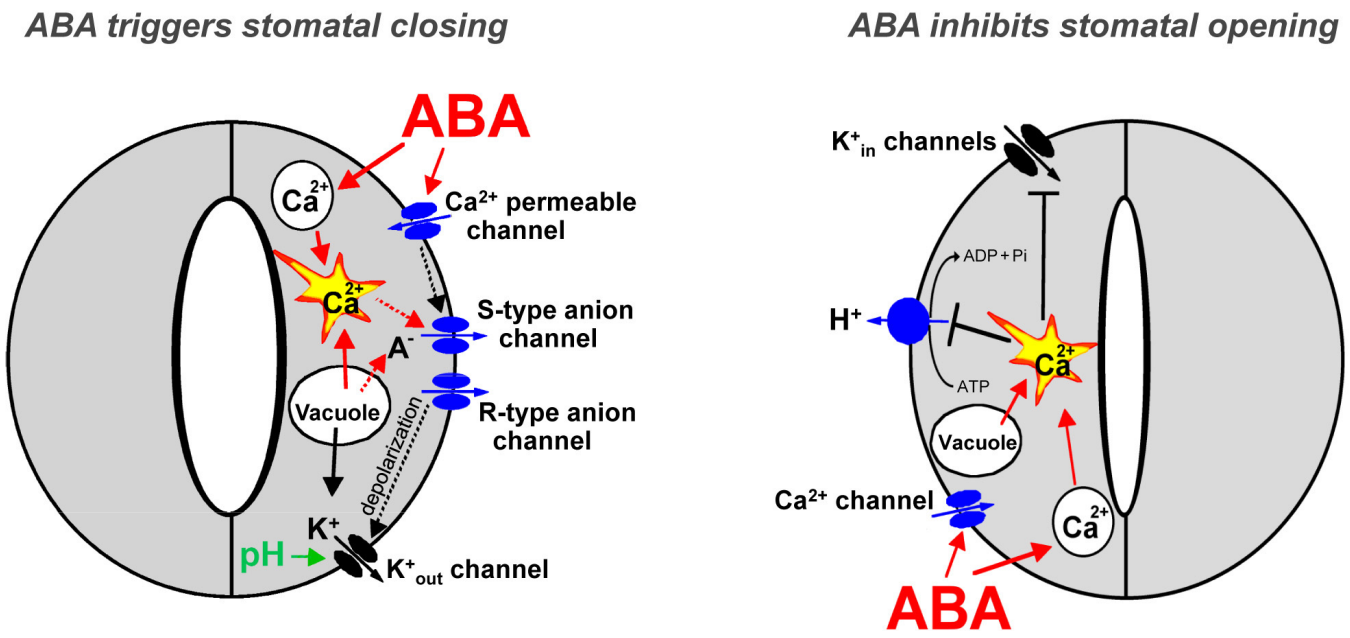

Figure 1. Simplified model for roles of ion channels and pumps in regulating stomatal movements.

Note that other 2nd messengers in addition to Ca^{2+} also function in stomatal movements (see text for details). Mouse-over for explanations (may not work on all browsers). See online version of the figure for explanations.

Guard cells allow quantitative dissection of the functions of individual genes and proteins within signaling cascades because:

1. Guard cells can respond to physiological stimuli even after they are isolated, allowing cell biological and time-resolved analyses of stomatal opening and closure in response to various stimuli.

2. Guard cells respond to several of the classically known hormonal and light stimuli in plants, illustrating that plant receptors and early signaling mechanisms function in these cells.

3. A network of ion channels in the plasma membrane and tonoplast of guard cells has been characterized that, together with metabolic responses, controls stomatal movements (see Figure 1) (Ward et al., 1995). Being targets of early signaling branches, these ion channels provide effective functional signaling and quantitative analysis points to identify and characterize upstream regulators and identify the intermediate targets and signaling branches that are affected either directly or indirectly by these regulators.

4. Stomatal aperture regulates plant CO_2 intake and water loss, thus critically influencing growth and water stress responsiveness (Pei et al., 1998; Hugouvieux et al., 2001).

5. Patch clamp, Ca^{2+} imaging and genomic techniques have been developed and adapted to Arabidopsis guard cell signal transduction studies., This allows for molecular genetic, cell biological, biophysical, physiological, and functional genomics analyses of single cell signaling responses (Pei et al., 1997; Pei et al., 1998; Allen et al., 1999; Wang et al., 2001; Hosy et al., 2003; Leonhardt et al., 2004).

THE ROLES OF ION CHANNELS IN STOMATAL MOVEMENTS

A pair of guard cells surrounds each stomatal pore, located in the leaf epidermis of plants, and controls the aperture of this pore. Stomatal movements are regulated by turgor pressure of guard cells. Stomatal opening is achieved by uptake of solutes into the cytosol and vacuoles resulting in water uptake into the guard cells and, thus, turgor increase drives opening of the stomatal pore. During stomatal opening, H^+-ATPases in the plasma membrane mediate proton efflux from the cytoplasm, leading to membrane hyperpolarization (Assmann et al., 1985; Shimazaki et al., 1986), which in turn activates inward-rectifying K^+ channels (Schroeder et al., 1984). Moreover, malate is produced from starch and functions as one of the major osmotica causing an increase in turgor pressure in guard cells. Anions including Cl^- and NO_3^- are transported into the guard cell to further increase turgor pressure.

Stomatal closure is mediated by turgor reduction in guard cells, which is caused by efflux of K^+ and anions from guard cells, sucrose removal, and a parallel conversion of the organic acid malate to osmotically inactive starch (MacRobbie, 1998; Schroeder et al., 2001). Membrane depolarization during stomatal closure is mediated by anion channel activation and inhibition of H^+-ATPases in the plasma membrane (Keller et al., 1989; Schroeder and Hagiwara, 1989; Shimazaki et al., 2007). An increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) occurs via Ca^{2+} influx through plasma membrane Ca^{2+} permeable channels and Ca^{2+} release from internal stores, leading to activation of anion channels (Schroeder and Hagiwara, 1989) that cause positive shifts (depolarization) in the membrane potential of guard cells. This change in membrane potential down-regulates inward-recti-

fying K^+ (K^+_{in}) channels (Schroeder et al., 1987; Pilot et al., 2001) and activates outward-rectifying K^+ (K^+_{out}) channels (Schroeder et al., 1987; Hosy et al., 2003), resulting in a net K^+ efflux from guard cells (Figure 1). In addition, during stomatal closure ABA causes an alkalization of the guard cell cytosol (Blatt and Armstrong, 1993) which directly enhances K^+_{out} channel activity (Blatt, 1992; Blatt and Armstrong, 1993; Ilan et al., 1994; Miedema and Assmann, 1996), but also down-regulates the transient R-type anion channels (Schulz-Lessdorf et al., 1996). The efflux of both anions and K^+ from guard cells via anion and K^+_{out} channels contributes to loss of guard cell turgor, which leads to stomatal closing (Schroeder and Hagiwara, 1989; Pei et al., 1997; Vahisalu et al., 2008). Figure 1 shows an extension of early models of the roles of ion channels and transporters in stomatal opening and closing (Schroeder and Hedrich, 1989; McAinsh et al., 1990; Ward et al., 1995).

The contribution of plasma membrane H^+-ATPases to stomatal movements was assessed by studies with transgenic and mutant plants. Fusicoccin-induced stomatal opening is significantly reduced in transgenic tobacco plants in which the tobacco H^+-ATPase *PMA4* is co-suppressed (Zhao et al., 2000) (Figure 2). Recently, two dominant mutations in the *OST2* gene that encodes the AHA1 H^+-ATPase were isolated from a genetic screen in Arabidopsis (Merlot et al., 2007). This study found that these dominant mutations caused constitutive activation of the AHA1/OST2 H^+-ATPase, and thus, impaired stomatal closure induced by ABA. These new findings helped shape the model that continuous membrane hyperpolarization by constitutively-activated H^+-ATPase mutants counteracts ABA-induced stomatal closure (Merlot et al., 2007) (Figure 3).

Vacuolar Channels

As vacuoles can take up over 90% of the guard cell's volume, over 90% of the ions exported from the cell during stomatal closing must first be transported from vacuoles into the cytosol (MacRobbie, 1995, 1998). $[Ca^{2+}]_{cyt}$ elevation activates vacuolar K^+ (VK) channels, which are proposed to provide a pathway for Ca^{2+}-induced K^+ release from the vacuole (Ward and Schroeder, 1994) (Figure 3). At resting $[Ca^{2+}]_{cyt}$, K^+ efflux from guard cell vacuoles can be mediated by fast vacuolar (FV) channels, allowing for versatile vacuolar K^+ efflux pathways (Allen and Sanders, 1996). The Arabidopsis genome contains five TPK/KCO genes which encode "two-pore" K^+ channel proteins. A recent study with *tpk1* knockout mutants showed that VK channel currents are absent in the mutant indicating that TPK1/KCO1 encodes a major component of the vacuolar VK channels (Gobert et al., 2007). Expression of TPK1 in yeast led to appearance of VK-like channels in yeast vacuoles (Bihler et al., 2005). 14-3-3 proteins physically interact with TPK1/KCO1 and activate TPK1/KCO1 in a dose-dependent manner (Latz et al., 2007). Furthermore, ABA-induced stomatal closure was impaired by the *tpk1* mutation (Gobert et al., 2007), providing genetic evidence for the model that VK channels function in mediating vacuolar K^+ release for stomatal closing (Ward and Schroeder, 1994; Gobert et al., 2007).

It has been proposed that calcium release is induced by inositol hexakisphosphate (Lemtiri-Chlieh et al., 2003), and that a protein phosphorylation event upstream of Ca^{2+} release is required for ABA signaling (Sokolovski et al., 2005). Slow vacuolar (SV) channels are voltage-dependent and slowly activated at elevated $[Ca^{2+}]_{cyt}$ (Hedrich and Neher, 1987). SV channels were first thought to mediate anion transport (Hedrich et al., 1986), but were later shown to be cation channels that are permeable to Ca^{2+} ions (Ward and Schroeder, 1994; Ward et al., 1995; Allen and Sanders, 1996) (Figure 3). The vacuolar Ca^{2+}-dependent channel TPC1 has been identified as an essential subunit of the vacuolar SV channels, because SV type currents are absent in the *tpc1* knockout mutant (Peiter et al., 2005). ABA-induced stomatal closure was not affected in *tpc1* mutants, whereas external Ca^{2+}-inhibition of stomatal opening was impaired in *tpc1* mutants (Peiter et al., 2005). Absence of Ca^{2+}-activated SV currents was confirmed in an independent study (Ranf et al., 2008). SV channels are down-regulated by vacuolar Ca^{2+} (Pottosin et al., 1997; Pei et al., 1999), but can allow Ca^{2+} transport from the vacuole to the cytoplasm (Bewell et al., 1999), which is for example illustrated by "tail" currents recorded in pure $CaCl_2$ gradients (Ward et al., 1995). SV channels are ubiquitous and have been found in all plant vacuoles, but in spite of their broad distribution in plant vacuoles, their physiological functions are not yet understood. Further studies are required to address the physiological role for this SV channel protein in plants.

CLC family genes function as vacuolar anion (nitrate) uptake transporters (De Angeli et al., 2006), and Cl^- channels and malate channels can mediate low-affinity vacuolar anion uptake (Pei et al., 1996; Hafke et al., 2003; Kovermann et al., 2007), but the pathways for anion release from vacuoles still remain elusive.

LIGHT-INDUCED STOMATAL OPENING

Guard cells respond to a multitude of signals including temperature, partial CO_2 pressure, light, humidity, and hormones. For the majority of signals the molecular identity of the sensors is not known, with the notable exception of blue light: The phototropins PHOT1 and PHOT2 were shown to function as blue light receptors in Arabidopsis guard cells (Kinoshita et al., 2001). PHOT1 in deetiolated seedlings and PHOT2 in leaves were reported to mediate blue light-induced $[Ca^{2+}]_{cyt}$ increases (Baum et al., 1999; Harada et al., 2003). Furthermore, PHOT1 was recently shown to genetically interact with an inositol polyphosphate 5- phosphatase that negatively regulates PHOT1-mediated $[Ca^{2+}]_{cyt}$ increases in root hairs (Chen et al., 2008). Thus, it would be interesting to test whether or not PHOT1 and PHOT2 receptors lead to an increase in cytosolic calcium in guard cells in response to blue light (Figure 2). The RPT2 (Root Phototropism 2) protein is a plant specific protein containing a putative protein-protein interaction domain (Sakai et al., 2000). PHOT1 was shown to form a complex with RPT2 in vivo and to mediate stomatal opening in a RPT2-dependent manner (Inada et al., 2004) (Figure 2).

