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Invited Expert Review

Abscisic Acid Receptors: Past, Present and Future

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Abstract

Abscisic acid (ABA) is the key plant stress hormone. Consistent with the earlier studies in support of the presence of both membrane- and cytoplasm-localized ABA receptors, recent studies have identified multiple ABA receptors located in various subcellular locations. These include a chloroplast envelope-localized receptor (the H subunit of Chloroplast Mg^{2+}-chelatase/ABA Receptor), two plasma membrane-localized receptors (G-protein Coupled Receptor 2 and GPCR-type G proteins), and one cytosol/nucleus-localized Pyrabactin Resistant (PYR)/PYR-Like (PYL)/Regulatory Component of ABA Receptor 1 (RCAR). Although the downstream molecular events for most of the identified ABA receptors are currently unknown, one of them, PYR/PYL/RCAR was found to directly bind and regulate the activity of a long-known central regulator of ABA signaling, the A-group protein phosphatase 2C (PP2C). Together with the Sucrose Non-fermentation Kinase Subfamily 2 (SnRK2s) protein kinases, a central signaling complex (ABA-PYR-PP2Cs-SnRK2s) that is responsible for ABA signal perception and transduction is supported by abundant genetic, physiological, biochemical and structural evidence. The identification of multiple ABA receptors has advanced our understanding of ABA signal perception and transduction while adding an extra layer of complexity.

Keywords: ABA; ChlH/ABAR/CCH/GUN5; FCA; GCR2; GTG1/GTG2;PYL/PYL/RCAR.

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Introduction

Plant hormone abscisic acid (ABA) is an isoprenoid. Both the synthesis and metabolism of ABA are under tight control in response to environmental cues (Nambara and Marion-Poll 2005). ABA not only plays pivotal roles in plant adaption to environmental challenges but also regulates diverse aspects of plant growth and development, such as seed dormancy and early seedling development (Zhu 2002). In recent years, considerable progress has been made on the identification and characterization of components of the ABA signaling pathway, including both positive and negative regulators. In particular, much progress has been made on the identification of ABA receptors and the understanding of the molecular and biochemical mechanisms regarding ABA perception and transduction. In this review, we will focus on the recent

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developments of ABA receptor identification. For ABA biosynthesis and metabolism, signal transduction and its physiological functions, readers should refer to the following review articles (Finkelstein et al. 2002; Finkelstein and Gibson 2002; Nambara and Marion-Poll 2005; Finkelstein et al. 2008; Wang and Zhang 2008; Wasilewska et al. 2008; Cutler et al. 2010; Graeber et al. 2010; Hubbard et al. 2010; Kim et al. 2010; Kline et al., 2010; Klingler et al. 2010; Lumba et al. 2010; Melcher et al. 2010; Umezawa et al. 2010; Weiner et al. 2010).

Earlier Evidence Supporting the Presence of Multiple ABA Binding Sites

A pioneering study by Hartung (1983) provided the initial evidence for the presence of ABA binding and perception sites at the cell surface in *Valerianella locusta*. The author explored the property that protonated ABA can diffuse through the plasma membrane while ABA^- cannot, and observed that both forms of ABA were able to promote stomatal closure. These findings suggested that passing through plasma membrane may be not necessary for ABA-triggered response. Later studies isolated ABA-binding proteins from the plasma membrane fractions, providing further evidence for the presence of ABA binding proteins at the plasma membrane (Hornberg and Weiler 1984; Pedron et al. 1998). It should be noted that some membrane-localized ABA-binding proteins isolated earlier could actually function as ABA transporters identified recently (Kang et al. 2010; Kuromori et al. 2010). Using the cell membrane impermeable ABA-bovine serum albumin conjugate, independent studies found that the plasma membrane-perception sites are sufficient to trigger the expression of ABA-responsive genes in rice (Schultz and Quatrano 1997) and *Arabidopsis* suspension cells (Jeannette et al. 1999; Ghelis et al. 2000), and alter the anion current and K^+ efflux in *Arabidopsis* suspension cells (Jeannette et al. 1999; Ghelis et al. 2000). Later on, Yamazaki et al. (2003) visualized the binding of ABA on the surface of plasma membrane by utilizing the biotin-ABA conjugate in combination with fluorescence-labeled avidin and fluorescence microscopic technique. These studies all pointed to the presence of ABA binding/perception sites at the plasma membrane.

