Front. Biol. 2012, 7(2): 96–112
DOI 10.1007/s11515-012-1193-3

REVIEW

The stomata frontline of plant interaction with the environment-perspectives from hormone regulation

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Abstract **Plants have evolved elaborate mechanisms to perceive and integrate signals from various environmental conditions. On leaf surface, stomata formed by pairs of guard cells mediate gas exchange, water transpiration as well as function in response to abiotic and biotic stresses. Stomatal closure could be induced by drought, salt, pathogen and other adverse conditions. This constitutes an instant defense response to prevent further damage to plants. Abscisic acid (ABA) is a major plant hormone involved in stress responses. Stress-activated ABA synthesis causes stomatal closure and prevents opening to reduce water loss and cell dehydration. Key regulatory receptor complex and other important components in the ABA signaling pathway have been identified. However, our knowledge of ABA signal transduction in guard cells is far from complete. Jasmonates are a group of phytohormones generally known to be important for plant defense against insects and necrotrophic pathogens. The increased levels of methyl jasmonate (MeJA) induced by herbivory and pathogen invasion show a similar effect on stomatal movement associated with ROS production as ABA. Investigation of guard cell signaling networks involving the two important phytohormones is significant and exciting. Information about protein and metabolite components and how they interact in guard cells is lacking. Here we review recent advances on hormone signaling networks in guard cells and how the networks integrate environmental signals to plant physiological output.**

Keywords stomata, guard cells, hormone, signaling, molecular networks

Introduction

The importance of water to terrestrial plants can never be over emphasized just by simply looking at the molecular composition of a typical plant cell. Water makes up a large portion of the cell volume, directly participates in essential biochemical reactions and physiological processes. However, land plants are constantly challenged by dehydration, which led to the evolution of vascular plant structures, including an extensive root system to extract water from the soil, a low resistance pathway through the xylem for water transport, a hydrophobic cuticle covering the leaf surface to reduce evaporation, and microscopic stomatal structure to control gas exchange and transpiration (Taiz and Zeiger, 2006). The need for water conservation and carbon dioxide (CO_2) uptake poses a dilemma for plants, especially when the two processes are controlled by the same structure, stomata on leaf surface. On one hand, plants need ready access to the atmosphere to obtain CO_2 for photosynthesis, a process of organic carbon production for growth, development and yield. On the other hand, the large leaf surface area aggravates the problem of water loss and dehydration through stomatal transpiration. To survive, plants must delicately modulate stomatal movement to balance water conservation and carbon sequestration, especially under the current challenges of climate change and global warming.

Stomata are microscopic pores located in greater numbers on the lower side of the leaf, serving as the outward water gate and inward CO_2 valve. The evolution of stomata could be retrospected to over 400 million years ago (Hetherington and Woodward, 2003). Considering the change of atmospheric

Received October 24, 2011; accepted November 24, 2011
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components, i.e., CO_2 concentration increase and emergence of new plant groups such as ferns and angiosperms, guard cells are showing considerable morphological diversity. The two major distinctive types include dumbbell-shaped guard cells typically found in grasses and a few monocots, whereas the kidney-shaped guard cells are commonly seen in dicotyledonous plants and non-grass monocots. It is noteworthy that the subsidiary cells are often absent in the species with kidney-shaped guard cells, in which case the guard cells are surrounded by ordinary epidermal cells. One of the guard cell structural features includes the specialized alignment of cellulose microfibrils, which contributes to its function. The microfibrils are arranged radically from the pore, making the cell girth reinforced like a steel-belted radial tire. This organization of microfibrils offers the least resistance when guard cells curve outward during stomatal opening (Taiz and Zeiger, 2006). In addition, multiple ion channels, such as K^+ inward- and outward-rectifying channels, Ca^{2+} channels and anion channels distributed on the plasma membrane and vacuole membrane are coordinated to regulate the ion influx and efflux, thus the 'turgidity' of guard cells (Assmann, 1993). Furthermore, previous studies that focused on guard cells metabolism and response to environmental signals have revealed important features of functional differentiation of guard cells (Assmann, 1993; Vavasseur and Raghavendra, 2005; Zhu et al., 2009). Compared to mesophyll cells, guard cells contain fewer chloroplasts with limited structures and thus possess very low photosynthetic capability. The Calvin cycle in guard cells only assimilates 2%–4% of CO_2 fixed in mesophyll cells (Outlaw and De Vlieghere-He, 2001). In contrast, guard cells contain abundant mitochondria and display a high respiratory rate, suggesting that oxidative phosphorylation is an important source of ATP to fuel the guard cell machinery (Parvathi and Raghavendra, 1997). Such distinguished features, e.g. high activities of energy metabolism and solute transport are consistent with the guard cell specific functions. Guard cells clearly possess a robust machinery to perceive and transduce environmental signals and regulate stomatal movement.

Given the fact that the guard cells play an essential role in plant terrestrial adaptation, it is obviously important for us to understand the mechanisms within the tiny cells for improving agricultural productivity, especially considering our current grand challenges of water shortage, global warming and climate change. Since guard cells do not have plasmadesmata, the adaptive responses to the environment are thus cell-autonomous (Sirichandra et al., 2009). These properties, together with the correlation between stomatal closure and many environmental conditions, such as water availability, make the stomatal guard cells an ideal system for investigating molecular mechanisms underlying plant response to environmental factors. Here we review recent advances on homorne networks in guard cells and how they integrate environmental signals to plant physiological output.

Hormone signaling and crosstalk in guard cells

ABA signaling in guard cells

Great effort has been made in the last decades to identify the molecular components in ABA signal transduction in guard cells because ABA is a well documented stress hormone in plants that regulates water conservation through promoting stomatal closure (Sirichandra et al., 2009) and readjustment of cellular osmotic pressure to cope with prolonged dehydration (Shinozaki and Yamaguchi-Shinozaki, 2006). ABA is a terpenoid synthesized from carotenoid precursors and is inducible by drought, salinity and cold. ABA was first isolated from cotton and sycamore in the 1960s and is now known to be present in all plant species (Wasilewska et al., 2008). Besides functioning as a key player in drought response, ABA plays important roles in plant developmental processes, including cell division, seed maturation, seed dormancy and germination, and post-germination seedling growth (Leung and Giraudat, 1998; Finkelstein and Gibson, 2002).

Water deficiency triggers ABA synthesis, accumulation, and redistribution in plants, e.g. water transport from roots to shoots. In addition, drought-induced pH increase in the apoplast favors extracellular retention of the anionic form of ABA, which may facilitate ABA delivery to guard cells through the efficient apoplast pathway (Wilkinson and Davies, 2002). ABA reduces transpirational water loss by triggering stomatal closure and preventing stomatal opening. This modulation of stomatal movement is associated with multiple cascades of cellular events (Fig. 1). Briefly, ABA is perceived by ABA receptors (Razem et al., 2006; Shen et al., 2006; Liu et al., 2007; Ma et al., 2009; Park et al., 2009) and promotes stomatal closure via messengers including reactive oxygen species (ROS), cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) and pH increase (Irving et al., 1992; Allen et al., 2000; Pei et al., 2000; Murata et al., 2001). $[Ca^{2+}]_{cyt}$ increase is due to Ca^{2+} influx from the outside of guard cells and its release from internal stores such as endoplasmic reticulum (ER). Ca^{2+} influx is mediated by Ca^{2+}-permeable channels and prompted by ROS (Pei et al., 2000; Zhang et al., 2001). ABA-induced ROS production relies on NADPH oxidases downstream of the ABA-activated protein kinase, OPEN STOMATA 1 (OST1) (Mustilli et al., 2002; Kwak et al., 2003). ROS also promotes the synthesis of nitric oxide (NO), which in turn elicits Ca^{2+} release from internal stores (Desikan et al., 2002; Neill et al., 2002; Garcia-Mata et al., 2003; Sokolovski et al., 2005; Bright et al., 2006). Downstream components responding to cytosolic Ca^{2+} increase include vacuolar K^+-permeable channels, plasma membrane K^+-influx channels and anion efflux channels for malate, chloride and nitrate. An increase in cytosolic pH promotes the opening of anion and K^+ efflux channels in the plasma membrane (Colcombet et al., 2005; Li