In guard cells, blue light activates the plasma membrane H^+-ATPase by phosphorylation (Kinoshita and Shimazaki, 1999). Because of their importance in stomatal opening, H^+-ATPases seem to be a target of multiple modulators. 14-3-3 proteins are conserved in all eukaryotic cells and bind to a variety of signaling proteins. In guard cells, 14-3-3 proteins bind to the phosphorylated plasma membrane H^+-ATPase. A 14-3-3 protein was also shown to bind to light-activated, autophosphorylated phototropins in Vicia

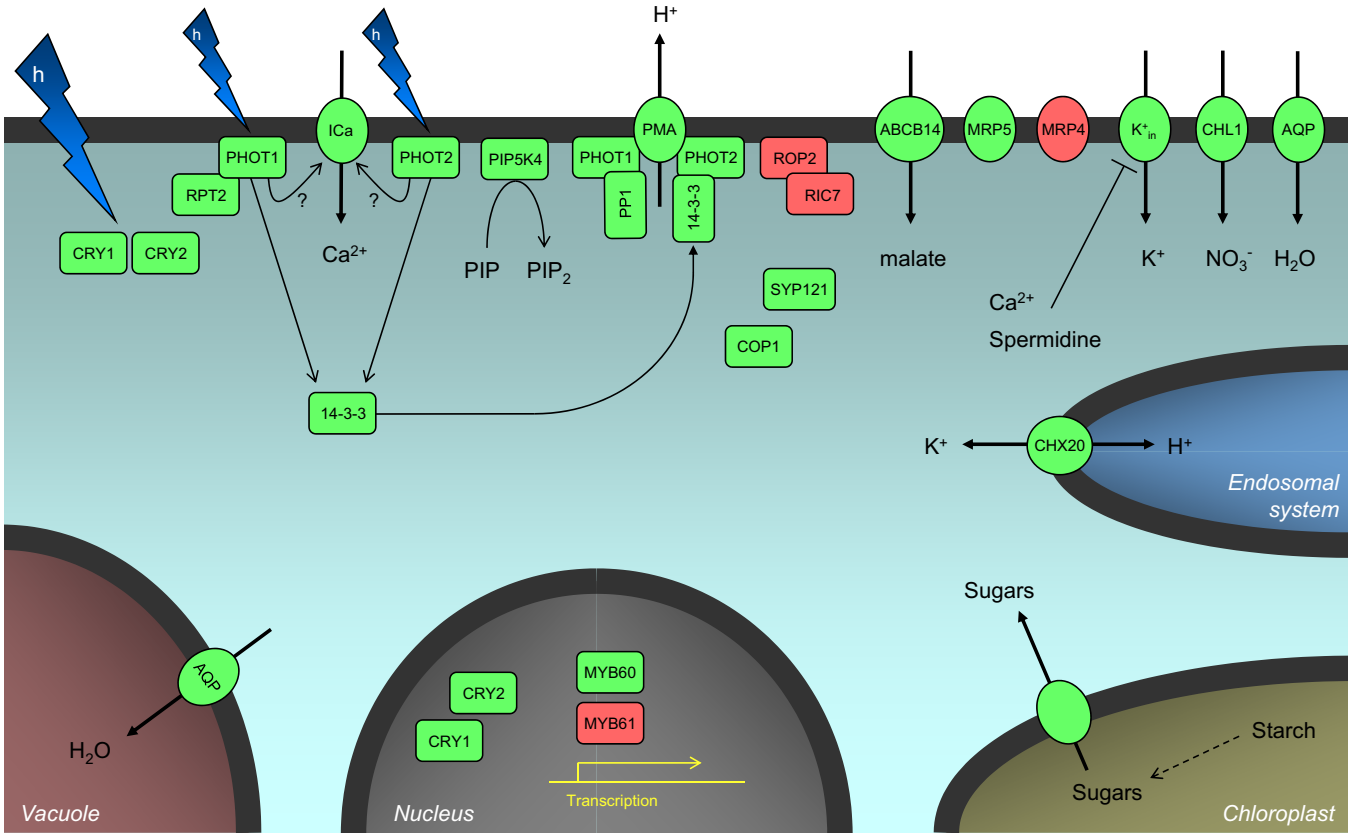

Figure 2. Blue light signaling pathways in guard cells.

Positive signaling regulators are shown in green, negative regulators in red. Pointed arrows, activation; blunt arrows, inhibition; filled arrows indicate substrate transport across membranes. Note that arrows have not been added to all experimentally examined links in this simplified model. Further links are discussed through out the text and the publications.

guard cells (Kinoshita et al., 2003). Thus, it appears that phototropins form a complex with the H^+-ATPase via 14-3-3 proteins that possibly maintain an active state (Figure 2). In addition, another protein, VfPIP, interacting with *Vicia* phototropin was identified from a yeast two-hybrid screen (Emi et al., 2005). This protein shares a high similarity with the dynein light chain, yet its role remains to be determined. Recently, type 1 protein phosphatase (PP1) genes that are expressed in *Vicia faba* guard cells have been identified as positive regulators of blue light-induced stomatal opening (Takemiya et al., 2006) (Figure 2). Introduction of a dominant non-functional mutant PP1 into guard cells inhibited light-induced stomatal opening whereas wild type PP1 had no effect. Furthermore, the PP1 inhibitor tautomycin impaired blue light-induced H^+ pumping, phosphorylation of the H^+-ATPase, and stomatal opening without affecting the blue light-induced phosphorylation of phototropins. This suggests that PP1 functions downstream of phototropins and upstream of H^+-ATPases (Takemiya et al., 2006).

During light-induced stomatal opening, activation of the plasma membrane H^+-ATPase results in membrane hyperpolarization, which cannot be achieved without inactivation of anion channels that provide a large conductance leading to membrane depolarization. Recently it was shown that blue light inhibits the S-type anion channels that are activated by 700 ppm CO_2 (Marten et al., 2007a). Furthermore, blue light inhibition of anion channels was not seen in guard cells of the *phot1phot2* double mutants, indicating that phototropins also function in this response (Marten et al., 2007a).

In addition to phototropins, cryptochromes (CRY1 and CRY2), COP1, and NPQ1 also appear to participate in blue light-regulation of stomatal movements (Mao et al., 2005) (Figure 2). Compared to wild type, *cry1cry2* and *phot1phot2* double mutant guard cells are less responsive to blue light, whereas guard cells of a *cry1cry2phot1phot2* quadruple mutant did not respond to blue light at all (Mao et al., 2005). Guard cells of COP1 over-expressing plants are hypersensitive to blue light (Mao et al., 2005).

K^+_{in} Channels Mediate K^+ Uptake During Stomatal Opening

Inward rectifying K^+ channels were characterized and proposed to contribute to K^+ influx during stomatal opening (Schroeder et al., 1984; Schroeder et al., 1987). The guard cell K^+ channel subunit *KAT1* and the root-expressed *AKT1* homologue were the first cloned Arabidopsis K^+_{in} channel genes (Anderson et al., 1992;

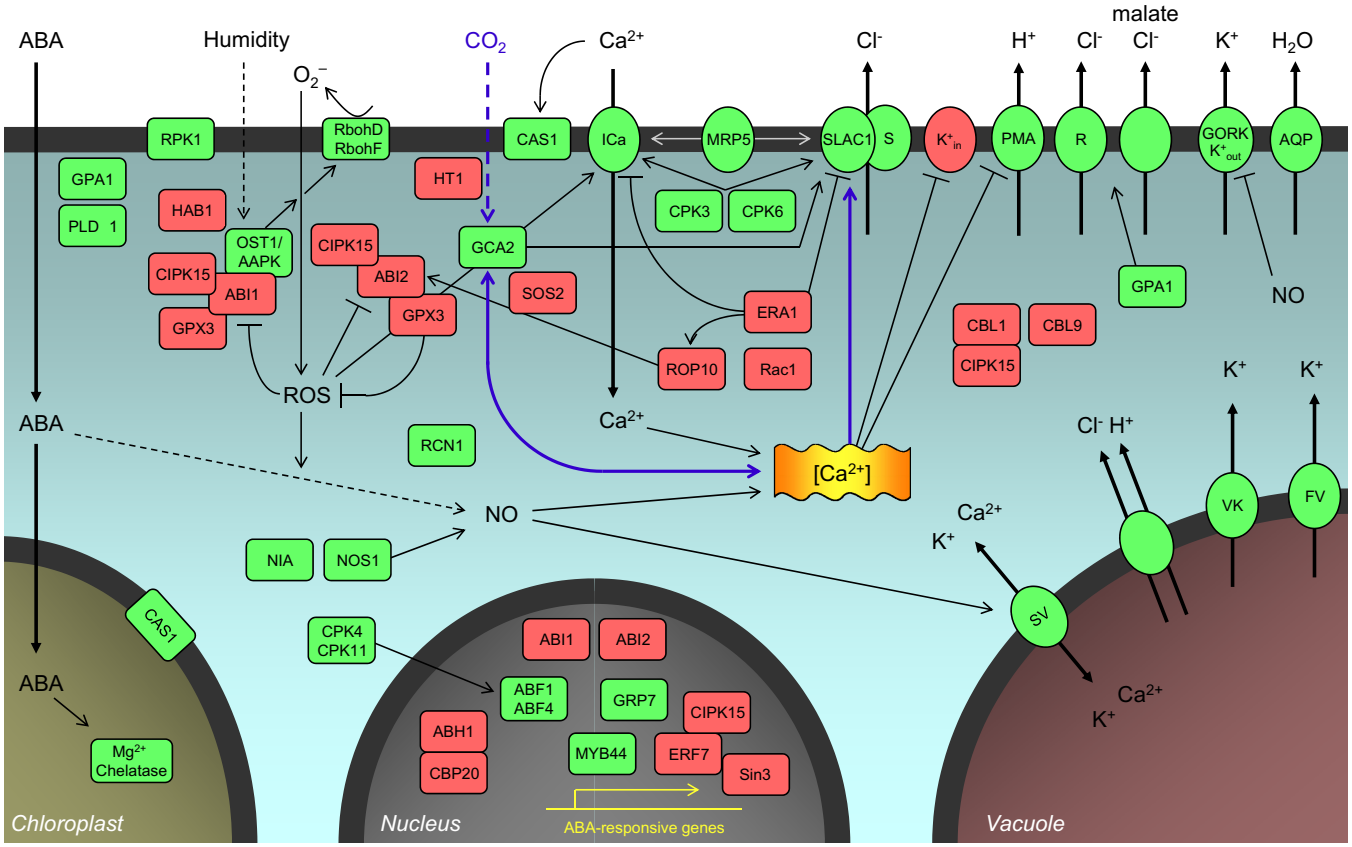

Figure 3. ABA signaling pathways in guard cells.

Positive regulators of ABA signaling are shown in green, negative regulators in red. The convergence between ABA signaling and CO_2 signaling is approximated in blue (for details, see (Israelsson et al., 2006). Pointed arrows, activation; blunt arrows, inhibition; filled arrows indicate substrate transport across membranes. Note that the illustrated interactions and locations correspond in many cases to physiological and genetic signal transduction data, and further cell biological and protein-protein interaction analyses are needed to characterize protein interactions. Note that arrows have not been added to all experimentally examined links in this simplified model. Further links are discussed through out the text and the publications.

Sentenac et al., 1992). *KAT1* was characterized as the first eukaryotic inward-rectifying K^+ channel gene (Schachtman et al., 1992). *KAT1* is expressed in guard cells (Nakamura et al., 1995) and expression of dominant negative mutants, as well as knockout mutant analyses support the model that KAT1 and its homologues play an important role in stomatal opening (Kwak et al., 2001; Lebaudy et al., 2008). *KAT2* is also expressed in guard cells (Pilot et al., 2001). Furthermore, expression analysis in Xenopus oocytes and yeast suggests that KAT1 and KAT2 form heteromultimeric channel proteins that are probably responsible for K^+ influx into guard cells (Pilot et al., 2001). K^+_{in} channel activity is regulated by various factors including $[Ca^{2+}]_{cyt}$ and protein de/phosphorylation. In addition, spermidine was shown to inhibit stomatal opening and induce stomatal closure (Liu et al., 2000) (Figure 2). Spermidine is a polyamine that functions as a growth signal in both eukaryotes and prokaryotes. The levels of polyamines increase under stress conditions, suggesting their roles in stress responses (Slocum and Flores, 1991). Addition of spermidine inhibited K^+_{in} channel currents in guard cells in a dose-dependent manner, and also inward K^+ currents in mesophyll cells expressing KAT1, indicating a link between stress response and the regulation of K^+_{in} channel activity (Liu et al., 2000).

Phopholipid kinases also modulate stomatal opening. Phosphatidylinositol (PI) 4,5-bisphosphate produced from either PI4P or PI5P was reported to inhibit both light-induced stomatal opening and ABA-induced stomatal closure (Jung et al., 2002) (Figure 2). The PI3 kinase and PI4 kinase inhibitors wortmannin and LY294002 impaired ABA-induced $[Ca^{2+}]_{cyt}$ increases and stomatal closure (Jung et al., 2002). PI 4,5-bisphosphate was also shown to inhibit S-type anion channel current in guard cells (Lee et al., 2007). Furthermore, a mutation in the Arabidopsis PIP5 kinase 4 causes reduced stomatal opening in the light (Lee et al., 2007). Stomatal opening of the *pip5k4* mutant was rescued by exogenous PI 4,5-bisphosphate, indicating that PIP5K4 is a positive regulator of light-induced stomatal opening (Lee et al., 2007) (Figure 2).

The Arabidopsis genome encodes 11 ROP-type small G proteins that function as a molecular switch in various cellular responses in plants. It was recently shown that ROP2 is expressed in guard cells and translocates to the plasma membrane upon illumination (Jeon et al., 2008). Expression of a constitutively ac-

tive form of ROP2 negatively modulated stomatal opening in both Vicia and Arabidopsis (Jeon et al., 2008) (Figure 2). Furthermore, the guard cell-expressed RIC7 (ROP-Interactive CRIB motif-containing protein 7) physically interacts with ROP2 and translocates to the plasma membrane and cytosol from the nucleus upon illumination, which is dependent on ROP2 activation (Jeon et al., 2008). Expression of RIC7 in *Vicia faba* guard cells suppresses light-induced stomatal opening (Jeon et al., 2008). These results suggest that RIC7 negatively regulates light-induced stomatal opening by working downstream of ROP2 (Figure 2).

Transcriptional modulation contributes to the regulation of guard cell signaling cascades, positively or negatively. Two MYB transcription factors have been identified as regulators of stomatal opening. *MYB60* was shown to be specifically expressed in Arabidopsis guard cells, and the expression level of *MYB60* was reduced by ABA and desiccation (Cominelli et al., 2005). A null mutation in *MYB60* leads to a reduction in light-induced stomatal opening and to an enhanced drought tolerance, without affecting ABA sensitivity of guard cells (Cominelli et al., 2005). Furthermore, only 36 genes were shown to be down- or up-regulated in the *atmyb60* mutant, suggesting that MYB60 specifically regulates a limited number of genes (Cominelli et al., 2005) (Figure 2). In contrast, another MYB transcriptional factor, *MYB61*, was reported to be highly expressed in guard cells (Liang et al., 2005). Overexpression of *MYB61* results in a higher leaf surface temperature (indicative of smaller stomatal apertures), whereas *atmyb61* null mutants have a lower leaf surface temperature (larger stomatal apertures), compared to wild type plants (Liang et al., 2005). Similar to the situation with *atmyb60*, neither overexpression or knockout of *MYB61* appears to alter ABA sensitivity of the plants in stomatal movements (Liang et al., 2005). However, dark-induced stomatal closure is partially impaired in *atmyb61* (Liang et al., 2005), suggesting that MYB61 is a negative regulator of light-induced stomatal opening and/or a positive regulator of dark-induced stomatal closure (Figure 2).

In addition to the cytosolic factors, protein trafficking plays a role in regulating K^+_{in} channel activity during stomatal movements. During swelling of guard cell protoplasts, an increase in the membrane surface area was reported to be associated with an increase in both K^+_{in} channel and K^+_{out} channel currents without affecting the activation kinetics of K^+ channels (Homann and Thiel, 2002). This result suggests that vesicles containing both K^+_{in} and K^+_{out} channels are delivered to the plasma membrane during guard cell swelling, resulting in the increase in the membrane surface area and K^+ currents. SYP121 is the Arabidopsis homolog of the tobacco syntaxin SYP121/Syr1 that was previously shown to function in ABA-activation of Cl^- currents and ABA inhibition of KAT1-mediated K^+ currents in oocytes (Leyman et al., 1999). A cell biological study using the dominant-negative Sp2 fragment of SYP121 showed that the Arabidopsis SYP121 protein is responsible for delivery of KAT1 K^+_{in} channel to the plasma membrane (Sutter et al., 2006). Furthermore, it was shown that ABA induces specific endocytosis of KAT1 K^+_{in} channels in an endosomal pool in guard cells leading to a reduction in the number of KAT1 K^+_{in} channel subunits residing in the plasma membrane (Sutter et al., 2007). The endocytosed KAT1 K+in channels were almost entirely recycled to the plasma membrane 8hr after removal of ABA (Sutter et al., 2007). These studies indicate that trafficking of K^+ channels is involved in stomatal movements (Figure 2).