Meanwhile, a number of investigations provided evidence in support of the presence of cytosolic perception sites for ABA. For example, microinjection of ABA into *Commelina* guard cells was found to be sufficient to induce stomatal closure (Allan et al. 1994; Schwartz et al. 1994), although a similar study from another group provided contradictory evidence (Anderson et al. 1994). Levechenko et al. (2005) found that although externally-applied ABA was able to activate the guard cell anion channels, injection of ABA into cytosol activates the anion channel faster and more pronounced, indicating the presence of an internal ABA perception site.

In accordance with these earlier findings, recent studies have identified two types of plasma membrane-localized ABA receptors (Liu et al. 2007a; Pandey et al. 2009), one chloroplast envelop-localized ABA receptor (Shen et al. 2006), and one cytosol/nucleus-localized ABA receptor (Ma et al. 2009; Park et al. 2009).

Recent Discoveries of Multiple ABA Receptors

Although the traditional forward genetics approach has been successful in receptor discovery for other plant hormones, such as ethylene (Chang et al. 1993) and brassinosteroids (Li and Chory 1997), it has failed to identify any ABA receptors. This led to the assumption that either ABA receptors belong to a gene family with redundant functions, or that mutations in the ABA receptors confer lethality (McCourt 1999). The identification of multiple ABA receptors via other strategies later supported both assumptions.

FCA

Flowering time control protein A (FCA) is an RNA-binding protein that regulates flowering time in *Arabidopsis* (Macknight et al. 1997). Razem et al. (2006) reported that FCA functions as an ABA receptor. However, the binding property of FCA to ABA was questioned (Risk et al. 2008) and the original article claiming FCA as an ABA receptor was retracted (Razem et al. 2008).

ChlH/ABAR/CCH/GUN5

The magnesium protoporphyrin-IX chelatase (Mg^{2+}-chelatase) catalyzes the insertion of Mg^{2+} into protoporphyrin IX in the process of chlorophyll synthesis (Walker and Willows 1997). The putative H subunit of the chloroplast Mg^{2+}-chelatase (ChlH) was initially isolated as an ABA-binding protein from the epidermis of broad bean (*Vicia faba*) via affinity chromatography (Zhang et al. 2002). *Arabidopsis* ChlH/ABAR (Abscisic Acid Receptor)/CCH (Conditional Chlorina)/GUN5 (Genome Uncoupled 5)(Mochizuki et al. 2001) was later found to specifically bind (+)-ABA with high affinity and mediate all major aspects of ABA responses including seed germination, early seedling development and stomatal movement (Shen et al. 2006). Recently, a key regulator of circadian clock, TOC1 (timing of CAB expression 1), was found to bind to the promoter of ChlH/ABAR and modulate its circadian expression (Legnaioli et al. 2009), raising the possibility that ChlH/ABAR might serve as a link between ABA signaling and the circadian clock. Biochemical and genetic evidence indicated that the C-terminal half (amino acid residues 631–999) of ChlH/ABAR

is the core ABA binding domain and plays a central role in mediating ABA responses (Wu et al. 2009). Since ChlH/ABAR is localized at the chloroplast envelope, an intriguing question is how ChlH/ABAR perceives and transduces ABA signal to regulate the ABA-mediated massive transcriptional responses. To address this question, a more recent study from the same group identified several WRKY (WRKYGQK-containing) transcription factors (WRKY40, WRKY18 and WRKY60) that could interact with the cytosolic C-terminus of the ChlH/ABAR (Shang et al. 2010). The authors demonstrated that under low cellular ABA levels, WRKY40, via binding to the W-BOX *cis*-elements, could inhibit the expression of several key transcription factors, such as ABI5 and DREB2A that had been previously shown to be responsible for the activation of the ABA responsive genes. High ABA concentrations, on the other hand, promote the ChlH/ABAR to sequester WRKY40 to the cytosol, thus allowing the expression of these transcription factors and they, in turn, could activate the expression of ABA-responsive genes (**Figure 1**). Consistent with this view, single, double and triple mutants among these three *WRKY* genes displayed ABA hypersensitivity (Shang et al. 2010). Notably, a recent study from another research group (Chen et al. 2010) did not observe the increased expression of *ABI5* and *DREB2A* in *wrky40* mutants, nor did they observe the hypersensitive response of *wrky40* mutants to ABA. Their genetic studies with mutants and overexpression lines supported WRKY18 and WRKY60 as positive regulators of ABA responses, contradictory to what Shang et al. (2010) had observed. In addition, conflicting experimental evidence was presented on whether barley ChlH/ABAR homolog could function as an ABA receptor (Muller and Hansson 2009; Wu et al. 2009; Shang et al. 2010). Therefore, the exact role of ChlH/ABAR and WRKY18, 40 and 60 in ABA signaling requires further investigation.