et al., 2006). Guard cell volume reduction and stomatal closure occur upon water efflux induced by K^+ and anion efflux, sucrose removal, and conversion of malate to osmotically inactive starch (Schroeder and Hedrich, 1989; MacRobbie, 1998). Phosphatidic acid (PA) and ROS negatively regulate a protein phosphatase 2C (PP2C), which plays a role in inhibiting anion efflux and ROS production (Leung et al., 1997; Gosti et al. 1999; Merlot et al., 2001) (Fig. 1).

Recent studies have revealed new ABA signaling components in guard cells including ABA receptors. Hormone signaling is initiated by the specific receptor recognition of the hormone molecules. Although the search for the ABA receptors in plants was launched over 27 years ago since the report of ABA binding proteins in the plasma membrane of *Vicia faba* guard cells (Hornberg and Weiler, 1984), almost all the early reported receptors are controversial. For example, the Mg-chelatase H subunit (CHLH) was identified to be an ABA receptor in 2006 (Shen et al., 2006). However, recently it was disputed that the Mg-chelatase complex only affects ABA signaling, but not serving the role as a receptor (Tsuzuki et al., 2011). Another ABA receptor under dispute is G-protein-coupled receptor (GPCR) homolog (GCR2) (Johnston et al., 2007; Liu et al., 2007; Chen, 2008; Guo et al., 2008). Recently, two GPCR-type G proteins (GTG1 and GTG2) were reported ABA receptors on guard cell plasma membrane with GTPase activity (Pandey et al., 2009). Another breakthrough in guard cell ABA signal transduction is the identification of soluble ABA receptors, pyrabactin (4-Bromo-N-[pyridin-2-ylmethyl] naphthalene-1-sulfonamide) resistance/pyrabactin resistance-like/regulatory component of ABA receptor family (PYR/PYL/RCAR) and the elucidation of their biochemical mode of action, so called double negative regulatory core (Ma et al., 2009; Park et al., 2009) (Fig. 1).

One of the strategies used to isolate novel components of

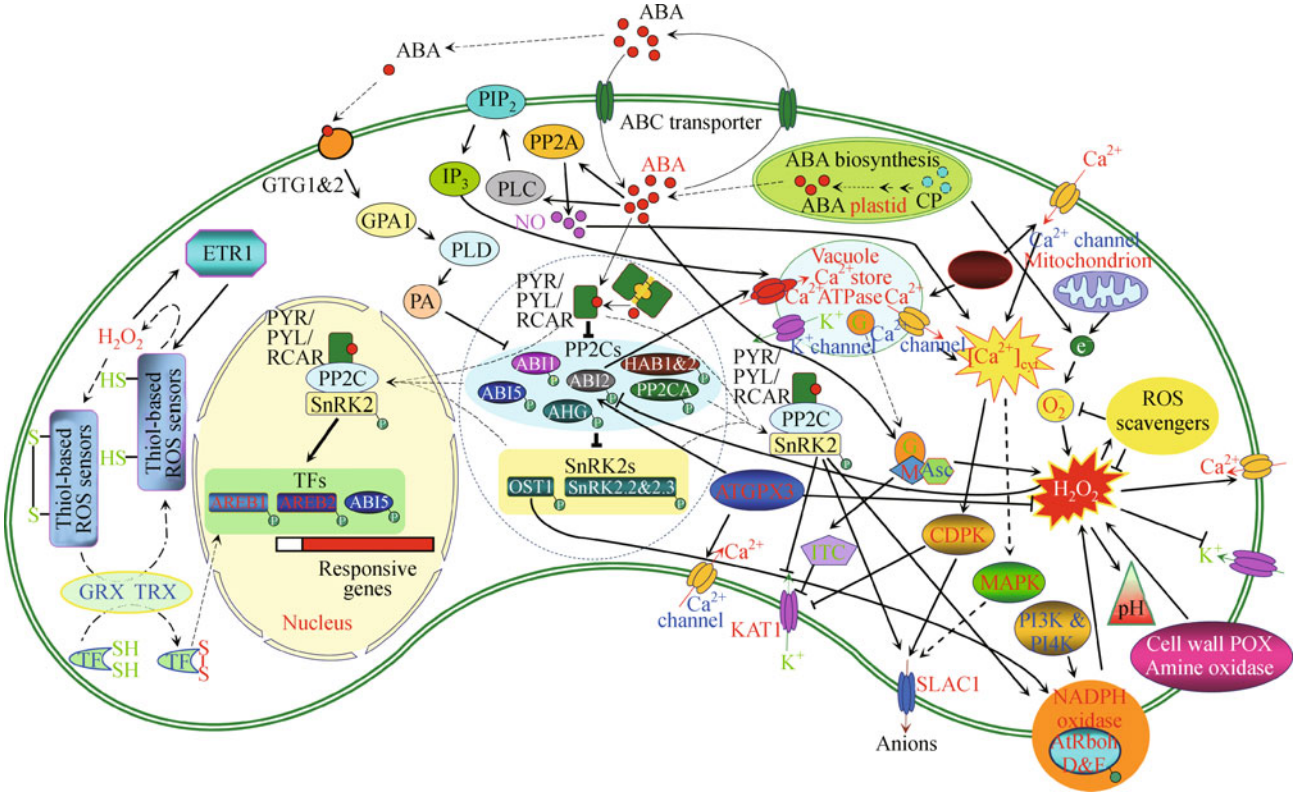

Figure 1 Overview of the ABA signaling networks in guard cells. $[Ca^{2+}]_{cyt}$, cytosolic free Ca^{2+} concentration; ABA, abscisic acid; ABC, ATP binding cassette; ABI1, ABA insensitive 1; ABI2, ABA insensitive 2; ABI5, ABA insensitive 5; AREB 2, ABA responsive element binding protein 2; Asc, ascorbic acid; ATGPX3, *Arabidopsis* glutathione peroxidase 3; AtRboh, *A. thaliana* respiratory burst oxidase protein; CDPK, calcium-dependent protein kinase; CP, carotenoid precursor; ETR1, ethylene response 1; G, glucosinolate; GCA2, growth controlled by abscisic acid 2; GCR2, G protein-coupled receptor; GPA1, *Arabidopsis* α-subunit of the trimeric G protein; GRX, glutaredoxin; HAB1&2, homology to ABI1 1&2; IP_3, inositol trisphosphate; ITC, isothiocyanate; KAT1, potassium channel 1; M, myrosinase; GTG, G protein coupled receptor (GPCR) type protein; MAPK, mitogen-activated protein kinase; OST1, open stomata 1; PA, phosphatidic acid; PI3K, phosphatidylinositol-3-kinase; PI4K, phosphatidylinositol-4-kinase; PIP_2, phosphatidylinositol-4,5-bisphosphate; PLC, phospholipase C; PLD, phospholipase D; POX, peroxidase; PP2A, protein phosphatase 2A; PP2C, protein phosphatase 2C; PYL, pyrabactin resistance-like; PYR, pyrabactin resistance; RCAR, regulatory component of ABA receptor; ROS, reactive oxygen species; SLAC1, slow anion channel 1; SnRK2, sucrose non-fermenting 1-related protein kinase 2; TF, transcription factor; TRX, thioredoxin.