In addition to K^+_{in} channels, other transporters have been suggested to contribute to stomatal opening. AtCHX20, a proton antiporter that localizes to endomembranes, was reported to positively regulate light-induced stomatal opening (Padmanaban et al., 2007). The CHX family is comprised of 28 members, and their functions are largely unknown (Sze et al., 2004). *AtCHX20* is highly and preferentially expressed in guard cells, and *Atchx20* T-DNA insertional mutants exhibited a significant reduction in light-induced stomatal opening, implying that the endomembrane cation/H^+ exchanger contributes to K^+ uptake into vacuoles during stomatal opening (Padmanaban et al., 2007) (Figure 2). During stomatal opening, K^+ influx occurs which balances an influx of anions, mainly Cl^-. Because nitrate is also abundant in soil and transported to plant cells by the nitrate transporter AtNRT1.1/CHL1, it was tested whether AtNRT1.1/CHL1 has a role in stomatal movements (Guo et al., 2003). *chl1* mutant plants have reduced stomatal opening, reduced nitrate accumulation, and impaired nitrate-induced membrane depolarization in the light, suggesting that nitrate uptake is accompanying K^+ influx and required for stomatal opening (Guo et al., 2003) (Figure 2).

Figure 2 illustrates the signaling cascade from activation of PHOT1, 2 to stomatal opening in terms of known genes and their relative position in the signal transduction pathway. For a detailed description of each gene please see the on-line version of this figure where the figure is clickable and linked to an updated version of (Schroeder et al., 2001).

THE ROLE OF CO_2 AND HUMIDITY IN REGULATION OF STOMATAL MOVEMENTS

CO_2 and humidity are environmental stimuli that regulate stomatal movements. The continual rise in atmospheric pCO_2 causes an increase in leaf CO_2 and a reduction of stomatal apertures in many plant species on a global scale (Medlyn et al., 2001). However, the mechanisms mediating CO_2-induced stomatal closing are still much less understood than those triggered by ABA, and until recently no genetic mutants had been identified that robustly impaired high pCO_2-induced stomatal closing. CO_2-induced stomatal closure is mediated by $[Ca^{2+}]_{cyt}$ (Schwartz et al., 1988; Webb et al., 1996; Young et al., 2006). Moreover, the ABA-insensitive mutant *gca2* was identified as a first recessive mutation that shows a strong impairment in high CO_2-induced stomatal closing (Young et al., 2006) (Figure 3). Mutations in the *SLAC1* gene encoding a plasma membrane protein with similarity to a bacterial malate transporter exhibit impairment in CO_2-induced stomatal closing (Negi et al., 2008; Vahisalu et al., 2008). *SLAC1* is highly expressed in guard cells and localized at the plasma membrane (Negi et al., 2008; Vahisalu et al., 2008). SLAC1 shows homology to malate transporters and S-type anion currents are strongly impaired in *slac1* mutant alleles (Vahisalu et al., 2008) and consistent with this malate contents are significantly higher in guard cells of *slac1* null mutant compared to wild type guard cells (Negi et al., 2008). Thus *gca2* and *slac1* mutants represent first recessive CO_2-insensitive mutants that are strongly impaired in stomatal closing (Young et al., 2006; Negi et al., 2008; Vahisalu et al. 2008). These mutants are also impaired in their ABA response, suggesting that the encoded proteins function downstream of a convergence point between CO_2- and ABA-induced stomatal closing (Figure 3).

ABC (ATP-binding cassette) transporters are a large family of multi-spanning membrane proteins. An ABC transporter AtABCB14 was shown to mediate malate uptake when expressed in *E. coli* and HeLa cells (Lee et al., 2008). *AtABCB14* is predominantly expressed in guard cells and localized to the plasma membrane. *Atabcb14* mutant plants show slightly more rapid high CO_2-induced reduction in stomatal conductance (Lee et al., 2008). This study suggests that AtABCB14 functions during stomatal opening in mediating malate uptake into guard cells, which was released via anion channels during stomatal closing responses and possibly also from mesophyll cells. AtABCB14 is proposed to osmotically enhance stomatal opening and to remove extracellular malate which is known to enhance activation of anion channels (Lee et al., 2008).

The HT1 protein kinase was identified as a signaling component that mediates a strong negative regulation of CO_2-induced stomatal closing (Hashimoto et al., 2006). Thus *ht1* mutant alleles that show impaired HT1 kinase activity cause a constitutive high CO_2 stomatal closing response, even when CO_2 concentrations are clamped to low levels (Hashimoto et al., 2006) (Figure 3). *ht1* mutant alleles exhibit largely functional responses to blue light and ABA (Hashimoto et al., 2006). In this respect, it is interesting to note that the CO_2-hypersensitive response in *ht1* mutant alleles, continued to show blue light-induced stomatal opening and ABA-induced stomatal closing, even though the degree of stomatal responses differed quantitatively from wild type (Hashimoto et al., 2006), suggesting that HT1 functions as a central negative regulator of early CO_2 signaling (Figure 3). Note that cross talk occurs among stomatal movement responses and therefore any mutation that greatly impairs one pathway is expected to have a secondary effect on other responses. For example a blue-light insensitive mutant will show reduced stomatal opening under many conditions and therefore may be expected to quantitatively affect the degree of ABA-induced stomatal closing. Thus, the blue light receptors are presently not added as mechanisms to the primary ABA signaling cascade. Several genes that affect CO_2-regulated stomatal movements have been identified, but the CO_2 sensing mechanisms remain unknown, in spite of their importance for global plant gas exchange regulation in light of the continuing increase in atmospheric CO_2 (Medlyn et al., 2001).

The humidity response of stomata is interesting for a number of reasons: (i) Stomatal closure occurs very rapidly in response to a reduction in relative humidity. (ii) A previous study with the *abi1-1*, *abi2-1*, and *aba1* mutants suggests that humidity signaling is not ABA-dependent (Assmann et al., 2000). (iii) High humidity enhances the stomatal sensitivity to CO_2 (Talbott et al., 2003). Despite its role in stomatal movements, molecular components of the humidity signaling cascade largely remain unknown. Thermal imaging of leaf temperatures allows one to directly measure leaf surface temperature and thus identify mutants with altered stomatal behavior because plants with larger stomatal apertures have a lower leaf surface temperature due to a higher transpiration rate (Mustilli et al., 2002). In a forward genetic screen, mutants were screened for reduced stomatal closing in response to stepwise reductions in relative humidity of the air surrounding plants (Xie et al., 2006). Interestingly, *OST1* and *ABA2* were identified as positive modulators of relative humidity-controlled stomatal closure (Xie et al., 2006). This result suggests, in addition to a proposed ABA-independent pathway (Assmann et al., 2000), that humidity signaling is mediated by ABA biosynthesis and signaling, indicating a convergence between ABA and humidity signaling (Xie et al., 2006) (Figure 3). Further research on this response should be interesting to understand how plants sense humidity changes and then turn on the above signaling mechanisms.

Furthermore, it was recently shown that Arabidopsis *slac1* mutants are impaired in rapid stomatal closure when induced by low humidity, suggesting an important role of the SLAC1-associated anion channel in low humidity-induced stomatal closing (Vahisalu et al., 2008) (Figures 1, 2). The residual and slowed stomatal closing response in *slac1* plants may be due to R-type anion channels, which remained intact in *slac1* guard cells (Vahisalu et al., 2008) (Figures 1, 2). Thus initial insights have been obtained into genetic and physiological mechanisms that mediate low-humidity induced stomatal closing, but more information regarding this important response is needed and early humidity sensing mechanisms remain unknown.

ABA-INDUCED STOMATAL CLOSING

One of the best understood plant signaling networks is the one triggered by ABA in guard cells, causing stomatal closure. As shown in Figure 3, the ion channels which control this response and many genes encoding positive as well as negative regulators of guard cell ABA signaling have been identified in Arabidopsis. Recently, three proposed ABA receptor genes have been cloned from Arabidopsis, encoding the RNA-binding protein FCA, a Mg-chelatase H subunit, and the proposed G-protein coupled receptor GCR2, respectively (Razem et al., 2006; Shen et al., 2006; Liu et al., 2007b). The Mg-chelatase H subunit and GCR2 but not FCA were reported to function in ABA regulation of stomatal movements (Razem et al., 2006; Shen et al., 2006; Liu et al., 2007b), although the *GCR2* results could not be produced and have been questioned (Gao et al., 2007; Johnston et al., 2007; Liu et al., 2007a). In addition, a weak mutant allele in the Mg-chelatase subunit, *cch1* (Harper et al., 2004) shows strong recessive ABA insensitivity phenotypes in ABA-induced stomatal closing and in ABA inhibition of seed germination and root elongation (Shen et al., 2006). It is therefore surprising that mutations in this gene have not been identified in conventional forward genetic screens (Koornneef et al., 1984). In contrast to the *cch1* allele, known *gun5* mutant alleles in the same Mg-chelatase subunit gene did not affect ABA signaling (Shen et al., 2006). It is interesting to note, in contrast to other plant hormone receptors, that these three proposed ABA receptors do not share any primary structural features.

ABA-Regulated Calcium Signaling

ABA triggers cytosolic calcium ($[Ca^{2+}]_{cyt}$) increases (Figure 3) (McAinsh et al., 1990; Allan et al., 1994; Grabov and Blatt, 1998; Allen et al., 2001; Webb et al., 2001; Klusener et al., 2002; Li et al., 2004; Israelsson et al., 2006; Nomura et al., 2008). Cytosolic Ca^{2+} elevations in guard cells down-regulate both K^+_{in} channels (Schroeder and Hagiwara, 1989) and plasma membrane H^+-ATPases (Kinoshita et al., 1995), providing a mechanistic basis for ABA and Ca^{2+} inhibition of K^+ uptake during stomatal opening (Figure 1).

Hyperpolarization-activated Ca^{2+}-permeable "I_{Ca}" channels in the plasma membrane contribute to the increase in $[Ca^{2+}]_{cyt}$ (Hamilton et al., 2000; Pei et al., 2000; Kwak et al., 2003) (Figure 3). ABA was shown to activate these plasma membrane Ca^{2+} channels in both *Vicia faba* and Arabidopsis (Hamilton et al., 2000; Pei et al., 2000; Murata et al., 2001). Protein phosphorylation plays a role in the activation of Ca^{2+} channels (Kohler and Blatt, 2002). Two Ca^{2+}-dependent protein kinases, CPK3 and CPK6, are required for ABA activation of S-type anion and Ca^{2+}-permeable channels (Mori et al., 2006). Two other CDPKs, CPK4 and CPK11 also help mediate ABA responses (Zhu et al., 2007). In addition, Ca^{2+} channels are regulated by hydrogen peroxide leading to increases in $[Ca^{2+}]_{cyt}$ (Pei et al., 2000; Kohler et al., 2003; Kwak et al., 2003; Lemtiri-Chlieh and Berkowitz, 2004) (Figure 3). A hyperpolarization-activated Ca^{2+}-permeable current was also activated by cyclic AMP (Lemtiri-Chlieh and Berkowitz, 2004). Further research is needed to determine whether the cAMP-activated Ca^{2+} channels are the same channels as ABA-regulated I_{Ca} channels.

$[Ca^{2+}]_{cyt}$ elevations activate two different types of anion channels in the plasma membrane of guard cells: Slow-activating sustained (S-type; Schroeder and Hagiwara, 1989) and rapid transient (R-type; Hedrich et al., 1990; Roelfsema et al., 2004) anion channels. Both mediate anion release from guard cells, causing depolarization (Figure 1).

Early intracellular Ca^{2+} imaging studies showed that a significant number of guard cells do not show measurable $[Ca^{2+}]_{cyt}$ elevations in response to ABA (Schroeder and Hagiwara, 1990; Gilroy et al., 1991; McAinsh et al., 1992). These findings led to the model that both Ca^{2+}-dependent and Ca^{2+}-independent pathways mediate ABA responses (Allan et al., 1994). Based on cells showing no ABA-induced Ca^{2+} increases the Ca^{2+}-independent pathway has also been proposed and discussed in further studies (Allan et al., 1994; Ward et al., 1995; Romano et al., 2000; Levchenko et al., 2005; Marten et al., 2007b). However, the relative contribution of the proposed Ca^{2+}-independent pathway to ABA-induced stomatal closing has not yet been quantified, and questions of whether known ABA signaling mutations transduce or bypass this pathway remains unanswered. In addition, recent research on CO_2 and ABA signaling has led to the hypothesis that these physiological stomatal closing stimuli sensitize guard cells to cytosolic Ca^{2+} (Young et al., 2006; Yang et al., 2008). This proposed Ca^{2+} sensitivity priming might also mediate Ca^{2+}-dependent signaling at lower cytosolic Ca^{2+} levels (Israelsson et al., 2006). Further work, beyond the "negative" results from multiple laboratories that led to the Ca^{2+}-independent signaling model, is needed to characterize the Ca^{2+}-independent pathway.

In addition to promotion of stomatal closure, ABA also inhibits stomatal opening by negatively regulating the plasma membrane H^+-ATPases in a dose-dependent manner (Goh et al., 1996). Increases in $[Ca^{2+}]_{cyt}$ were shown to inhibit the ATP hydrolysis and H^+ pumping by plasma membrane H^+-ATPase, suggesting that $[Ca^{2+}]_{cyt}$ mediates this ABA inhibition of H^+-ATPase activity (Kinoshita et al., 1995). Increases in $[Ca^{2+}]_{cyt}$ also inhibit K^+_{in} channels and thus contribute to ABA inhibition of stomatal opening (Schroeder and Hagiwara, 1989; Wang et al., 1998).