early ABA signaling was to identify proteins interacting with the PP2C ABA-INSENSITIVE 1 (ABI1), which is known to function upstream of all known rapid signaling events. The dominant negative mutant *abi1-1* shows ABA-insensitive stomatal conductance (Koornneef et al., 1989; Leung et al., 1994), whereas loss-of-function recessive mutants of *ABI1* show hypersensitivity in ABA-mediated stomatal response, leading to the conclusion that *ABI1* is a negative regulator of ABA signaling. In response to ABA, dominant *abi1-1* mutants lost the ability to generate ROS, but the dominant ABA-insensitive *abi2-1* mutant could generate ROS. Thus it has been proposed that *ABI1* acts upstream of ROS production and *ABI2* downstream of ROS production in ABA signaling (Murata et al., 2001) (Fig. 2). RCAR1/PYR1-LIKE9 (PYL9) was identified in a yeast two-hybrid screen using the PP2C ABI2 as a bait (Ma et al., 2009), and a similar strategy using HOMOLOGY TO ABI1 (HAB1) as a bait identified PYL5, PYL6, and PYL8 (Santiago et al., 2009). With an alternative strategy, *PYR1* gene was identified using chemical genetics based on insensitivity to the synthetic ABA agonist pyrabactin (Park et al., 2009). Purification of *in vivo* ABI1 complex from *Arabidopsis* led to the identification of nine of the 14 PYR/PYL/RCARs as the major interactors of ABI1 in planta (Nishimura et al., 2010). And the *pyr1/pyl1/pyl2/pyl4* quadruple mutants showed a strong ABA insensitive phenotype in double-blinded ABA-induced stomatal closing and ABA inhibition of stomatal opening analyses (Nishimura et al., 2010). These multiple independent lines of evidence indicated that the previously uncharacterized PYR/PYL/RCAR proteins are major early ABA signaling components (Fig. 1). PYR/PYL/RCARs are small soluble proteins belonging to the START/Bet v I super family and they contain a central hydrophobic ligand binding pocket (Iyer et al., 2001). The *Arabidopsis thaliana* genome encodes 14 PYR/PYL/RCAR proteins that are highly conserved at the protein sequence level. The identification of this new class of ABA signaling proteins has shed light in the plant hormone signaling field, providing new avenues of research into ABA signal transduction. For example, after the crystallization of the ABA receptor, the mechanism of action has started to emerge. Direct ABA binding to PYR/PYL/RCARs was subsequently established through the elucidation of PYR1, PYL1, and PYL2 crystal structures in the presence of ABA (Melcher et al., 2009; Miyazono et al., 2009; Santiago et al., 2009; Yin et al., 2009; Nishimura et al., 2010). It was established that PYR/PYL/RCARs consist of homodimers with each subunit binding to ABA. The binding of ABA results in the dissociation of the dimer, introducing conformational changes of PYR/PYL/RCARs. This creates a new surface for PP2Cs to interact with the receptor, and consequently the interaction inhibits the phosphatase activity of PP2Cs by blocking the access of their substrates to the catalytic center since the acting interface between ABA-bound PYR/PYL/RCARs and PP2Cs is located at the PP2C active site (Fig. 1).

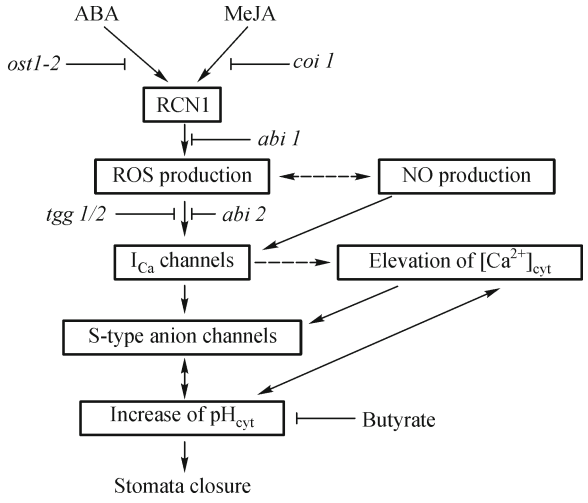

Figure 2 **S**implified model of crosstalk between ABA and MeJA signaling in stomatal closure. $[Ca^{2+}]_{cyt}$, cytosolic calcium concentration; ABA, abscisic acid; *abi 1,ABA-insensitive1*; *coi 1*, *coronatine insensitive 1*; I_{Ca} channels, Ca^{2+} ion channel; MeJA, methyl jasmonate; *ost1-2*, *open stomata 1-2*; pH_{cyt}, cytosolic pH; RCN1, root curling in n-naphthylphthalamic acid 1; ROS, reactive oxygen species; *tgg 1/2, thioglucoside glucohydrolase 1/2*.

As we understand how PYR/PYL/RCARs function through ABA-dependent inhibition of PP2C activity, the targets of PP2Cs in the signaling pathway become intriguing. To date, one of the best characterized PP2C targets is OST1, a serine/threonine kinase with high homology to ABA-activated protein kinases (AAPKs) found in *V. faba* (Li and Assmann, 1996). OST1 is a positive regulator in the ABA signaling pathway under the regulation of the complex formed by ABA receptor and phosphatase PP2C (Lee et al., 2009). With the presence of the hormone molecule binding to the receptor PYR/PYL/RCAR, the phosphatase is deactivated, which in consequence keep the phosphorylated status of OST1 in an active form (Fig. 1). The substrates of OST1 identified so far include NADPH oxidase located on plasma membrane, S-type anion channel (SLAC1), inward-rectifying potassium channel (KAT1) and transcription factors such as ABSCISIC ACID RESPONSIVE ELEMENTS BINDING FACTOR 3 (ABF3) (Geiger et al., 2009; Sato et al., 2009; Sirichandra et al., 2009; Sirichandra et al., 2010). Overall, the receptor, PP2C and OST1 form a double negative regulatory complex at the initial stage of guard cell ABA signaling pathway to convey the signal to the downstream components, leading to the physiological output of stomatal movement.

Although the key regulatory events in the ABA signaling pathway have been unraveled (Table 1), the complete molecular networks, including nodes, edges and the regulatory mechanisms remain to be determined. For example, the structural details of the receptors imply the formation of homodimer without binding to ABA but the interaction with PP2C occurs on the receptor monomer with an ABA molecule, which implies the correlation of the complex formation with the dimer dissociation. However, it is not clear

Table 1 Protein components of guard cell ABA and MeJA signaling pathways in *Arabidopsis*