Essential Function of Anion Channels in Stomatal Closing

Anion channels in the plasma membrane of guard cells were proposed to provide a central control mechanism for stomatal closing (Schroeder and Hagiwara, 1989). Genetic evidence for this model was recently obtained. *SLAC1* encodes a plasma membrane protein in Arabidopsis guard cells and is required for the function of S-type anion channels and for CO_2-, ABA- and ozone-induced stomatal closing (Negi et al., 2008; Vahisalu et al., 2008) (Figure 3). The SLAC1 protein has distant similarity to a bacterial malate transporter. Since S-type anion channels have a permeability to malate anions (Schmidt and Schroeder, 1994), and *slac1* mutants lack S-type anion currents in guard cells (Vahisalu et al., 2008), SLAC1 encodes a long sought subunit of these guard cell anion channels. *slac1* mutant alleles show residual stomatal closing responses to low-humidity, light-dark transitions and ABA, which correlates with the finding that R-type anion channels remain functional in *slac1* guard cells (Vahisalu et al., 2008). R-type channels are thus modeled to function in parallel to S-type anion channels (Keller et al., 1989; Schroeder and Keller, 1992) (Figures 1 and 3). *slac1* mutant alleles show greatly reduced responses in ABA-, CO_2-, low-humidity, ozone- and Ca^{2+}-induced stomatal closing (Negi et al., 2008; Vahisalu et al., 2008), supporting the model that S-type anion channels are major control mechanisms of stomatal closing (Schroeder and Hagiwara, 1989; Schroeder et al., 1993).

ABC transporters have been suggested to regulate S-type anion channels or to serve as the channel themselves (Leonhardt et al., 1997; Leonhardt et al., 1999). Two ABC transporters AtMRP4 and AtMRP5 were reported to have opposite regulatory roles in stomatal opening. Null mutations in *AtMRP4* resulted in enhanced stomatal opening in response to light, increased transpirational water loss, and enhanced drought sensitivity, indicating that AtMRP4 is a negative regulator of stomatal opening (Klein et al., 2004). Another ABC transporter, AtMRP5, is expressed in guard cells and localizes to the plasma membrane (Suh et al., 2007) (Figure 3). *Atmrp5-1* knockout mutants show reduced stomatal opening, in response to the light, when compared wild type plant responses (Klein et al., 2003). *Atmrp5-1* mutants also display other phenotypes consistent with the observed stomatal behavior, including a reduced transpiration rate, reduced water loss in detached leaves, enhanced drought tolerance, indicating that AtMRP5 positively regulates stomatal opening (Klein et al., 2003). Furthermore, the *atmrp5* null mutant shows reduced sensitivity to ABA in stomatal closing (Gaedeke et al., 2001; Klein et al., 2003). *atmrp5* gene disruption impairs Ca^{2+}-activated S-type anion channel currents (Suh et al., 2007). However, in contrast to *slac1* alleles, *atmrp5* mutations impair both S-type anion channel and Ca^{2+} channel regulation (Suh et al., 2007) (Figure 3), indicating that AtMRP5 may function as a transmembrane ion channel regulator, analogous to it's mammalian family members, the cystic fibrosis transmembrane conductance regulator (CFTR) and sulfonylurea receptor (SUR) (Ashcroft, 2006; Gadsby et al., 2006). Whether AtMRP5 directly interacts with ion channel proteins remains presently unknown.

Modulators of ABA Signaling Strength

The gene *ERA1* encodes a β-subunit of farnesyltransferase, an enzyme that modifies specific target proteins by attaching a hy-

drophobic prenyl (farnesyl) group to their C terminus. ERA1 is a negative regulator of ABA signaling (Cutler et al., 1996) and a deletion of *ERA1* causes hypersensitive ABA-induced stomatal closing and a reduced water loss of plants during drought stress (Pei et al., 1998). *era1* guard cells show ABA hypersensitive activation of S-type anion channels and I_{Ca} Ca^{2+} channels, suggesting that ERA1 negatively regulates early ABA signaling and ion channels (Pei et al., 1998; Allen et al., 2002) (Figure 3). Prenylation of target proteins leads to localization to the plasma membrane. Thus ERA1 may recruit negative regulators of ABA signaling to the plasma membrane of guard cells. Two small G proteins ROP10 and AtRac1 are putative targets of prenylation and show C-terminal sequences that may be prenylated. Interestingly, both small G proteins, ROP10 and AtRac1, are negative regulators of ABA signaling and stomatal closing (Lemichez et al., 2001; Zheng et al., 2002) (Figure 3). Transgenic Arabidopsis plants expressing a constitutively active mutant of AtRac1 showed an impaired ABA-induced actin reorganization and stomatal closure (Lemichez et al., 2001). The null *rop10* mutant shows enhanced ABA response in stomatal closure, and a dominant-negative mutant of ROP10 partially suppresses the *abi2-1* mutant phenotype in seed germination and root elongation (Zheng et al., 2002). Cytosolic Ca^{2+} also mediates ABA-triggered actin reorganization, which in turn activates mechanosensitive Ca^{2+} channels (Hwang and Lee, 2001; Zhang et al., 2007a).

The extracellular Ca^{2+} sensor CAS was cloned from Arabidopsis using a functional expression assay, combined with Ca^{2+} imaging, in HEK293 mammalian cells (Han et al., 2003). *CAS* encodes a plant-specific protein that has a single transmembrane domain. The C-terminal region of CAS shares a sequence similarity with a rhodanese-like domain (Pfam00581) that might be involved in protein-protein interactions, and several acidic amino acids reside in the N-terminus that was shown to bind to Ca^{2+} (Han et al., 2003). *CAS* is mainly expressed in aerial parts of plants including guard cells and mediates external Ca^{2+}-induced $[Ca^{2+}]_{cyt}$ increases by increasing IP3 concentration (Han et al., 2003; Tang et al., 2007; Nomura et al., 2008). In *cas* knockout mutants and *CAS* antisense plants, external Ca^{2+}-induced stomatal closure is impaired whereas *CAS* overexpression triggers stomatal closure in the absence of external Ca^{2+} (Han et al., 2003; Nomura et al., 2008). The cellular targeting of CAS is presently a matter of debate, since there are data indicating plasma membrane and/or thylakoid membrane localizations (Han et al., 2003; Nomura et al., 2008; Weinl et al., 2008) (Figure 3).

Phospholipids, phospholipid kinases, and phospholipid lipases function in guard cell ABA signaling. The phospholipase C (PLC) blocker U-73122 partially inhibits ABA-induced stomatal closure, suggesting that PLC positively regulates ABA signaling (Staxen et al., 1999). Transgenic tobacco with reduced levels of PLC showed a reduction in ABA inhibition of stomatal opening, further indicating that PLC is a positive regulator of the signaling cascade (Hunt et al., 2003) (Figure 3). Phospholipase PLDα1 was reported to also function as a positive regulator by producing phophatidic acid (PA) that binds to and inhibits the negative regulator PP2C ABI1 (Zhang et al., 2004a) (Figure 3). While ABI1 binding of PA would stimulate ABA promotion of stomatal closure, PA and PLDα1 interact with the GPA1 subunit (see below) to mediate ABA inhibition of stomatal opening, indicating a dual function of PA (Mishra et al., 2006). *Pldα1* knock out mutants were reported to be disrupted in ABA-induced stomatal closing, and this disruption was alleviated in *abi1* and *plda1* double knockouts, suggesting that PLDα1 is not required for ABA responses in the absence of ABI1. The strong suppressor effect of *abi1* knockout on the *pldα1* phenotype appears interesting and unique, since many close homologues to the ABI1 protein phosphatase have been shown to have similar functions to ABI1 and are co-expressed in guard cells (Leonhardt et al., 2004; Saez et al., 2004; Kuhn et al., 2006; Saez et al., 2006; Yoshida et al., 2006b). Thus further investigation of the reported interaction of PLDα1 and ABI1 would be interesting.

Phosphatidylinositol 3- and 4-phosphate, sphingosine-1-phosphate (S1P), and phytosphingosine-1-phosphate (phyS1P) are additional phospholipids that positively regulate ABA signaling, indicating that PI3 kinase, PI4 kinase, and sphingosine kinase are positive effectors of the ABA signaling cascade (Ng et al., 2001; Jung et al., 2002; Coursol et al., 2003; Coursol et al., 2005). In the *gpa1* mutant, S1P-induced stomatal closure and S-type anion channel activation are impaired, suggesting that S1P and phyS1P function upstream of GPA1 (Coursol et al., 2003). A pharmacological study combined with calcium imaging analysis suggests that PI3P and PI4P function upstream of $[Ca^{2+}]_{cyt}$ increases (Jung et al., 2002).

Genetic evidence suggests that the heterotrimeric G protein α subunit (GPA1) mediates ABA signaling. Both ABA inhibition of inward K+ currents and stomatal opening are reduced by the *gpa1-1* and *gpa1-2* T-DNA insertional mutations (Wang et al., 2001) (Figure 3). GPA1 physically interacts with GCR1, a G protein coupled receptor (Pandey and Assmann, 2004). In contrast to the *gpa1* mutants, *gcr1* mutant guard cells are hypersensitive to ABA and S1P, suggesting that GCR1 is a negative regulator of this ABA response (Pandey and Assmann, 2004). Analysis of ABA responses in seed germination, compared to guard cells, show differential effects of G protein mutants. Both *gpa1* and *gcr1* mutants show ABA hypersensitivity in seed germination and early seedling development, indicating that GPA1 negatively regulates this signaling pathway (Pandey et al., 2006). Thus G proteins affect ABA signaling and modulate ABA responses in a cell- and tissue-specific manner (Pandey et al., 2006; Pandey et al., 2007).

Reactive Oxygen Signaling Mechanisms in Guard Cells

Reactive oxygen species (ROS) and the gas nitric oxide (NO) function as second messengers in guard cell ABA signaling. ABA induces ROS production in guard cells (Pei et al., 2000; Zhang et al., 2001). ROS lead to activation of hyperpolarization-activated Ca^{2+} channels and stomatal closure (Pei et al., 2000; Murata et al., 2001). NADPH oxidases are multi spanning membrane proteins that produce extracellular superoxide (Keller et al., 1998; Sagi and Fluhr, 2001; Sagi and Fluhr, 2006). Guard cell microarray experiments showed that of the 10 NADPH oxidase genes in the Arabidopsis genome, AtRbohD and AtRbohF are highly expressed in guard cells (Kwak et al., 2003). Analysis of *AtrbohD/F* double knock out mutant guard cells, showed that these NADPH oxidases contribute to ABA-triggered ROS production, and function in ABA-activation of plasma membrane Ca^{2+} channels, and ABA-induced stomatal closure (Kwak et al., 2003) (Figure 3). Compared to the mammalian counterpart, one of the unique structural features of plant NADPH oxidases is that there are two EF-

hand Ca^{2+} binding domains in their N-terminus, suggesting the regulation of the protein by Ca^{2+} (Keller et al., 1998; Torres et al., 1998). Plant NADPH oxidases are activated by Ca^{2+} (Sagi and Fluhr, 2001; Ogasawara et al., 2008). Furthermore, NADPH oxidases appear to be positively regulated by protein kinases (Kobayashi et al., 2007; Nuhse et al., 2007; Ogasawara et al., 2008).

The ethylene receptor ETR1 was reported to mediate ethylene- and H_2O_2-induced stomatal closure, and AtrbohF functions in this pathway (Desikan et al., 2005; Desikan et al., 2006), indicating a signaling convergence between ABA and ethylene signaling pathways. However, ethylene was also shown to counteract and delay ABA-induced stomatal closure (Tanaka et al., 2005). Thus further research would be helpful to illuminate these apparently counter-acting physiological ethylene responses in stomatal regulation.

Hydrogen peroxide also functions in ABA inhibition of blue light-induced H^+ pumping by triggering dephosphorylation of H^+-ATPase in the plasma membrane (Zhang et al., 2004b). Hydrogen peroxide was reported to inhibit ATP hydrolysis by the plasma membrane H^+-ATPase and blue light-induced phosphorylation of the H^+-ATPase, whereas the cellular H_2O_2 scavenger ascorbate partially rescues ABA inhibition of H^+ pumping, indicating that H_2O_2 is another molecular player mediating ABA inhibition of stomatal closure (Zhang et al., 2004b). Cytoplasmic alkalization and PI3P appear to function upstream of ROS, whereas ABA-induced NO production and stomatal closure rely on ABA-triggered ROS production (Park et al., 2003; Suhita et al., 2004; Bright et al., 2006). NO triggers Ca^{2+} release from intracellular stores contributing to an increase in the cytosolic Ca^{2+} concentration (Figure 3), resulting in inhibition of K^+_{in} channels and activation of anion channels (Garcia-Mata et al., 2003). The glutathione peroxidase AtGPX3 physically interacts with ABI1 and ABI2 and contributes to the regulation of redox states in guard cells (Miao et al., 2006) (Figure 3). In addition, the redox state of the H_2O_2 scavenger ascorbate plays a role in stomatal movements. Overexpression of dehydroascorbate reductase results in increased ascorbate reduction, a lower H_2O_2 level in guard cells, and increased stomatal conductance and water transpiration (Chen and Gallie, 2004). Together, these studies show a key role for ROS in modulating the mechanisms that mediate stomatal closure, including Ca^{2+} channel activation (Pei et al., 2000; Murata et al., 2001; Kwak et al., 2003; Kwak et al., 2006), H^+ pump inhibition (Zhang et al., 2004b), and, via NO production, K^+_{in} channel de-activation and anion channel activation (Garcia-Mata et al., 2003).

Protein Kinases and Phosphatases in ABA Signaling

Protein de/phosphorylation plays a key regulatory role in guard cell ABA signaling. The dominant ABA insensitive *abi1-1* and *abi2-1* loci were isolated (Koornneef et al., 1984) and identified as closely related type 2C protein phosphatases (Leung et al., 1994; Meyer et al., 1994; Leung et al., 1997; Rodriguez et al., 1998). Hydrogen peroxide inhibits the activities of the negative regulatory type 2C protein phosphatases ABI1 and ABI2 (Meinhard and Grill, 2001; Meinhard et al., 2002). Localization of the mutant abi1-1 protein into the nucleus is required for the ABA-insensitive response (Moes et al., 2008). When the nuclear localization sequence in abi1-1 is disrupted, the transcription of genes regulated by ABA is rescued to wild-type levels, thus, suggesting that abi1-1 functions as a hypermorphic allele (Moes et al., 2008). Two additional PP2Cs, HAB1/AtP2C-HAand AtPP2CA, were identified to function as negative regulators of ABA signaling (Leonhardt et al., 2004; Kuhn et al., 2006; Yoshida et al., 2006b) (Figure 3). Moreover, the stomatal response to ABA is enhanced when the two negatively regulating PP2C ABI1 and HAB1 are inactivated simultaneously (Saez et al., 2006). The dominant mutation *abi1-1* and *abi2-1* impair ABA activation of I_{Ca} Ca^{2+} channels (Murata et al., 2001) and S-type anion channels (Pei et al., 1997). ABI2 physically interacts with the SOS2 protein kinase, and this interaction is disrupted by the *abi2-1* mutation (Ohta et al., 2003) (Figure 3). Note that no pharmacological inhibitors for PP2Cs are known. Therefore, the dominant natures of the *abi1-1* and *abi2-1* alleles (Koornneef et al., 1984) do not allow to distinguish whether the phenotypes of these mutants occur through direct protein-protein interactions in early ABA signaling, or via less direct, possibly neomorphic or hypermorphic modulation of the early ABA signaling pathway (For detailed previous discussions, see (Pei et al., 1997; Murata et al., 2001; Yoshida et al., 2006b). A recent study showed that ABI1 physically interacts with the OST1/SnRK2E protein kinase, and ABA activation of OST1/SRK2E is inhibited in *abi1-1* but not in *abi2-1* mutants (Yoshida et al., 2006a). The PKS3 kinase interacts with the calcium binding protein ScaBP5/CBL1 and the PP2C ABI2 and ABI1, and RNAi-mediated silencing of ScaBP5 causes ABA hypersensitivity (Guo et al., 2002) (Figure 3).