Protein	Gene locus	Gene	References	
			ABA signaling	MeJA signaling
PYR/PYL/RCAR (14) Bet v I domain protein	At4g17870	*PYR1*	Ma et al., 2009	
	At5g46790	*PYL1*	Park et al., 2009	
	At2g26040	*PYL2*	Santiago et al., 2009	
	At2g38310	*PYL4*	Nishimura et al., 2010	Lackman et al., 2011
	At5g53160	*PYL8/RCAR3*		
	At1g01360	*PYL9/RCAR1*		
PP2C Group A (9) Mg^{2+}-dependent Ser/Thr protein phosphatase	At4g26080	*ABI1*	Leung et al., 1994	Munemasa et al., 2007
	At5g57050	*ABI2*	Saez et al., 2004	Islam et al., 2009
	At1g72770	*HAB1*	Saez et al., 2004	
	At1g17550	*HAB2*	Leonhardt et al., 2004	
	At3g11410	*AtPP2CA*	Yoshida et al., 2005	
	At5g51760	*AHG1*	Nishimura et al., 2007	
SnRK2 (10) Ser/Thr protein kinase	At3g50500	*SnRK2.2*	Merlot et al., 2002	
	At5g66880	*SnRK2.3*	Fujii et al., 2007	
	At4g33950	*SnRK2.6/OST1*	Mustilli et al., 2002	Suhita et al., 2004
CaM/CML (57) Calmodulin (-like)	At3g51920	*CML9*	Delk et al., 2005	
	At5g37770	*CML24*	Magnan et al., 2008	
CDPK (34) Ca^{2+}-dependent Ser/Thr kinase	At4g23650	*CPK3*	Choi et al., 2005	
	At4g09570	*CPK4*	Mori et al., 2006	
	At2g17290	*CPK6*	Ma and Wu, 2007	
	At1g35670	*CPK11*	Zhu et al., 2007	
	At4g04720	*CPK21*	Geiger et al., 2011	
	At4g07470	*CPK23*		
	At3g57530	*CPK32*		
F-box protein (> 568)	At2g39940	*COI1*		Xie et al., 1998 Katsir et al., 2008
Jasmonate-ZIM domain protein (12)	At1g19180	*JAZ1*		Sheard et al., 2010 Thines et al., 2007
CBL (10) Calcineurin-B like	At4g17615	*CBL1/SCABP5*	Cheong et al., 2003	
	At5g47100	*CBL9*	Pandey et al., 2008	
CIPK/SnRK3 (25) Ser/Thr protein kinase	At5g01810	*CIPK15/PKS3*	Cheong et al., 2007	
	At1g30270	*CIPK23*	Pandey et al., 2008	
Rboh (10) NADPH oxidase	At5g47910	*AtRbohD*	Kwak et al., 2003	Suhita et al., 2004
	At1g64060	*AtRbohF*	Suhita et al., 2004	
PP2A (26) Protein phosphatase regulatory subunit	At1g25490	*RCN1*	Kwak et al., 2002	Saito et al., 2008 Murata et al., 2001
Myrosinase (6)	At5g26000	*TGG1*	Zhao et al., 2008	Islam et al., 2009
	At5g25980	*TGG2*	Islam et al., 2009	
Transcription factors (> 1500)	At1g32640	*MYC2/JAI1/JIN1*	Abe et al., 2003	Lorenzo et al., 2004
	At2g47190	*MYB2*		

The numbers in the parenthesis indicate the number of protein homologs, some of which have been characterized and are listed with references.

whether the receptor-PP2C complex formation precedes the homodimer dissociation or *vice versa*. The protein/hormone ratio or concentration *in vivo* could be balanced to tune the equilibrium for homodimer association/dissociation, to appropriately respond to the environmental stimulus (Hubbard et al., 2010). In addition, the *in vitro* interaction of PYR/PYL/RCARs-PP2C has been intensively investigated and the interactions observed, for instance, ABI1 forms complexes with PYR1, PYL1, PYL8 and PYL9 (Ma et al., 2009; Miyazono et al., 2009; Park et al., 2009; Yin et al., 2009), may not represent those *in vivo*. The specificity of PYR/PYL/RCAR and PP2C complexes need to be determined in relation

to different biochemical/physiological processes since many different homologs exist in the genome. Additionally, PP2C is known to target OST1 to regulate the activity of the kinase for downstream activation. However, other targets of PP2C other than OST1 need to be explored. The observation that OST1 can be activated by osmotic stress in the PP2C dominant negative mutants indicates the inhibition of the dephosphorylation activity is not the only strategy to activate OST1 (Yoshida et al., 2005). Other models, e.g. the existence of upstream kinases should be further tested. Other questions to be addressed include, but not exclusively, the relationships between the central regulatory module and other signaling intermediates, such as ion channels, ROS/NO production, Ca^{2+} oscillation and transcriptional responses. Recently Wang and his colleagues applied "omics" approach to discover common and unique elements of the ABA-regulated transcriptome of *Arabidopsis* guard cells and found 1173 ABA responsive genes (696 ABA-induced and 441 ABA-repressed) out of around 24000 profiled genes (Wang et al., 2011). This work set the stage for targeted gene functional characterization and further biotechnological manipulation to improve plant water use efficiency.

Jasmonate signaling and crosstalk in guard cells

ABA is often categorized as a phytohormone closely related to abiotic stresses, while jasmonate (JA) is often recognized more as a biotic stress hormone since this group of plant hormones mediate plant defense responses against necrotrophic pathogen and insects (Liechti and Farmer, 2002; Fujita et al., 2006). For example, JA was found to be accumulated in suspension cells when challenged with fungal elicitors (Blechert et al., 1995). Besides the roles in biotic stress, the lipid-derived plant hormone also participates in the regulation of vegetative and reproductive growth, and defense responses against abiotic stresses, e.g. UV light and ozone (Katsir et al., 2008). Since the first report of MeJA-induced stomatal closure (Gehring et al., 1997), multiple lines of evidence including JA accumulation during drought (Creelman and Mullet, 1997), JA mediated stomatal closure under ozone stress (Gomi et al., 2005), as well as guard cell location of a JA-responsive promoter, *Picea glauca defensin 1*(*PgD1*) (Germain et al., 2011) support the function of JA signaling in stomatal movement.

Although ABA and MeJA are positive regulators of stomatal closure, knowledge on how MeJA and ABA signaling pathways interact and function in guard cells is lacking. The function of the jasmonate coreceptor *CORONATINE INSENSITIVE 1* (*COI1)*, encoding an F-box protein, together with JASMONATE ZIM-DOMAIN (JAZ) co-receptor were known for efficient ligand binding (Xie et al., 1998; Thines et al., 2007; Katsir et al., 2008; Sheard et al., 2010). Due to the receptor specificity of each hormone, the divergence between the ABA and MeJA signal pathways seems apparent. However, the MeJA-mediated stomatal closure has been found to involve guard cell cytoplasmic alkalinization, ROS production via NADPH oxidase subunits AtrbohD/F (Suhita et al., 2004), NO production (Munemasa et al., 2007; Saito et al., 2008), and activation of K^+ efflux channels (Evans, 2003) and slow anion channels (Gehring et al., 1997; Suhita et al., 2003), which are processes common to ABA signaling. For example, NAD(P)H oxidase-mediated ROS production is necessary for activation of calcium permeable non-selective cation channels (I_{Ca} channels) by ABA and MeJA. In addition, cytosolic Ca^{2+} elevation is involved in ABA and MeJA-induced stomatal closure via S-type anion channels. Furthermore, a regulatory subunit of protein phosphatase 2A and root curling in n-naphthylphthalamic acid 1 (RCN1) regulate both MeJA signaling and ABA signaling in guard cells (Murata et al., 2001). These lines of evidence suggest crosstalks between ABA and MeJA signaling pathways in guard cells (Table 1and Fig. 2). This hypothesis is further supported by the observation of MeJA hyposensitivity of stomatal closure in the *ost1* (ABA hyposensitive) mutant, and reduced ABA-mediated stomatal closure in the *jar1 (jasmonate resistant 1*, MeJA insensitive) mutant (Suhita et al., 2004). MeJA-induced stomatal closure was also studied in the ABA-insensitive PP2C mutant, *abi2-1*. In this mutant, no stomatal closure was observed in response to either MeJA or ABA, but production of ROS and NO were retained (Munemasa et al., 2007). All together, COI1 functions upstream of ROS and NO in MeJA but not ABA signaling, while ABI2 functions downstream of ROS and NO after the MeJA and ABA-signaling pathways have converged. A downstream event in ABA signal transduction is the activation of transcription factors, e.g. the basic helix-loop-helix (bHLH) transcription factor AtMYC2. AtMYC2 was also demonstrated to involve in JA signal transduction (Abe et al., 2003; Lorenzo et al., 2004).

Interestingly, myrosinase-glucosinolate system has recently been demonstrated to be a novel component in ABA signaling in guard cells (Zhao et al., 2008). TGG1 that encodes a myrosinase highly expressed in guard cells (Husebye et al., 2002; Zhao et al., 2008). TGG1 was found to be involved in inhibition of light-induced stomatal opening by ABA, but not in ABA-induced stomatal closure (Zhao et al., 2008). Two functional myrosinase genes, *TGG1* (At5g26000) and *TGG2* (At5g25980), have been found to express at high levels in *Arabidopsis* shoots and they have redundant functions in glucosinolate hydrolysis (Barth and Jander, 2006). Recent work by Murata group suggests that the two myrosinases function downstream of ROS production and upstream of cytosolic Ca^{2+} elevation in ABA and MeJA signaling in guard cells (Islam et al., 2009). They have also provided evidence showing one of the glucosionlate degradation products, isothiocyanate can induce stomatal closure, which requires MeJA priming, but not ABA priming (Khokon et al., 2011). Additionally, Lackman and colleagues have identified a tobacco gene, *NtPYL4* encoding a protein with high homology to the PYR/PYL/RCAR family ABA

receptors in *Arabidopsis* and it is involved in guard cell JA signaling (Lackman et al., 2011). Furthermore, recent transcriptomic analysis of ABA responsive genes in *Arabidopsis* guard cells has also provided evidence for the crosstalks at transcriptional level between ABA and MeJA (Wang et al., 2011).

The evidence for the signaling crosstalk between ABA and MeJA is not limited on the molecular basis but also found in genetic and physiological experiments. Drought is known to alter the balance of different hormones (Dodd and Davies, 2010). To address the role of ABA in JA-mediated stomatal regulation, the JA-mediated stomatal response has been studied in tomato ABA-biosynthetic mutant *sitiens*. When the petioles of the *sitiens* leaves were incubated in JA, no stomatal movement was observed; however, stomatal closure is triggered by JA when the petioles were pretreated with ABA (Herde et al., 1997). This suggests that ABA is required by the JA-mediated stomatal response in tomato. In barley, water stressed seedlings pretreated with JA showed more than 4-fold accumulation of ABA in comparison to water stressed barley seedlings without the pretreatment with JA. This indicates a role for JA in ABA biosynthesis under water stress conditions (Bandurska et al., 2003). Many drought-responsive genes are regulated by MeJA (Huang et al., 2008) and several MeJA-regulated, drought-responsive genes are also regulated by ABA with similar expression dynamics (Nemhauser et al., 2006; Huang et al., 2008). These data support that common signaling components are employed by both ABA and MeJA. However, unique components and regulatory mechanisms underlying each pathway of hormone signaling need to be further studied.

Other hormone functions in guard cells

Several other hormones, including salicylic acid (SA), auxin, cytokinin, ethylene and brassinosteroid (BR) are also involved in the regulation of stomatal movement but the modes of action vary. Generally, BRs and SA are positive regulators triggering stomatal closure like ABA and JA, whereas cytokinins and auxins are positive regulators for stomatal opening (Lohse and Hedrich, 1992; Manthe et al., 1992; Lee, 1998; Mori et al., 2001; Wilkinson and Davies, 2002; Haubrick and Assmann, 2006). Interestingly, ethylene alone promotes stomatal closure, whereas ethylene in concert with other hormones opposes stomatal closure. This dual regulatory role of ethylene on stomatal movement can be observed in a species and/or condition dependent manner (Jackson, 2002; Dat et al., 2004; Acharya and Assmann, 2009). Although gibberellin (GA) modulates the expression levels of drought-related genes, little or no effect was observed on stomatal movement in *Arabidopsis* when GA was applied exogenously (Nemhauser et al., 2006; Tanaka et al., 2006; Huang et al., 2008). Additional evidence also suggests GA may not play critical roles in controlling stomatal aperture under water stress conditions (Cramer et al., 1995).

Although the effect of each hormone on stomatal biology was often studied independently, it is believed that different hormones regulate plant responses to biotic and abiotic stresses via synergistic and antagonistic mechanisms (Fujita et al., 2006). For instance, interaction of auxin, cytokinin, and ethylene with ABA inhibits ABA-induced stomatal closure (Tanaka et al., 2006). Additionally, interaction of ABA and SA positively regulates stomatal closure to impede invasion of bacterial pathogens (Melotto et al., 2006). However, such observations using exogenous hormone treatment may not reflect the effects of changes in endogenous hormone levels. In addition, despite recent progress on hormonal control of stomatal function, many questions remain to be answered. Many plant hormonal responses are developmental in nature, whereas hormonal regulation of stomatal movement is a reversible, non-developmental process. Whether the subcellular targets of hormones discovered in guard cells (e.g., ion channels) are also cellular targets of hormonal regulation in the developmental processes and whether they are cell type specific are not known. What are the molecular switches in regulation that allow quick response of guard cells to changes in the environment remain to be a key question. Additional questions include whether stomatal regulatory mechanisms, which to date have been explored primarily in dicot species, prevail in other species as well, and in particular whether they occur in the graminaceous species with their unique guard cell morphology and dominance in agroecosystems. The concerted application of molecular, genetic, cell biology and biochemical approaches including modern "omics" technologies is anticipated to significantly advance our knowledge of guard cell signal transduction. The improved knowledge will contribute positively toward future biotechnology of enhanced plant yield and bioenergy.

Molecular switch based regulatory mechanisms underlying stomatal movement

Phosphorylation/dephosphorylation switches

Evolution of protein kinases/phosphatases is significant because protein phosphorylation/dephosphorylation switch dominates signal transduction processes in plants and animals (Stone and Walker, 1995; Luan, 2003). The reaction of transferring phosphate groups from high-energy donor molecules, such as ATP, to specific substrates is referred as phosphorylation, which is catalyzed by a kinase, alternatively known as a phosphotransferase. The reversed process, i.e., the removal of the phosphate group, known as dephosphorylation is catalyzed by another groups of enzymes named phosphatases (Burnett and Kennedy, 1954). These reactions occur on certain amino acid residues, such as histidine and aspartic

acid in the two-component phosphotransfer system commonly seen in prokaryotes, as well as serine, threonine and tyrosine typical for eukaryotes (Hanks and Hunter, 1995; West and Stock, 2001). Undoubtedly such reversible protein modifications play a significant role since it could activate or deactivate target proteins with diverse functions, in a way similar to "on or off switches" (Mundy and Schneitz, 2002).