In addition to PP2Cs, type2A protein phosphatases function in ABA signaling. The *RCN1* gene encoding the PP2A regulatory subunit A is a positive regulator of the ABA signaling cascade in guard cells and seed germination (Kwak et al., 2002). RCN1 was previously shown to increase PP2A enzyme activity (Deruère et al., 1999). T-DNA insertional mutation in *RCN1* causes impairment in both ABA-induced stomatal closure and ABA activation of anion channels, a reduced sensitivity of ABA-triggered cytosolic Ca^{2+} increases, ABA-insensitive gene expression, and ABA-insensitive seed germination (Kwak et al., 2002) (Figure 3). Furthermore, the PP2A inhibitor okadaic acid phenocopies the rcn1 phenotypes in wild type plants (Kwak et al., 2002).

The putative protein tyrosine phosphatase gene *PHS1* was also suggested to negatively regulate ABA signaling (Quettier et al., 2006), suggesting a role for protein tyrosine phosphatases in stomatal movements (MacRobbie, 2002).

mRNA Metabolism and ABA Signaling

Transcriptional and post-transcriptional regulation provides cellular mechanisms through which ABA signaling is modulated. For reviews on transcription factors that function in ABA signaling, readers are referred to the following reviews (Giraudat, 1995; Finkelstein et al., 2002; Zhu, 2002; Kuhn and Schroeder, 2003; Yamaguchi-Shinozaki and Shinozaki, 2006) Mutations in either of the nuclear mRNA cap binding protein subunits *ABH1* and *CBP20* confer ABA hypersensitivity in Arabidopsis, indicating that specific mRNA processing events may be needed for expression of negative regulators of ABA signaling (Hugouvieux et al., 2001; Papp et al., 2004) (Figure 3). Expression of the negative regulator AtPP2CA is greatly reduced in *abh1* mutants (Hugouvieux et al.,

2001) and *atpp2ca* mutants were identified in two independent forward genetic screens for causing ABA hypersensitivity (Kuhn et al., 2006; Yoshida et al., 2006b). Further analyses of *AtPP2CA* transcripts in *abh1* showed that this effect can contribute to the *abh1* phenotype, but detailed analyses also demonstrated that additional mechanisms are required for generating the ABA hypersensitivity of *abh1* (Kuhn et al., 2006). The recent finding that the nuclear mRNA CAP binding proteins, including ABH1, function in generating small non-coding RNAs, may provide further insights into mechanisms by which *abh1* causes ABA hypersensitivity (Gregory et al., 2008). Other mRNA binding proteins, for which mutations cause ABA hypersensitivity, include the dsRNA-binding protein HYL1, the poly(A)-specific RNase AHG2/AtPARN, and an Sm-like protein (Lu and Fedoroff, 2000; Xiong et al., 2001; Gregory et al., 2008). These results imply that RNA processing and turnover is utilized by plant cells to regulate ABA signaling or that rate-limiting genes that function in ABA signaling are affected in these mRNA processing mutants (for more details, see Kuhn and Schroeder, 2003).

Another guard cell-expressed RNA-binding protein GRP7 functions in mRNA export to the cytoplasm and positively regulates ABA-induced stomatal closure (Kim et al., 2008) (Figure 3). The AP2 type AtERF7 interacts with the PKS3 kinase and a putative transcription repressor AtSin3 to negatively regulate ABA signaling (Song et al., 2005). Studies with transgenic plants showed that over-expression of the MYB44 transcription factor enhances ABA sensitivity in stomatal closure (Jung et al., 2008) (Figure 3). Transgenic plants overexpressing *MYB44* have reduced levels of negatively acting PP2Cs including *ABI1*, *ABI2*, *AtPP2CA*, *HAB1*, and *HAB2* (Jung et al., 2008). Two calcium-dependent protein kinase genes *CPK4* and *CPK11* were also reported to positively regulate ABA signaling possibly through ABF1 and ABF4 transcription factors (Zhu et al., 2007) (Figure 3).

The syntaxin protein OSM1/SYP61 and the RING finger E3 ligase protein SDIR1 were also found to positively control guard cell ABA signaling (Zhu et al., 2002; Zhang et al., 2007b).

CONCLUSIONS AND FUTURE OUTLOOK

Many molecular players of guard cell and ABA signaling have been identified, suggesting a complex ABA signaling network in guard cells. Some mechanisms are central to ABA signaling and stomatal closing, such as the different classes of guard cell ion channels and H^+ pumps (Ward et al., 1995; Kinoshita and Shimazaki, 1999; Pei et al., 2000), the OST1 kinase (Mustilli et al., 2002; Yoshida et al., 2002), the SLAC1 anion channel membrane protein (Negi et al., 2008; Vahisalu et al., 2008), cytosolic Ca^{2+} (Schroeder and Hagiwara, 1989; McAinsh et al., 1990; Mori et al., 2006; Zhu et al., 2007) and as negative regulators, the clade of PP2Cs that include ABI1 and ABI2. These data indicate that there are signaling branches within ABA signaling, but that there are also essential signaling nodes, including the above mechanisms (Hetherington and Woodward, 2003). Thus it appears likely that some mechanisms more directly mediate ABA signaling, and other mechanisms may be peripheral modulators. However, more research is needed to distinguish such mechanisms. An important aspect in this respect is the detailed quantification of the degree of ABA-induced stomatal closing that occurs in each experimental treatment, which can be best analyzed in "blind" analyses of stomatal signaling (Murata et al., 2001; Mori et al., 2006). Moreover, many genes and mechanisms are waiting to be identified, including the mechanisms that mediate CO_2 sensing, humidity sensing as well as understanding the diverse proposed ABA receptors.

Guard cells use a complex signaling network to create a "graded binary" output that can readily be observed under the microscope: stomatal 'opening' or 'closing'. The study of guard cell signaling provides insights into how the many cellular processes assemble together to create a quantifiable single cell output. Thus, identification and characterization of genes that are highly and specifically expressed in guard cells using various approaches, including gene trap screens and cell-type specific microarray analyses (Leonhardt et al., 2004; Galbiati et al., 2008; Yang et al., 2008), would help to provide a further understanding of guard cell, ABA and blue light signal transduction networks. Furthermore, dynamic modeling and computational simulations of ABA signaling as done by Li et al. (2006) could lead to comprehensive and quantitative information on ABA signaling in guard cells.

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References

Allan, A.C., Fricker, M.D., Ward, J.L., Beale, M.H., and Trewavas, A.J. (1994). Two transduction pathways mediate rapid effects of abscisic acid in Commelina guard cells. Plant Cell **6:** 1319-1328.

Allen, G.J., and Sanders, D. (1996). Control of ionic currents guard cell vacuoles by cytosolic and luminal calcium. Plant J. **10,** 1055-1069.

Allen, G.J., Murata, Y., Chu, S.P., Nafisi, M., and Schroeder, J.I. (2002). Hypersensitivity of abscisic acid-induced cytosolic calcium increases in the Arabidopsis farnesyltransferase mutant *era1-2*. Plant Cell **14:** 1649-1662.

Allen, G.J., Kwak, J.M., Chu, S.P., Llopis, J., Tsien, R.Y., Harper, J.F., and Schroeder, J.I. (1999). Cameleon calcium indicator reports cytoplasmic calcium dynamics in Arabidopsis guard cells. Plant J. **19:** 735-747.

Allen, G.J., Chu, S.P., Harrington, C.L., Schumacher, K., Hoffmann, T., Tang, Y.Y., Grill, E., and Schroeder, J.I. (2001). A defined range of guard cell calcium oscillation parameters encodes stomatal movements. Nature **411:** 1053-1057.

Anderson, J.A., Huprikar, S.S., Kochian, L.V., Lucas, W.J., and Gaber, R.F. (1992). Functional expression of a probable *Arabidopsis thaliana* potassium channel in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **89:** 3736-3740.

Ashcroft, F.M. (2006). From molecule to malady. Nature **440,** 440-447.

Assmann, S.M., Simoncini, L., and Schroeder, J.I. (1985). Blue light activates electrogenic ion pumping in guard cell protoplasts of *Vicia faba*. Nature **318:** 285-287.

Assmann, S.M., Snyder, J.A., and Lee, J.Y.-R. (2000). ABA-deficient (*aba1*)

and ABA-insensitive (*abi1-1*, *abi2-1*) mutants of Arabidopsis have a wild-type stomatal response to humidity. Plant Cell Environ **23,** 387-395.

Baum, G., Long, J.C., Jenkins, G.I., and Trewavas, A.J. (1999). Stimulation of the blue light phototropic receptor NPH1 causes a transient increase in cytosolic Ca^{2+}. Proc. Natl. Acad. Sci. USA **96:** 13554-13559.

Bewell, M.A., Maathuis, F.J., Allen, G.J., and Sanders, D. (1999). Calcium-induced calcium release mediated by a voltage-activated cation channel in vacuolar vesicles from red beet. FEBS Lett. **458:** 41-44.

Bihler, H., Eing, C., Hebeisen, S., Roller, A., Czempinski, K., and Bertl, A. (2005). TPK1 is a vacuolar ion channel different from the slow-vacuolar cation channel. Plant Physiol. **139:** 417-424.

Blatt, M.R. (1992). K^+ channels of stomatal guard cells - Characteristics of the inward-rectifier and its control by pH. J. Gen. Physiol. **99:** 615-644.

Blatt, M.R., and 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.

Bright, J., Desikan, R., Hancock, J.T., Weir, I.S., and Neill, S.J. (2006). ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H2O2 synthesis. Plant J. **45:** 113-122.

Chen, X., Lin, W.H., Wang, Y., Luan, S., and Xue, H.W. (2008). An inositol polyphosphate 5-phosphatase functions in PHOTOTROPIN1 signaling in Arabidopis by altering cytosolic Ca^{2+}. Plant Cell **20:** 353-366.

Chen, Z., and Gallie, D.R. (2004). The ascorbic acid redox state controls guard cell signaling and stomatal movement. Plant Cell **16:** 1143-1162.

Cominelli, E., Galbiati, M., Vavasseur, A., Conti, L., Sala, T., Vuylsteke, M., Leonhardt, N., Dellaporta, S.L., and Tonelli, C. (2005). A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance. Curr. Biol. **15:** 1196-1200.

Coursol, S., Fan, L.M., Le Stunff, H., Spiegel, S., Gilroy, S., and Assmann, S.M. (2003). Sphingolipid signalling in Arabidopsis guard cells involves heterotrimeric G proteins. Nature **423:** 651-654.

Coursol, S., Le Stunff, H., Lynch, D.V., Gilroy, S., Assmann, S.M., and Spiegel, S. (2005). Arabidopsis sphingosine kinase and the effects of phytosphingosine-1-phosphate on stomatal aperture. Plant Physiol. **137:** 724-737.

Cutler, S., Ghassemian, M., Bonetta, D., Cooney, S., and McCourt, P. (1996). A protein farnesyl transferase involved in abscisic acid signal transduction in Arabidopsis. Science **273:** 1239-1241.

De Angeli, A., Monachello, D., Ephritikhine, G., Frachisse, J.M., Thomine, S., Gambale, F., and Barbier-Brygoo, H. (2006). The nitrate/proton antiporter AtCLCa mediates nitrate accumulation in plant vacuoles. Nature **442:** 939-942.

Deruère, J., Jackson, K., Garbers, C., Söll, D., and DeLong, A. (1999). The *RCN1*-encoded A subunit of protein phosphatase 2A increases phosphatase activity *in vivo*. Plant J. **20:** 389-399.

Desikan, R., Hancock, J.T., Bright, J., Harrison, J., Weir, I., Hooley, R., and Neill, S.J. (2005). A role for ETR1 in hydrogen peroxide signaling in stomatal guard cells. Plant Physiol. **137:** 831-834.

Desikan, R., Last, K., Harrett-Williams, R., Tagliavia, C., Harter, K., Hooley, R., Hancock, J.T., and Neill, S.J. (2006). Ethylene-induced stomatal closure in Arabidopsis occurs via AtrbohF-mediated hydrogen peroxide synthesis. Plant J. **47:** 907-916.

Emi, T., Kinoshita, T., Sakamoto, K., Mineyuki, Y., and Shimazaki, K. (2005). Isolation of a protein interacting with Vfphot1a in guard cells of Vicia faba. Plant Physiol. **138:** 1615-1626.

Finkelstein, R.R., Gampala, S.S.L., and Rock, C.D. (2002). Abscisic acid signaling in seeds and seedlings. Plant Cell **14:** S15-S45.

Gadsby, D.C., Vergani, P., and Csanady, L. (2006). The ABC protein turned chloride channel whose failure causes cystic fibrosis. Nature **440:** 477-483.

Gaedeke, N., Klein, M., Kolukisaoglu, U., Forestier, C., Muller, A., Ansorge, M., Becker, D., Mamnun, Y., Kuchler, K., Schulz, B., Mueller-Roeber, B., and Martinoia, E. (2001). The *Arabidopsis thaliana* ABC transporter AtMRP5 controls root development and stomata movement. EMBO J. **20:** 1875-1887.

Galbiati, M., Simoni, L., Pavesi, G., Cominelli, E., Francia, P., Vavasseur, A., Nelson, T., Bevan, M., and Tonelli, C. (2008). Gene trap lines identify Arabidopsis genes expressed in stomatal guard cells. Plant J. **53:** 750-762.