Prior to the identification of kinases or phosphatases with functions in guard cells, early evidence has already suggested ion channels and H^+ pumps are targets of phosphorylation/dephosphorylation regulation in guard cells. For example, it has been reported the plasma membrane H^+ pump in *V. faba* guard cells, which was activated by blue light, was inhibited by an inhibitor of myosin light-chain kinase (Shimazaki et al., 1992). The activities of inward- and outward-rectifying K^+ channels were affected by protein phosphatase inhibitors (Li et al., 1994; Thiel and Blatt, 1994; Armstrong et al., 1995). In the late 1990s, direct evidence of the presence of protein kinases and phosphatases in guard cells was obtained through identification of an ABA-activated protein kinase (AAPK) in *V. faba* guard cells (Li and Assmann, 1996; Mori and Muto, 1997). The 48 kDa ABA-activated and Ca^{2+}-independent protein kinase was discovered using in gel kinase assay and the peptide information was obtained using mass spectrometry (Li et al., 2000). The functional characterization of AAPK suggests that the kinase is activated by ABA *in vivo* and it regulates the anion channels to induce stomatal closure (Li et al., 2000). It is noteworthy that a 53 kDa ABA-activated, Ca^{2+}-dependent protein kinase was proposed to be the activator of AAPK (Mori and Muto, 1997). Ever since, more kinases have been found to function in guard cells. The discovery of an *Arabidopsis* serine/threonine kinase, known as *OST1*, acting upstream of ROS production in ABA signaling is significant (Mustilli et al., 2002). OST1 belongs to the sucrose non-fermenting 1 (SNF1) related kinase 2 (SnRK2) subfamily and the members in the SnRK2 family are mainly involved in plant stress response and tolerance (Harmon, 2003; Halford and Hey, 2009). Intensive studies have been conducted to characterize OST1 function, including its upstream activating kinase, downstream phosphorylation targets and interacting partners. To date, the upstream activating kinase remains elusive. However, the 53 kDa ABA-activated, Ca^{2+}-dependent protein kinase identified from *V. faba* guard cells could be a likely candidate since the AAPK is highly homologous to OST1 (Mori and Muto, 1997; Assmann, 2003). In contrast, studies on the OST1 targets have been fruitful. To date, the identified OST1 substrates include plasma membrane NADPH oxidase, S-type anion channel (SLAC1), inward-rectifying potassium channel (KAT1) and transcription factors such as ABF3 (Geiger et al., 2009; Sato et al., 2009; Sirichandra et al., 2009, 2010). The regulatory domain of OST1 was found to interact with phosphatase ABI1 and integrate ABA and osmotic stress signals to regulate stomatal closure in *Arabidopsis* (Yoshida et al., 2005; Vlad et al., 2009). When ABA binds its receptor PYR/PYL/RCAR, the PP2C phosphatases are deactivated, thus maintain the phosphorylated status of OST1 in an active form (Lee et al., 2009). In addition, the involvement of OST1 in JA, ROS and CO_2 signaling pathways has been unraveled (Suhita et al., 2004; Vahisalu et al., 2010; Xue et al., 2011). All current results suggest that the central role of OST1 in hormone signaling and stress response is mediated by the phosphorylation/dephosphorylation switches.

S-type anion channels contribute to chloride and nitrate export from guard cells, which in turn initiates stomatal closure. The first identified component of the guard cell S-type anion channel SLAC1 is a target of OST1 in a Ca^{2+}-independent manner (Geiger et al., 2009). However, since ABA-induced stomatal closure involves increased cytosolic Ca^{2+} levels, it is not known whether the S-type anion channels are also regulated by a Ca^{2+}-dependent mechanism (Li et al., 2006). Impairment of ABA activation of S-type anion channels in *cpk3cpk6* mutants implies the role of calcium-dependent protein kinase (CDPK) CPK3 and CPK6 function in ABA regulation of guard cell S-type anion channels and Ca^{2+}-permeable channels in stomatal closure (Mori et al., 2006). Recently *in vitro* evidence of direct interaction between CPK21 and SLAC1 homolog 3 (SLAH3) and between CPK21 and ABI1/2 suggests a Ca^{2+}-dependent activation of S-type anion channels in ABA signal transduction in *Arabidopsis* through the receptor RCAR1-PP2C complex, which may parallel the Ca^{2+}-independent activation by OST1(Geiger et al., 2011). The identification of the two types of kinases, SnRK2 and CDPK in the guard cell functions demonstrates the existence of Ca^{2+}-dependent and independent ABA activation pathways. However, the specificity and redundancy of the kinases in regulating downstream targets is worthy of further investigation considering there are 34 *CDPK* and 10 *SnRK2* genes in the *Arabidopsis* genome.

Another important signaling cascade is mediated by mitogen-activated protein kinases (MAPKs), including MAP4K, MAP3K, MAP2K, MAPK that are sequentially activated in the cascade (Taj et al., 2010). MAP kinases MAPK9 and MAPK12 were found to express preferentially in guard cells and positively regulate ROS-mediated ABA signaling (Jammes et al., 2009). Additionally, stomatal closure caused by increased levels of ABA under drought involves MKK1, MPK3 and MPK6 (Hamel et al., 2006; Gudesblat et al., 2007). Pathogen-induced stomatal closure restricts the invasion of bacteria and thus constitutes an important part of the plant innate immune response. The stomata of *MPK3* antisense plants act normally upon ABA treatment, but are not responsive to bacteria, indicating the unique function of MPK3 in the stomatal innate immunity response (Gudesblat et al., 2007). It is interesting to investigate whether pathogen-induced stomatal closure and ABA-induced stomatal closure are mediated via a common MAPK cascade or different MAPKs are involved in the stomatal movement.

Besides the three common kinase groups mentioned above,

other types of kinases have been shown to function guard cell signal transduction. A mutant (*ahk5*) of *Arabidopsis* histidine kinase AHK5 localized in cytosol and plasma membrane has shown reduced stomatal closure in response to abiotic stimuli, pathogen treatment, as well as exogenous H_2O_2 (Desikan et al., 2008). However, ABA-induced stomatal closure, H_2O_2 production induced by dark adaptation and H_2O_2 induced NO synthesis were preserved in the mutant. This observation suggests that AHK5 integrates multiple signaling pathways via H_2O_2 homeostasis and may be independent of ABA signaling in guard cells. In addition, a calcineurin B-like-interacting protein kinase (CIPK) belonging to the SnRK3 subfamily was isolated from *V. faba* guard cells and found to be negatively regulated by cytosolic Ca^{2+} through calcineurin B-like calcium binding protein (CBL). The VfCIPK1 may be related to the mitochondrial functions in guard cells but the detailed mechanisms await further analysis (Tominaga et al., 2010). In summary, all the identified guard cell protein kinases participate in the signaling processes that regulate proper stomatal movement in response to environmental stimuli. Future work needs to map their functions in the sophisticated guard cell molecular networks to achieve an ultimate understanding of stomatal movement in response to internal and external stimuli.

The opposite reaction of phosphorylation is dephosphorylation catalyzed by protein phosphatases. Similar to kinases, phosphatases are ubiquitous enzymes in all eukaryotes and could be classified based on their substrate specificity into serine/threonine phosphatases, tyrosine phosphatases, dual specificity phosphatases, histidine phosphatases and lipid phosphatases (Barford, 1996; Camps et al., 2000; Bäumer et al., 2007). To date, the first two groups, especially the serine/threonine phosphatases have been found to function in guard cells. The serine/threonine phosphatases can be classified into four major subgroups, protein phosphatase (PP) type 1 (PP1), PP2A, PP2B and PP2C based on their substrate specificity, divalent cation requirement and inhibitor sensitivity (Cohen et al., 1989). The PP1 group phosphatases, utilizing the β subunits of phosphorylase kinases as substrate, are potently inhibited by okadaic acid and independent of divalent cations for activity. The PP2 groups generally use the subunits of phosphorylase kinases as substrate, but their inhibitor and cation dependence vary. For example, PP2As are okadaic acid sensitive but not dependent on divalent ions. PP2Bs (calcineurin) are dependent on Ca^{2+} and stimulated by calmodulin, but not inhibited by okadaic acid. PP2Cs are dependent on Mg^{2+} and not sensitive to okadaic acid (Cohen et al., 1989).

With the knowledge of the known inhibitors to each subgroup of the serine/threonine phosphatases, early pharmacological studies suggested the involvement of all the groups in guard cell signal transduction. The PP1/PP2A inhibitor okadaic acid can partially impair activation of anion channels and stomatal closure in *Arabidopsis* (Ler) (Schmidt et al., 1995). The inhibitor disruption of the PP2A regulatory subunit RCN1 confers ABA insensitivity in *Arabidopsis* (WS), suggesting a role of RCN1 as a positive transducer of ABA-elicited $[Ca^{2+}]_{cyt}$ transients in guard cells (Kwak et al., 2002) (Fig. 2). In *Arabidopsis rcn1* mutants, MeJA failed to induce stomatal closure. ROS production and suppression of inward-rectifying K^+ channel activities were not observed in *rcn1* when treated with ABA or MeJA, suggesting RCN1 is a shared component between the two hormone pathways and is functioning upstream of ROS production and downstream of the branching point of hormone signal reception (Saito et al., 2008) (Fig. 2). These results suggest that PP1s/PP2As act as positive regulators of MeJA or ABA signal transduction in Ler and WS guard cells. Inconsistently, however, the application of okadaic acid promotes anion channel activation and ABA-induced stomatal closure in *Vicia* and *Commelina*, and activates ABA-responsive promoters in tomato hypocotyls (Pei et al., 1997; Wu et al., 1997). Therefore, PP1s and PP2As can be either positive or negative regulators in guard cell signaling, dependent upon plant species.

Ca^{2+} oscillation is an essential process in guard cell ABA signaling (Li et al., 2006). The Ca^{2+}-dependent PP2B (calcineurin) was found to deactivate the plasma membrane inward-rectifying K^+ channels in fava bean guard cells and the effect was blocked by PP2B specific inhibitor (Luan et al., 1993). This observation links the increase of cytosolic Ca^{2+} to the inhibition of inward-rectifying K^+ channels in guard cells, and in consequence, stomatal closure by changing the phosphorylation status of plasma membrane inward K^+ channels. Additionally, ABA responses in pea epidermal peels, including mRNA accumulation of ABA-induced dehydrin and stomatal closure were reduced by an inhibitor of PP2B (Hey et al., 1997). Furthermore, a Ca^{2+}-permeable slow vacuolar channel in guard cells was shown to be modulated by calcineurin (Allen and Sanders, 1995). Since no catalytic PP2B subunits are found in the *A. thaliana* genome, whether the participation of PP2B in the guard cell signal transduction is universal in plants is not known (Kerk et al., 2002).

Due to the unavailability of pharmacological inhibitors to PP2Cs, initial discovery of PP2Cs in the ABA pathway came from genetic studies of the ABA deficient mutant *abi1* (Leung et al., 1994; Meyer et al., 1994). To date, it has been demonstrated that at least four *Arabidopsis* PP2Cs (ABI1, ABI2, AtP2C-HAB1 and PP2CA) are negative regulators of the ABA signaling pathway in guard cells (Rodriguez et al., 1998; Gosti et al., 1999; Merlot et al., 2001; Tähtiharju and Palva, 2001). Armstrong and colleagues reported that ABI1, a putative ABA-insensitive PP2C, regulates inward- and outward-rectifying K^+ channels (Armstrong et al., 1995). In response to ABA, dominant *abi1-1* mutants lost the ability to generate ROS, but the dominant ABA-insensitive *abi2-1* mutant could generate ROS. Thus, it has been proposed that ABI1 acts upstream of ROS production and ABI2 downstream of ROS in guard cell ABA signaling (Murata et al., 2001). The aforementioned double negative regulatory core

shown in Figure 1 composed of the three key elements, the receptor, the phosphatase and the kinase, highlights the phosphorylation/dephosphorylation switch in the guard cell signaling. In addition, AtP2C-HAB1 is one of the closest relatives of ABI1 and ABI2 and overexpression of AtP2C-HAB1 impaired stomatal closure (Rodriguez et al., 1998). Similar to the kinases identified in the stomatal response, ion channels are targets of PP2Cs. For example, PP2CAs bind to *ARABIDOPSIS* POTASSIUM TRANSPORT 2 (AKT2), a K^+ channel and regulate the AKT2 to control K^+ channel activity and membrane polarization under stress conditions (Chérel et al., 2002). Compared to other subgroups of serine/threonine phosphatases, 76 *Arabidopsis* genes are identified as PP2C-type phosphatase candidates, more than the 26 PP1s and PP2As and there are no representatives of PP2Bs (Schweighofer et al., 2004; Farkas et al., 2007). Apparently, PP2Cs form a major class of phosphatases with important functions in plant stress signal transduction.

Another class of phosphatases, protein tyrosine phosphatase (PTPase) has also been shown to control ion efflux from guard cell vacuoles during stomatal closure. PTPase specific inhibitor prevents stomatal closure caused by ABA, high external Ca^{2+}, H_2O_2 and dark, but promotes reopening of the closed stomata, implying that protein tyrosine dephosphorylation must occur at downstream of the Ca^{2+} signal responsible for ion efflux from the vacuoles (MacRobbie, 2002). This conclusion is solely based on pharmacological studies. Genetic studies and other lines of evidence would be desirable.

The recurrent theme is that protein phosphatases negatively regulate signaling pathways activated by the action of particular protein kinases. The diversity of plant phosphatases suggests that individual phosphatase may have specificity in substrate recognition, as the case of kinases described before. The coordination between specific protein kinases and phosphatases, as well as their interaction with their diverse targets highlights sophisticated and orchestrated signaling networks. It has become clear that the regulatory mechanism of phosphorylation/dephosphorylation, best exemplified by research on guard cell ABA signaling, plays a pivotal role in plant stomatal function.

Thiol-based redox switches

Under stress conditions, metabolic imbalance between energy generation and consumption can induce oxidative stress in cells, as often indicated by the generation of ROS, including H_2O_2, superoxide ion (O^{2-}) and hydroxyl radical (OH^-) (Valko et al., 2007; Foyer and Noctor, 2009). A common property of ROS is that they can cause oxidative damage to proteins, DNA, and lipids. Oxidized biomolecules have been traditionally recognized as markers for oxidative stress (Suzuki et al., 2011). However, it remains unclear whether cysteines are involved and whether the redox regulation is a direct effect of sensing cellular redox state (Fig. 3).