Gao, Y., Zeng, Q., Guo, J., Cheng, J., Ellis, B.E., and Chen, J.G. (2007). Genetic characterization reveals no role for the reported ABA receptor, GCR2, in ABA control of seed germination and early seedling development in Arabidopsis. Plant J. **52:** 1001-1013.

Garcia-Mata, C., Gay, R., Sokolovski, S., Hills, A., Lamattina, L., and Blatt, M.R. (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.

Gilroy S., Fricker, M.D., Read, N.D., Trewavas, A.J. (1991). Role of calcium in signal transduction of Commelina guard cells. Plant Cell **3:** 333-344.

Giraudat, J. (1995). Abscisic acid signaling. Curr. Opin. in Cell Biol. **7:** 232-238.

Gobert, A., Isayenkov, S., Voelker, C., Czempinski, K., and Maathuis, F.J. (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.

Goh, C.-H., Kinoshita, T., Oku, T., and Shimazaki, K.-I. (1996). Inhibition of blue light-dependent H^+ pumping by abscisic acid in Vicia guard-cell protoplasts. Plant Physiol. **111:** 433-440.

Grabov, A., and Blatt, M.R. (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.

Gregory, B.D., O'Malley, R.C., Lister, R., Urich, M.A., Tonti-Filippini, J., Chen, H., Millar, A.H., and Ecker, J.R. (2008). A link between RNA metabolism and silencing affecting Arabidopsis development. Dev. Cell **14:** 854-866.

Guo, F.Q., Young, J., and Crawford, N.M. (2003). The nitrate transporter AtNRT1.1 (CHL1) functions in stomatal opening and contributes to drought susceptibility in Arabidopsis. Plant Cell **15:** 107-117.

Guo, Y., Xiong, L., Song, C.-P., Gong, D., Halfter, U., and Zhu, J.-K. (2002). A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signaling in Arabidopsis. Dev. Cell **3:** 233-244.

Hamilton, D.W.A., Hills, A., Kohler, B., and Blatt, M.R. (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.

Hafke, J.B., Hafke, Y., Smith, J.A., Luttge, U., and Thiel, G. (2003) Vacuolar malate uptake is mediated by an anion-selective inware rectifier. Plant J. **35:** 116-128.

Han, S., Tang, R., Anderson, L.K., Woerner, T.E., and Pei, Z.M. (2003). A cell surface receptor mediates extracellular Ca^{2+} sensing in guard cells. Nature **425:** 196-200.

Harada, A., Sakai, T., and Okada, K. (2003). phot1 and phot2 mediate blue light-induced transient increases in cytosolic Ca^{2+} differently in Arabidopsis leaves. Proc. Natl. Acad. Sci. USA **100:** 8583-8588.

Harper, A.L., von Gesjen, S.E., Linford, A.S., Peterson, M.P., Faircloth, R.S., Thissen, M.M., and Brusslan, J.A. (2004). Chlorophyllide a oxygenase mRNA and protein levels correlate with the chlorophyll a/b ratio in *Arabidopsis thaliana*. Photosynth. Res. **79:** 149-159.

Hashimoto, M., Negi, J., Young, J., Israelsson, M., Schroeder, J.I., and Iba, K. (2006). Arabidopsis HT1 kinase controls stomatal movements in response to CO_2. Nat. Cell Biol. **8:** 391-397.

Hedrich, R., and Neher, E. (1987). Cytoplasmic calcium regulates voltage-

dependent ion channels in plant vacuoles. Nature **329:** 833-836.

Hedrich, R., Flugge, U.I., and Fernandez, J.M. (1986). Patch-clamp studies of ion transport in isolated plant vacuoles. FEBS Lett. **204:** 228-232.

Hedrich, R., Busch, H., and Raschke, K. (1990). Ca^{2+} and nucleotide dependent regulation of voltage dependent anion channels in the plasma membrane of guard cells. EMBO J. **9:** 3889-3892.

Hetherington, A.M., and Woodward, F.I. (2003). The role of stomata in sensing and driving environmental change. Nature **424:** 901-908.

Homann, U., and Thiel, G. (2002). The number of K^+ channels in the plasma membrane of guard cell protoplasts changes in parallel with the surface area. Proc. Natl. Acad. Sci. USA **99:** 10215-10220.

Hosy, E., Vavasseur, A., Mouline, K., Dreyer, I., Gaymard, F., Poree, F., Boucherez, J., Lebaudy, A., Bouchez, D., Very, A.A., Simonneau, T., Thibaud, J.B., and 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.

Hugouvieux, V., Kwak, J.M., and Schroeder, J.I. (2001). An mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in Arabidopsis. Cell **106:** 477-487.

Hunt, L., Mills, L.N., Pical, C., Leckie, C.P., Aitken, F.L., Kopka, J., Mueller-Roeber, B., McAinsh, M.R., Hetherington, A.M., and Gray, J.E. (2003). Phospholipase C is required for the control of stomatal aperture by ABA. Plant J. **34:** 47-55.

Hwang, J.U., and Lee, Y. (2001). Abscisic acid-induced actin reorganization in guard cells of dayflower is mediated by cytosolic calcium levels and by protein kinase and protein phosphatase activities. Plant Physiol. **125:** 2120-2128.

Ilan, N., Schwartz, A., and Moran, N. (1994). External pH effects on the depolarization-activated K^+ channels in guard cell protoplasts of *Vicia faba*. J. Gen. Physiol. **103:** 807-831.

Inada, S., Ohgishi, M., Mayama, T., Okada, K., and Sakai, T. (2004). RPT2 is a signal transducer involved in phototropic response and stomatal opening by association with phototropin 1 in *Arabidopsis thaliana*. Plant Cell **16:** 887-896.

Israelsson, M., Siegel, R.S., Young, J., Hashimoto, M., Iba, K., and Schroeder, J.I. (2006). Guard cell ABA and CO_2 signaling network updates and Ca^{2+} sensor priming hypothesis. Curr. Opin. Plant Biol. **9:** 654-663.

Jeon, B.W., Hwang, J.U., Hwang, Y., Song, W.Y., Fu, Y., Gu, Y., Bao, F., Cho, D., Kwak, J.M., Yang, Z., and Lee, Y. (2008). The Arabidopsis small G protein ROP2 is activated by light in guard cells and inhibits light-induced stomatal opening. Plant Cell **20:** 75-87.

Johnston, C.A., Temple, B.R., Chen, J.G., Gao, Y., Moriyama, E.N., Jones, A.M., Siderovski, D.P., and Willard, F.S. (2007). Comment on "A G protein coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid". Science **318:** 914; author reply 914.

Jung, C., Seo, J.S., Han, S.W., Koo, Y.J., Kim, C.H., Song, S.I., Nahm, B.H., Choi, Y.D., and Cheong, J.J. (2008). Overexpression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic Arabidopsis. Plant Physiol. **146:** 623-635.

Jung, J.Y., Kim, Y.W., Kwak, J.M., Hwang, J.U., Young, J., Schroeder, J.I., Hwang, I., and Lee, Y. (2002). Phosphatidylinositol 3- and 4-phosphate are required for normal stomatal movements. Plant Cell **14,** 2399-2412.

Keller, B.U., Hedrich, R., and Raschke, K. (1989). Voltage-dependent anion channels in the plasma membrane of guard cells. Nature **341:** 450-453.

Keller, T., Damude, H.G., Werner, D., Doerner, P., Dixon, R.A., and Lamb, C. (1998). A plant homolog of the neutrophil NADPH oxidase $gp91^{phox}$ subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs. Plant Cell **10,** 255-266.

Kim, J.S., Jung, H.J., Lee, H.J., Kim, K.A., Goh, C.H., Woo, Y., Oh, S.H., Han, Y.S., and Kang, H. (2008). Glycine-rich RNA-binding protein7 affects abiotic stress responses by regulating stomata opening and closing in *Arabidopsis thaliana*. Plant J. **55:** 455-466.

Kinoshita, T., and Shimazaki, K. (1999). Blue light activates the plasma membrane H^+-ATPase by phosphorylation of the C-terminus in stomatal guard cells. EMBO J. **18:** 5548-5558.

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

Kinoshita, T., Doi, M., Suetsugu, N., Kagawa, T., Wada, M., and Shimazaki, K. (2001). phot1 and phot2 mediate blue light regulation of stomatal opening. Nature **414:** 656-660.

Kinoshita, T., Emi, T., Tominaga, M., Sakamoto, K., Shigenaga, A., Doi, M., and Shimazaki, K. (2003). Blue-light- and phosphorylation-dependent binding of a 14-3-3 protein to phototropins in stomatal guard cells of broad bean. Plant Physiol. **133:** 1453-1463.

Klein, M., Perfus-Barbeoch, L., Frelet, A., Gaedeke, N., Reinhardt, D., Mueller-Roeber, B., Martinoia, E., and Forestier, C. (2003). The plant multidrug resistance ABC transporter AtMRP5 is involved in guard cell hormonal signalling and water use. Plant J. **33:** 119-129.

Klein, M., Geisler, M., Suh, S.J., Kolukisaoglu, H.U., Azevedo, L., Plaza, S., Curtis, M.D., Richter, A., Weder, B., Schulz, B., and Martinoia, E. (2004). Disruption of AtMRP4, a guard cell plasma membrane ABCC-type ABC transporter, leads to deregulation of stomatal opening and increased drought susceptibility. Plant J. **39:** 219-236.

Klüsener, B., Young, J.J., Murata, Y., Allen, G.J., Mori, I.C., Hugouvieux, V., and Schroeder, J.I. (2002). Convergence of calcium signaling pathways of pathogenic elicitors and abscisic acid in Arabidopsis guard cells. Plant Physiol. **130:** 2152-2163.

Kobayashi, M., Ohura, I., Kawakita, K., Yokota, N., Fujiwara, M., Shimamoto, K., Doke, N., and Yoshioka, H. (2007). Calcium-dependent protein kinases regulate the production of reactive oxygen species by potato NADPH oxidase. Plant Cell **19:** 1065-1080.

Köhler, B., and Blatt, M.R. (2002). Protein phosphorylation activates the guard cell Ca^{2+} channel and is a prerequisite for gating by abscisic acid. Plant J. **32:** 185-194.

Köhler, B., Hills, A., and Blatt, M.R. (2003). Control of guard cell ion channels by hydrogen peroxide and abscisic acid indicates their action through alternate signaling pathways. Plant Physiol. **131:** 385-388.

Koornneef, M., Reuling, G., and Karssen, C.M. (1984). The isolation of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. Physiol. Plant. **61:** 377-383.

Kovermann, P., Meyer, S., Hortensteiner, S., Picco, C., Scholz-Starke, J., Ravera, S., Lee, Y., and Martinoia, E. (2007). The Arabidopsis vacuolar malate channel is a member of the ALMT family. Plant J. **52:** 1169-1180.

Kuhn, J.M., and Schroeder, J.I. (2003). Impacts of altered RNA metabolism on abscisic acid signaling. Curr. Opin. Plant Biol. **6:** 463-469.

Kuhn, J.M., Boisson-Dernier, A., Dizon, M.B., Maktabi, M.H., and Schroeder, J.I. (2006). The protein phosphatase AtPP2CA negatively regulates abscisic acid signal transduction in Arabidopsis, and effects of *abh1* on AtPP2CA mRNA. Plant Physiol. **140:** 127-139.

Kwak, J.M., Nguyen, V., and Schroeder, J.I. (2006). The role of reactive oxygen species in hormonal responses. Plant Physiol. **141:** 323-329.

Kwak, J.M., Moon, J.H., Murata, Y., Kuchitsu, K., Leonhardt, N., DeLong, A., and Schroeder, J.I. (2002). Disruption of a guard cell-expressed protein phosphatase 2A regulatory subunit, RCN1, confers abscisic acid insensitivity in Arabidopsis. Plant Cell **14:** 2849-2861.

Kwak, J.M., Murata, Y., Baizabal-Aguirre, V.M., Merrill, J., Wang, M., Kemper, A., Hawke, S.D., Tallman, G., and Schroeder, J.I. (2001). Dominant negative guard cell K^+ channel mutants reduce inward-rectifying K^+ currents and light-induced stomatal opening in *Arabidopsis*. Plant Physiol. **127:** 473-485.

Kwak, J.M., Mori, I.C., Pei, Z.M., Leonhardt, N., Torres, M.A., Dangl, J.L.,

Bloom, R.E., Bodde, S., Jones, J.D., and Schroeder, J.I. (2003). NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in Arabidopsis. EMBO J. **22:** 2623-2633.

Latz, A., Becker, D., Hekman, M., Muller, T., Beyhl, D., Marten, I., Eing, C., Fischer, A., Dunkel, M., Bertl, A., Rapp, U.R., and Hedrich, R. (2007). TPK1, a Ca^{2+}-regulated Arabidopsis vacuole two-pore K^+ channel is activated by 14-3-3 proteins. Plant J. **52:** 449-459.

Lebaudy, A., Vavasseur, A., Hosy, E., Dreyer, I., Leonhardt, N., Thibaud, J.B., Very, A.A., Simonneau, T., and Sentenac, H. (2008). Plant adaptation to fluctuating environment and biomass production are strongly dependent on guard cell potassium channels. Proc. Natl. Acad. Sci. USA **105:** 5271-5276.

Lee, M., Choi, Y., Burla, B., Kim, Y.Y., Jeon, B., Maeshima, M., Yoo, J.Y., Martinoia, E., and Lee, Y. (2008) The ABC transporter AtABCB14 is a malate importer and modulates stomatal responses to CO_2. Nat. Cell Biol. **10:** 1217-1223

Lee, Y., Kim, Y.W., Jeon, B.W., Park, K.Y., Suh, S.J., Seo, J., Kwak, J.M., Martinoia, E., and Hwang, I. (2007). Phosphatidylinositol 4,5-bisphosphate is important for stomatal opening. Plant J. **52:** 803-816.

Lemichez, E., Wu, Y., Sanchez, J.P., Mettouchi, A., Mathur, J., and Chua, N.H. (2001). Inactivation of AtRac1 by abscisic acid is essential for stomatal closure. Genes Dev. **15:** 1808-1816.

Lemtiri-Chlieh, F., and Berkowitz, G.A. (2004). Cyclic adenosine monophosphate regulates calcium channels in the plasma membrane of Arabidopsis leaf guard and mesophyll cells. J. Biol. Chem. **279:** 35306-35312.

Lemtiri-Chlieh, F., MacRobbie, E.A., Webb, A.A., Manison, N.F., Brownlee, C., Skepper, J.N., Chen, J., Prestwich, G.D., and Brearley, C.A. (2003). Inositol hexakisphosphate mobilizes an endomembrane store of calcium in guard cells. Proc. Natl. Acad. Sci. USA **100:** 10091-10095.

Leonhardt, N., Vavasseur, A., and Forestier, C. (1999). ATP binding cassette modulators control abscisic acid-regulated slow anion channels in guard cells. Plant Cell **11:** 1141-1151.

Leonhardt, N., Marin, E., Vavasseur, A., and Forestier, C. (1997). Evidence for the existence of a sulfonylurea-receptor-like protein in plants: Modulation of stomatal movements and guard cell potassium channels by sulfonylureas and potassium channel openers. Proc. Natl. Acad. Sci. USA **94:** 14156-14161.

Leonhardt, N., Kwak, J.M., Robert, N., Waner, D., Leonhardt, G., and Schroeder, J.I. (2004). Microarray expression analyses of Arabidopsis guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. Plant Cell **16:** 596-615.

Leung, J., Merlot, S., and Giraudat, J. (1997). The Arabidopsis *ABSCISIC ACID-INSENSITIVE 2* (*ABI2*) and *ABI1* genes encode homologous protein phosphatases 2C involved in abscisic acid signal transduction. Plant Cell **9:** 759-771.

Leung, J., Bouvier-Durand, M., Morris, P.-C., Guerrier, D., Chefdor, F., and Giraudat, J. (1994). Arabidopsis ABA response gene *ABI1*: Features of a calcium-modulated protein phosphatase. Science **264:** 1448-1452.

Levchenko, V., Konrad, K.R., Dietrich, P., Roelfsema, M.R., and Hedrich, R. (2005). Cytosolic abscisic acid activates guard cell anion channels without preceding Ca^{2+} signals. Proc. Natl. Acad. Sci. USA **102:** 4203-4208.

Leyman, B., Geelen, D., Quintero, F.J., and Blatt, M.R. (1999). A tobacco syntaxin with a role in hormonal control of guard cell ion channels. Science **283:** 537-540.

Li, J., Wang, X.-Q., Watson, M.B., and Assmann, S.M. (2000). Regulation of abscisic acid-induced stomatal closure and anion channels by guard cell AAPK kinase. Science **287:** 300-303.

Li, S., Assmann, S.M., and Albert, R. (2006). Predicting essential components of signal transduction networks: a dynamic model of guard cell abscisic acid signaling. PLoS Biol. **4:** e312.

Li, Y., Wang, G.-X., Xin, M., Yang, H.-M., Wu, X.-J., and Li, T. (2004). The parameters of guard cell calcium oscillation encodes stomatal oscillation and closure in *Vicia faba*. Plant Sci. **166:** 415-421.

Liang, Y.K., Dubos, C., Dodd, I.C., Holroyd, G.H., Hetherington, A.M., and Campbell, M.M. (2005). AtMYB61, an R2R3-MYB transcription factor controlling stomatal aperture in Arabidopsis thaliana. Curr. Biol. **15:** 1201-1206.

Liu, K., Fu, H., Bei, Q., and Luan, S. (2000). Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. Plant Physiol. **124:** 1315-1326.

Liu, X., Yue, Y., Li, W., and Ma, L. (2007a). Response to comment on "A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid". Science **318:** 914d.

Liu, X., Yue, Y., Li, B., Nie, Y., Li, W., Wu, W.H., and Ma, L. (2007b). A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid. Science **315:** 1712-1716.

Lu, C., and Fedoroff, N. (2000). A mutation in the Arabidopsis *HYL1* gene encoding a dsRNA binding protein affects responses to abscisic acid, auxin, and cytokinin. Plant Cell **12:** 2351-2366.

MacRobbie, E.A. (2002). Evidence for a role for protein tyrosine phosphatase in the control of ion release from the guard cell vacuole in stomatal closure. Proc. Natl. Acad. Sci. USA **99:** 11963-11968.

MacRobbie, E.A.C. (1995). ABA-induced ion efflux in stomatal guard cells: Multiple actions of ABA inside and outside the cell. Plant J. **7:** 565-576.

MacRobbie, E.A.C. (1998). Signal transduction and ion channels in guard cells. Phil. Trans. Roy. Soc. London **1374:** 1475-1488.

Mao, J., Zhang, Y.C., Sang, Y., Li, Q.H., and Yang, H.Q. (2005). A role for Arabidopsis cryptochromes and COP1 in the regulation of stomatal opening. Proc. Natl. Acad. Sci. USA **102:** 12270-12275.

Marten, H., Hedrich, R., and Roelfsema, M.R. (2007a). Blue light inhibits guard cell plasma membrane anion channels in a phototropin-dependent manner. Plant J. **50:** 29-39.

Marten, H., Konrad, K.R., Dietrich, P., Roelfsema, M.R., and Hedrich, R. (2007b). Ca2+-dependent and -independent abscisic acid activation of plasma membrane anion channels in guard cells of Nicotiana tabacum. Plant Physiol. **143:** 28-37.

McAinsh, M.R., Brownlee, C., and Hetherington, A.M. (1990). Abscisic acid-induced elevation of guard cell cytosolic Ca^{2+} precedes stomatal closure. Nature **343:** 186-188.

McAinsh, M.R., Brownlee, C., and Hetherington, A.M. (1992). Visualizing changes in cytosolic-free Ca^{2+} during the response of stomatal guard cells to abscisic acid. Plant Cell **4:** 1113-1122.

Medlyn, B.E., Barton, C.V., Broadmeadow, M.S.J., Ceulemans, R., De Angelis, P., Forstreuter, M., Freeman, M., Jackson, S.B., Kellomaki, S., Laitat, E., Rey, A., Roberntz, P., Sigurdsson, B.D., Strassemeyer, J., Wang, K., Curtis, P.S., and Jarvis, P.G. (2001). Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis. New Phytol. **149:** 247-264.

Meinhard, M., and Grill, E. (2001). Hydrogen peroxide is a regulator of ABI1, a protein phosphatase 2C from Arabidopsis. FEBS Lett. **508:** 443-446.

Meinhard, M., Rodriguez, P.L., and Grill, E. (2002). The sensitivity of ABI2 to hydrogen peroxide links the abscisic acid-response regulator to redox signalling. Planta **214:** 775-782.

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

Meyer, K., Leube, M.P., and Grill, E. (1994). A protein phosphatase 2C involved in ABA signal transduction in *Arabidopsis thaliana*. Science **264:** 1452-1455.

Miao, Y., Lv, D., Wang, P., Wang, X.C., Chen, J., Miao, C., and Song, C.P. (2006). An Arabidopsis glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress re-

sponses. Plant Cell **18:** 2749-2766.

Miedema, H., and Assmann, S.M. (1996). A membrane-delimited effect of internal pH on the K^+ outward rectifier of Vicia faba guard cells. J. Membr. Biol. **154:** 227-237.

Mishra, G., Zhang, W., Deng, F., Zhao, J., and Wang, X. (2006). A bifurcating pathway directs abscisic acid effects on stomatal closure and opening in Arabidopsis. Science **312:** 264-266.

Moes, D., Himmelbach, A., Korte, A., Haberer, G., and Grill, E. (2008). Nuclear localization of the mutant protein phosphatase abi1 is required for insensitivity towards ABA responses in Arabidopsis. Plant J. **54:** 806-819.

Mori, I.C., Murata, Y., Yang, Y., Munemasa, S., Wang, Y.F., Andreoli, S., Tiriac, H., Alonso, J.M., Harper, J.F., Ecker, J.R., Kwak, J.M., and Schroeder, J.I. (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:** e327.

Murata, Y., Pei, Z.-M., Mori, I.C., and Schroeder, J.I. (2001). Abscisic acid activation of plasma membrane Ca^{2+} channels in guard cells requires cytosolic NAD(P)H and is differentially disrupted upstream and downstream of reactive oxygen species production in *abi1-1* and *abi2-1* protein phosphatase 2C mutants. Plant Cell **13:** 2513-2523.

Mustilli, A.-C., Merlot, S., Vavasseur, A., Fenzi, F., and Giraudat, J. (2002). Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. Plant Cell **14:** 3089-3099.

Nakamura, R.L., McKendree, W.L., Hirsch, R.E., Sedbrook, J.C., Gaber, R.F., and Sussman, M.R. (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., and Iba, K. (2008). CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. Nature **452:** 483-486.

Ng, C.K., Carr, K., McAinsh, M.R., Powell, B., and Hetherington, A.M. (2001). Drought-induced guard cell signal transduction involves sphingosine-1-phosphate. Nature **410:** 596-599.

Nomura, H., Komori, T., Kobori, M., Nakahira, Y., and Shiina, T. (2008). Evidence for chloroplast control of external Ca^{2+}-induced cytosolic Ca^{2+} transients and stomatal closure. Plant J. **53:** 988-998.

Nuhse, T.S., Bottrill, A.R., Jones, A.M., and Peck, S.C. (2007). Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses. Plant J. **51:** 931-940.

Ogasawara, Y., Kaya, H., Hiraoka, G., Yumoto, F., Kimura, S., Kadota, Y., Hishinuma, H., Senzaki, E., Yamagoe, S., Nagata, K., Nara, M., Suzuki, K., Tanokura, M., and Kuchitsu, K. (2008). Synergistic activation of the Arabidopsis NADPH oxidase AtrbohD by Ca^{2+} and phosphorylation. J. Biol. Chem. **283:** 8885-8892.

Ohta, M., Guo, Y., Halfter, U., and Zhu, J.K. (2003). A novel domain in the protein kinase SOS2 mediates interaction with the protein phosphatase 2C ABI2. Proc. Natl. Acad. Sci. USA **100:** 11771-11776.

Padmanaban, S., Chanroj, S., Kwak, J.M., Li, X., Ward, J.M., and Sze, H. (2007). Participation of endomembrane cation/H^+ exchanger AtCHX20 in osmoregulation of guard cells. Plant Physiol. **144:** 82-93.

Pandey, S., and Assmann, S.M. (2004). The Arabidopsis putative G protein-coupled receptor GCR1 interacts with the G protein alpha subunit GPA1 and regulates abscisic acid signaling. Plant Cell **16:** 1616-1632.

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

Pandey, S., Chen, J.G., Jones, A.M., and Assmann, S.M. (2006). G-protein complex mutants are hypersensitive to abscisic acid regulation of germination and postgermination development. Plant Physiol. **141:** 243-256.

Papp, I., Mur, L.A., Dalmadi, A., Dulai, S., and Koncz, C. (2004). A mutation in the Cap Binding Protein 20 gene confers drought tolerance to Arabidopsis. Plant Mol. Biol. **55:** 679-686.

Park, K.Y., Jung, J.Y., Park, J., Hwang, J.U., Kim, Y.W., Hwang, I., and Lee, Y. (2003). A role for phosphatidylinositol 3-phosphate in abscisic acid-induced reactive oxygen species generation in guard cells. Plant Physiol. **132:** 92-98.

Pei, Z.-M., and Kuchitsu, K. (2005). Early ABA signaling events in guard cells. J. Plant Growth Regul. **24:** 296-307.

Pei, Z.-M., Kuchitsu, K., Ward, J.M., Schwarz, M., and Schroeder, J.I. (1997). Differential abscisic acid regulation of guard cell slow anion channels in *Arabdiopsis* wild-type and *abi1* and *abi2* mutants. Plant Cell **9:** 409-423.

Pei, Z.-M., Ghassemian, M., Kwak, C.M., McCourt, P., and Schroeder, J.I. (1998). Role of farnesyltransferase in ABA regulation of guard cell anion channels and plant water loss. Science **282:** 287-290.

Pei, Z.M., Ward, J.M., and Schroeder, J.I. (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.

Pei, Z.M., Ward, J.M., Harper, J.F., and Schroeder, J.I. (1996). A novel chloride channel in *Vicia faba* guard cell vacuoles activated by the serine/threonine kinase CDPK. EMBO J. **15:** 6564-6574.

Pei, Z.M., Murata, Y., Benning, G., Thomine, S., Klusener, B., Allen, G.J., Grill, E., and Schroeder, J.I. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature **406:** 731-734.

Peiter, E., Maathuis, F.J., Mills, L.N., Knight, H., Pelloux, J., Hetherington, A.M., and 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, J.B., and 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.

Pottosin, I.I., Tikhonova, L.I., Hedrich, R., and Schönknecht, G. (1997). Slowly activating vaculoar channels can not mediate Ca^{2+}-induced Ca^{2+} release. Plant J. **12:** 1387-1398.

Quettier, A.L., Bertrand, C., Habricot, Y., Miginiac, E., Agnes, C., Jeannette, E., and Maldiney, R. (2006). The *phs1-3* mutation in a putative dual-specificity protein tyrosine phosphatase gene provokes hypersensitive responses to abscisic acid in *Arabidopsis thaliana*. Plant J. **47:** 711-719.

Ranf, S., Wunnenberg, P., Lee, J., Becker, D., Dunkel, M., Hedrich, R., Scheel, D., and Dietrich, P. (2008). Loss of the vacuolar cation channel, AtTPC1, does not impair Ca^{2+} signals induced by abiotic and biotic stresses. Plant J. **53:** 287-299.

Razem, F.A., El-Kereamy, A., Abrams, S.R., and Hill, R.D. (2006). The RNA-binding protein FCA is an abscisic acid receptor. Nature **439:** 290-294.

Rodriguez, P.L., Benning, G., and Grill, E. (1998). ABI2, a second protein phosphatase 2C involved in abscisic acid signal transduction in Arabidopsis. FEBS Lett. **421:** 185-190.

Roelfsema, M.R., and Hedrich, R. (2005). In the light of stomatal opening: new insights into 'the Watergate'. New Phytol. **167:** 665-691.

Roelfsema, M.R., Levchenko, V., and Hedrich, R. (2004). ABA depolarizes guard cells in intact plants, through a transient activation of R- and S-type anion channels. Plant J. **37:** 578-588.

Romano, L.A., Jacob, T., Gilroy, S., and Assmann, S.M. (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.

Saez, A., Robert, N., Maktabi, M.H., Schroeder, J.I., Serrano, R., and Rodriguez, P.L. (2006). Enhancement of abscisic acid sensitivity and re-

duction of water consumption in Arabidopsis by combined inactivation of the protein phosphatases type 2C ABI1 and HAB1. Plant Physiol. **141:** 1389-1399.

Saez, A., Apostolova, N., Gonzalez-Guzman, M., Gonzalez-Garcia, M.P., Nicolas, C., Lorenzo, O., and Rodriguez, P.L. (2004). Gain-of-function and loss-of-function phenotypes of the protein phosphatase 2C HAB1 reveal its role as a negative regulator of abscisic acid signalling. Plant J. **37:** 354-369.