Recently, more and more evidence indicates that ROS can also serve as signaling molecules for the regulation of various physiological responses to environmental challenges including hormone-responsive stomatal closure (Buchanan and Balmer, 2005). ABA induced H_2O_2 production in guard cells promotes stomatal closure and inhibits stomatal opening (Pei et al., 2000). However, ROS production does not represent a unique mechanism in this pathway since JA- and SA-triggered stomatal closure is also associated with the elevation of ROS (Suhita et al., 2004; Vahisalu et al., 2010). ROS accumulation in guard cells was shown to be a biphasic event,

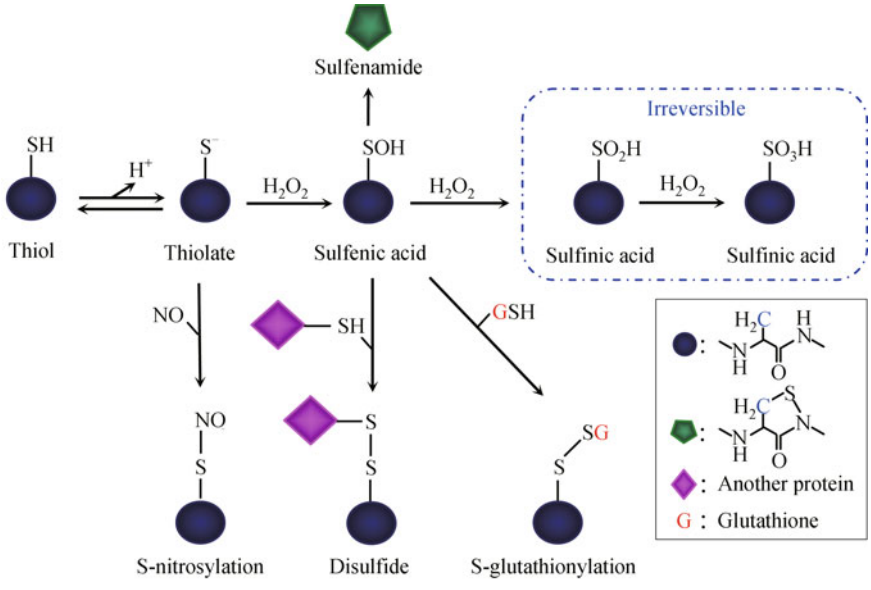

Figure 3 Diagram of potential modifications of redox sensitive cysteines in guard cell proteins. The reversible cysteine modifications may play important signaling roles in stomatal movement as redox switches.

in which the production was initiated from chloroplasts, followed by ROS production through plasma membrane NADPH oxidases (Joo et al., 2005; Vahisalu et al., 2010). Due to the dual function of ROS, tight regulation of ROS is essential in balancing oxidative damage and signaling activity, including reversible redox regulation of proteins, regulation of phosphoproteins, activation of ROS-responsive genes and buffering of ROS by ROS-scavenging enzymes and antioxidant molecules (Suzuki et al., 2011). There are a few key examples of guard cell protein redox regulation. The activities of protein phosphatase ABI1 and ABI2 in guard cells are sensitive to redox state (Meinhard and Grill, 2001; Meinhard et al., 2002). However, direct evidence for thiol-based redox regulation and a link between protein redox change and stomatal closure remain to be demonstrated. Great effort has been made to investigate the molecular mechanisms of ROS production and identify the ROS-regulated molecular components in guard cell signaling. Plasma membrane NADPH oxidase is important in ROS production in guard cells (Kwak et al., 2003). The evidence came from the observation that disruption of two partially redundant *Arabidopsis* guard cell NADPH oxidase catalytic subunit genes, *AtrbohD* and *AtrbohF*, impairs ABA-induced ROS production and stomatal closure (Kwak et al., 2003). Recently, the activity of the NADPH oxidase has been shown to be regulated by the upstream kinase OST1 through phosphorylation, linking the phosphorylation event with ROS production and redox regulation (Sirichandra et al., 2009). Exogenous H_2O_2 can rescue both Ca^{2+} channel activation and stomatal closure in the ABA-insensitive mutant *gca2*, indicating the correlation between the ROS production and Ca^{2+} channel activation (Pei et al., 2000). This was further confirmed by the observation that the ABA-induced cytosolic Ca^{2+} increase and activation of plasma membrane Ca^{2+}-permeable channels were impaired in the *AtrbohD*/*F* guard cells (Kwak et al., 2003).

Nitric oxide, one of the reactive nitrogen species (RNS), is an important signaling messenger in the guard cell ABA signaling pathway (Beligni and Lamattina, 2001). NO was shown to selectively regulate Ca^{2+}-sensitive ion channels of *Vicia* guard cells by promoting Ca^{2+} release from intracellular stores to raise cytosolic-free [Ca^{2+}] (Garcia-Mata et al., 2003). Together with ROS, RNS could alter the cellular redox environment. Two groups of ROS scavengers, non-enzymatic (e.g. ascorbate and glutathione) and enzymatic (e.g. superoxide dismutase, glutathione peroxidase, and ascorbate peroxidase), function together to adjust and balance the redox status within the cells. ROS, RNS and the scavenging system maintain the cellular redox homeostasis (Pitzschke et al., 2006). The redox status of guard cells is important in stomatal movement. Chen and Gallie (2004) observed that the levels of H_2O_2 and ascorbate redox in guard cells are diurnally regulated, i.e., the former increases in the afternoon whereas the latter decreases in the course of stomata closure. It is reasonable to predict the existence of signaling components in guard cells that are under redox regulation or interact with ROS and RNS.

Besides the interaction between Ca^{2+} channels and ROS, only a few redox regulated components in guard cell signaling are known. For example, one of the *Arabidopsis* annexins, known as the target of calcium signaling, has been shown to be susceptible to oxidation-driven S-glutathionylation on the two cysteines in the calcium reactive S3 cluster (Konopka-Postupolska et al., 2009). The S-glutathionylation occurs after ABA treatment, indicating the annexin could be regulated by the ABA induced ROS production. Recently, the activity of oxidized β-amylase (BAM1) was reported to be restored by thioredoxin isoform *f1* and partially by NADPH-thioredoxin reductase. This redox-regulated BAM1 plays a role in diurnal starch degradation important for stomatal opening (Valerio et al., 2011).

Phosphorylation/dephosphorylation and redox control are clearly important regulatory mechanisms in the guard cell signaling networks, where more components and interactions need to be discovered. In mammals, many signaling proteins have been shown to be redox regulated, including Ca^{2+}-ATPase, Ras-related GTPase, EGF growth factor, phosphorylase β kinase and voltage-dependent anion channel protein (Suzukawa et al., 2000; Adachi et al., 2004; Sethuraman et al., 2004). In guard cells, little is known about the interaction between the redox regulation and kinase/phosphatase signaling cascades. Recently, it was found that stomata of the ethylene receptor mutant *etr1* did not close in response to H_2O_2, and mutation of a cysteine residue in ETR1 disrupted H_2O_2-induced stomatal closure (Desikan et al., 2005). In maize, the ABA-induced H_2O_2 production activates a 46 kDa mitogen-activated protein kinase (p46MAPK) and the activation of p46MAPK regulates the production of H_2O_2, suggesting a positive feedback loop involving NADPH oxidase, H_2O_2, and ZmMAPK5 in ABA signaling (Lin et al., 2009). However, direct evidence for thiol modification and links between protein redox change in guard cells and stomatal closure remains to be demonstrated.

Conclusions

Although great progress has been made to elucidate hormone (e.g. ABA and MeJA) signal transduction pathways in guard cells, our knowledge of the pathways and the molecular networks detailing the interactions between different signaling and metabolic components and pathways are far from complete. Recent years have seen great progress in plant proteomics (Chen and Harmon, 2006). However, only a few proteomic analyses of guard cell signaling have been done (Zhao et al., 2008; Zhu et al., 2009; Zhao et al., 2010). High throughput proteomic approaches have shown utility in identifying hormone responsive proteins, and are powerful in characterizing protein posttranslational modifications including phosphorylation and thiol redox using isolated guard cells so that more novel nodes and edges in guard cell

molecular networks can be discovered. Moreover, Due to the presence of ROS as an important signaling messenger, the protein redox modification and the dynamic changes are critical regulatory mechanisms in ABA and MeJA signal transduction that need to be investigated.

Acknowledgements

The research on guard cells was supported by grants from the US National Science Foundation (MCB 0818051) and the National Institute of Health (1S10RR025418-01) to S. Chen, and grants from the National Natural Science Foundation of China (Grant No. 31071194) and Funds for Distinguished Young Scientists of Heilongjiang Province, China (No. JC201011) to S. Dai.

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