Sagi, M., and Fluhr, R. (2001). Superoxide production by plant homologues of the gp91^{phox} NADPH oxidase. Modulation of activity by calcium and by tobacco mosaic virus infection. Plant Physiol. **126:** 1281-1290.

Sagi, M., and Fluhr, R. (2006). Production of reactive oxygen species by plant NADPH oxidases. Plant Physiol. **141:** 336-340.

Sakai, T., Wada, T., Ishiguro, S., and Okada, K. (2000). RPT2. A signal transducer of the phototropic response in Arabidopsis. Plant Cell **12:** 225-236.

Schachtman, D., Schroeder, J.I., Lucas, W.J., Anderson, J.A., and Gaber, R.F. (1992). Expression of an inward-rectifying potassium channel by the Arabidopsis *KAT1* cDNA. Science **258:** 1654-1658.

Schmidt, C., and Schroeder, J.I. (1994). Anion-selectivity of slow anion channels in *Vicia faba* guard cells: Large nitrate permeability. Plant Physiol. **106:** 383-391.

Schroeder, J.I., and Hagiwara, S. (1989). Cytosolic calcium regulates ion channels in the plasma membrane of *Vicia faba* guard cells. Nature **338:** 427-430.

Schroeder, J.I., Hagiwara, S. (1990). Repetitive increases in cytoslic Ca^{2+} of guard cells by abscisic acid activation of non-selective Ca^{2+}-permeable channels. Proc. Natl. Acad. Sci. USA **87:** 9305-9309.

Schroeder, J.I., and Hedrich, R. (1989). Involvement of ion channels and active transport in osmoregulation and signaling of higher plant cells. Trends Biochem. Sci. **14:** 187-192.

Schroeder, J.I., and Keller, B.U. (1992). Two types of anion channel currents in guard cells with distinct voltage regulation. Proc. Natl. Acad. Sci. USA **89:** 5025-5029.

Schroeder, J.I., Hedrich, R., and Fernandez, J.M. (1984). Potassium-selective single channels in guard cell protoplasts of *Vicia faba*. Nature **312:** 361-362.

Schroeder, J.I., Raschke, K., and Neher, E. (1987). Voltage dependence of K^+ channels in guard cell protoplasts. Proc. Natl. Acad. Sci. USA **84:** 4108-4112.

Schroeder, J.I., Schmidt, R.C., and Sheaffer, J. (1993). Identification of high-affinity slow anion channel blockers and evidence for stomatal regulation by slow anion channels in guard cells. Plant Cell **5:** 1831-1841.

Schroeder, J.I., Allen, G.J., Hugouvieux, V., Kwak, J.M., and Waner, D. (2001). Guard cell signal transduction. Annu. Rev. Plant Physiol. Plant Mol. Biol. **52:** 627-658.

Schwartz, A., Ilan, N., and Grantz, D.A. (1988). Ca^{2+} modified stomatal responses to light, KCl and CO_2. Plant Physiol. **87:** 583-587.

Sentenac, H., Bonneaud, N., Minet, M., Lacroute, F., Salmon, J.M., Gaymard, F., and Grignon, C. (1992). Cloning and expression in yeast of a plant potassium ion transport system. Science **256:** 663-665.

Shen, Y.Y., Wang, X.F., Wu, F.Q., Du, S.Y., Cao, Z., Shang, Y., Wang, X.L., Peng, C.C., Yu, X.C., Zhu, S.Y., Fan, R.C., Xu, Y.H., and Zhang, D.P. (2006). The Mg-chelatase H subunit is an abscisic acid receptor. Nature **443:** 823-826.

Shimazaki, K., Iino, M., and Zeiger, E. (1986). Blue light-dependent proton extrusion by guard-cell protoplasts of *Vicia faba*. Nature **319:** 324-326.

Shimazaki, K., Doi, M., Assmann, S.M., and Kinoshita, T. (2007). Light regulation of stomatal movement. Annu. Rev. Plant. Biol. **58:** 219-247.

Slocum, R.D., and Flores, H.E. (1991). Biochemistry and Physiology of Polyamines in Plants. (Boca Raton, FL: CRC Press).

Sokolovski, S., Hills, A., Gay, R., Garcia-Mata, C., Lamattina, L., and Blatt, M.R. (2005). Protein phosphorylation is a prerequisite for intracellular Ca^{2+} release and ion channel control by nitric oxide and abscisic acid in guard cells. Plant J. **43:** 520-529.

Song, C.P., Agarwal, M., Ohta, M., Guo, Y., Halfter, U., Wang, P., and Zhu, J.K. (2005). Role of an Arabidopsis AP2/EREBP-type transcriptional repressor in abscisic acid and drought stress responses. Plant Cell **17:** 2384-2396.

Staxen, I., Pical, C., Montgomery, L.T., Gray, J.E., Hetherington, A.M., and McAinsh, M.R. (1999). Abscisic acid induces oscillations in guard-cell cytosolic free calcium that involve phosphoinositide-specific phospholipase C. Proc. Natl. Acad. Sci. USA **96:** 1779-1784.

Suh, S.J., Wang, Y.F., Frelet, A., Leonhardt, N., Klein, M., Forestier, C., Mueller-Roeber, B., Cho, M.H., Martinoia, E., and Schroeder, J.I. (2007). The ATP binding cassette transporter AtMRP5 modulates anion and calcium channel activities in Arabidopsis guard cells. J. Biol. Chem. **282:** 1916-1924.

Suhita, D., Raghavendra, A.S., Kwak, J.M., and Vavasseur, A. (2004). Cytoplasmic alkalization precedes reactive oxygen species production during methyl jasmonate- and abscisic acid-induced stomatal closure. Plant Physiol. **134:** 1536-1545.

Sutter, J.U., Campanoni, P., Tyrrell, M., and Blatt, M.R. (2006). Selective mobility and sensitivity to SNAREs is exhibited by the Arabidopsis KAT1 K^+ channel at the plasma membrane. Plant Cell **18:** 935-954.

Sutter, J.U., Sieben, C., Hartel, A., Eisenach, C., Thiel, G., and Blatt, M.R. (2007). Abscisic acid triggers the endocytosis of the arabidopsis KAT1 K^+ channel and its recycling to the plasma membrane. Curr. Biol. **17:** 1396-1402.

Sze, H., Padmanaban, S., Cellier, F., Honys, D., Cheng, N.H., Bock, K.W., Conejero, G., Li, X., Twell, D., Ward, J.M., and Hirschi, K.D. (2004). Expression patterns of a novel AtCHX gene family highlight potential roles in osmotic adjustment and K+ homeostasis in pollen development. Plant Physiol. **136:** 2532-2547.

Takemiya, A., Kinoshita, T., Asanuma, M., and Shimazaki, K. (2006). Protein phosphatase 1 positively regulates stomatal opening in response to blue light in Vicia faba. Proc. Natl. Acad. Sci. USA **103:** 13549-13554.

Talbott, L.D., Rahveh, E., and Zeiger, E. (2003). Relative humidity is a key factor in the acclimation of the stomatal response to CO_2. J. Exp. Bot. **54:** 2141-2147.

Tanaka, Y., Sano, T., Tamaoki, M., Nakajima, N., Kondo, N., and Hasezawa, S. (2005). Ethylene inhibits abscisic acid-induced stomatal closure in Arabidopsis. Plant Physiol. **138:** 2337-2343.

Tang, R.H., Han, S., Zheng, H., Cook, C.W., Choi, C.S., Woerner, T.E., Jackson, R.B., and Pei, Z.M. (2007). Coupling diurnal cytosolic Ca^{2+} oscillations to the CAS-IP3 pathway in Arabidopsis. Science **315:** 1423-1426.

Torres, M.A., Onouchi, H., Hamada, S., Machida, C., Hammond-Kosack, K.E., and Jones, J.D.G. (1998). Six *Arabidopsis thaliana* homologues of the human respiratory burst oxidase (gp91^{phox}). Plant J. **14:** 365-370.

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

Wang, X.-Q., Ullah, H., Jones, A.M., and Assmann, S.M. (2001). G protein regulation of ion channels and abscisic acid signaling in *Arabidopsis* guard cells. Science **292:** 2070-2072.

Wang, X.Q., Wu, W.H., and Assmann, S.M. (1998). Differential responses of abaxial and adaxial guard cells of broad bean to abscisic acid and calcium. Plant Physiol. **118:** 1421-1429.

Ward, J.M., and Schroeder, J.I. (1994). Calcium-activated K^+ channels and calcium-induced calcium release by slow vacuolar ion channels in

guard cell vacuoles implicated in the control of stomatal closure. Plant Cell **6:** 669-683.

Ward, J.M., Pei, Z.-M., and Schroeder, J.I. (1995). Roles of ion channels in initiation of signal transduction in higher plants. Plant Cell **7:** 833-844.

Webb, A.A., Larman, M.G., Montgomery, L.T., Taylor, J.E., and Hetherington, A.M. (2001). The role of calcium in ABA-induced gene expression and stomatal movements. Plant J. **26:** 351-362.

Webb, A.A.R., McAinsh, M.R., Mansfield, T.A., and Hetherington, A.M. (1996). Carbon dioxide induces increases in guard cell cytosolic free calcium. Plant J. **9:** 297-304.

Weinl, S., Held, K., Schlucking, K., Steinhorst, L., Kuhlgert, S., Hippler, M., and Kudla, J. (2008). A plastid protein crucial for Ca^{2+}-regulated stomatal responses. New Phytol. **179:** 675-686.

Xie, X., Wang, Y., Williamson, L., Holroyd, G.H., Tagliavia, C., Murchie, E., Theobald, J., Knight, M.R., Davies, W.J., Leyser, H.M., and Hetherington, A.M. (2006). The identification of genes involved in the stomatal response to reduced atmospheric relative humidity. Curr. Biol. **16:** 882-887.

Xiong, L., Gong, Z., Rock, C.D., Subramanian, S., Guo, Y., Xu, W., Galbraith, D., and Zhu, J.-K. (2001). Modulation of abscisic acid signal transduction and biosynthesis by an Sm-like protein in Arabidopsis. Dev. Cell **1:** 771-781.

Yamaguchi-Shinozaki, K., and Shinozaki, K. (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu. Rev. Plant Biol. **57:** 781-803.

Yang, Y., Costa, A., Leonhardt, N., Siegel, R.S., and Schroeder, J.I. (2008). Isolation of a strong Arabidopsis guard cell promoter and its potential as a research tool. Plant Methods **4:** 6.

Yoshida, R., Umezawa, T., Mizoguchi, T., Takahashi, S., Takahashi, F., and Shinozaki, K. (2006a). The regulatory domain of SRK2E/OST1/SnRK2.6 interacts with ABI1 and integrates abscisic acid (ABA) and osmotic stress signals controlling stomatal closure in Arabidopsis. J. Biol. Chem. **281:** 5310-5318.

Yoshida, R., Hobo, T., Ichimura, K., Mizoguchi, T., Takahashi, F., Aronso, J., Ecker, J.R., and Shinozaki, K. (2002). ABA-activated *SnRK2* protein kinase is required for dehydration stress signaling in Arabidopsis. Plant Cell Physiol. **43:** 1473-1483.

Yoshida, T., Nishimura, N., Kitahata, N., Kuromori, T., Ito, T., Asami, T., Shinozaki, K., and Hirayama, T. (2006b). ABA-hypersensitive germination3 encodes a protein phosphatase 2C (AtPP2CA) that strongly regulates abscisic acid signaling during germination among Arabidopsis protein phosphatase 2Cs. Plant Physiol. **140:** 115-126.

Young, J.J., Mehta, S., Israelsson, M., Godoski, J., Grill, E., and Schroeder, J.I. (2006). CO_2 signaling in guard cells: calcium sensitivity response modulation, a Ca^{2+}-independent phase, and CO2 insensitivity of the *gca2* mutant. Proc. Natl. Acad. Sci. USA **103:** 7506-7511.

Zhang, W., Fan, L.M., and Wu, W.H. (2007a). Osmo-sensitive and stretch-activated calcium-permeable channels in *Vicia faba* guard cells are regulated by actin dynamics. Plant Physiol. **143:** 1140-1151.

Zhang, W., Qin, C., Zhao, J., and Wang, X. (2004a). Phospholipase D alpha 1-derived phosphatidic acid interacts with ABI1 phosphatase 2C and regulates abscisic acid signaling. Proc. Natl. Acad. Sci. USA **101:** 9508-9513.

Zhang, X., Zhang, L., Dong, F., Gao, J., Galbraith, D.W., and Song, C.P. (2001). Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in Vicia faba. Plant Physiol. **126:** 1438-1448.

Zhang, X., Wang, H., Takemiya, A., Song, C.P., Kinoshita, T., and Shimazaki, K. (2004b). Inhibition of blue light-dependent H^+ pumping by abscisic acid through hydrogen peroxide-induced dephosphorylation of the plasma membrane H^+-ATPase in guard cell protoplasts. Plant Physiol. **136:** 4150-4158.

Zhang, Y., Yang, C., Li, Y., Zheng, N., Chen, H., Zhao, Q., Gao, T., Guo, H., and Xie, Q. (2007b). SDIR1 is a RING finger E3 ligase that positively regulates stress-responsive abscisic acid signaling in Arabidopsis. Plant Cell **19:** 1912-1929.

Zhao, R., Dielen, V., Kinet, J.M., and Boutry, M. (2000). Cosuppression of a plasma membrane H^+-ATPase isoform impairs sucrose translocation, stomatal opening, plant growth, and male fertility. Plant Cell **12:** 535-546.

Zheng, Z.L., Nafisi, M., Tam, A., Li, H., Crowell, D.N., Chary, S.N., Schroeder, J.I., Shen, J., and Yang, Z. (2002). Plasma membrane-associated ROP10 small GTPase is a specific negative regulator of abscisic acid responses in Arabidopsis. Plant Cell **14:** 2787-2797.

Zhu, J., Gong, Z., Zhang, C., Song, C.P., Damsz, B., Inan, G., Koiwa, H., Zhu, J.K., Hasegawa, P.M., and Bressan, R.A. (2002). OSM1/SYP61: a syntaxin protein in Arabidopsis controls abscisic acid-mediated and non-abscisic acid-mediated responses to abiotic stress. Plant Cell **14:** 3009-3028.

Zhu, J.-K. (2002). Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. **53:** 247-273.

Zhu, S.Y., Yu, X.C., Wang, X.J., Zhao, R., Li, Y., Fan, R.C., Shang, Y., Du, S.Y., Wang, X.F., Wu, F.Q., Xu, Y.H., Zhang, X.Y., and Zhang, D.P. (2007). Two calcium-dependent protein kinases, CPK4 and CPK11, regulate abscisic acid signal transduction in Arabidopsis. Plant Cell **19:** 3019-3036.