It should be noted that another ABA signaling component, ABI4, was also identified as a component of the plastid-nucleus retrograde signaling pathway (Koussevitzky et al. 2007). However, ABA may not be a plastid-derived retrograde signal since retrograde signaling was not influenced by either ABA-deficient mutant or exogenously-applied ABA (Koussevitzky et al. 2007).

GCR2

The identification of GCR2 (G-protein Coupled Receptor 2) as an ABA receptor began with a bioinformatic analysis in which the GCR2 protein was predicted to harbor seven transmembrane domains, a common structural feature for classical G-protein Coupled Receptors (GPCRs) in mammalian cells (Liu et al. 2007a). GCR2 was found to interact with the sole *Arabidopsis* heterotrimeric Gα subunit and is reported to be involved in all aspects of ABA-regulated physiological response including seed germination, stomatal closure and the expression of ABA-responsive genes. Through genetic, physiological and biochemical analysis, GCR2 was proposed to function as an ABA receptor (Liu et al. 2007a). However, Gao et al. (2007) could not observe the insensitivity of *gcr2* mutants to ABA in seed germination. Moreover, different combinations of mutants of *GCR2* and its homologs (GCR2-Like 1 and GCR2-Like 2) displayed mild or wild-type response to ABA in seed germination (Guo et al. 2008). Additional bioinformatic predictions did not detect seven transmembrane domains in GCR2 (Johnston et al. 2007; Liu et al. 2007b; Chen and Ellis 2008; Illingworth et al. 2008). Furthermore, the ABA-binding property of GCR2 is still controversial (Liu et al. 2007a; Risk et al. 2009), considering the fact that FCA, a falsely-identified ABA binding protein, was used as a positive control in GCR2's ABA binding assay (Liu et al. 2007a). Therefore, whether GCR2 functions as an ABA receptor remains elusive.

GTG1/GTG2

More recently, a novel class of GPCRs, GTGs (GPCR-type G proteins), were proposed as ABA receptors (Pandey et al. 2009). The predicted topology of GTG proteins is similar to that of GPCR. In addition, GTG proteins contain GTP binding/GTPase activity, which is not commonly present in classical GPCRs. GTGs interact with the sole *Arabidopsis* conical heterotrimeric G-protein α-subunit, GPA1, and the GTP-bound form of GPA1 inhibits the GTPase activity of GTG proteins. The GDP-bound GTGs bind ABA stronger than GTP-bound form, thus it might be the active form for perceiving and transducing ABA signal (**Figures 1**, **2**). The *gtg1*/*gtg2* double mutant displayed ABA hyposensitivity in seed germination, seedling growth and ABA-induction of stomatal closure. However, ABA responses were not abolished in the *gtg1*/*gtg2* mutant, implying the presence of other ABA signal perception sites. Due to the difficulty in isolating functionally active transmembrane protein, the observed stoichiometry of ABA binding was low ($\sim$0.01 mol ABA/mol protein), thereby requiring confirmation of binding activity with better quality protein (Christmann and Grill 2009; Jones and Sussman 2009; Pandey et al. 2009; Risk et al. 2009). It should also be noted that the GTG proteins were predicted to have nine transmembrane domains, unlike classical GPCRs, which contain seven transmembrane domains.

PYR/PYL/RCARs

Identification of PYR/PYL/RCARs as ABA receptors

While traditional genetic studies had failed to identify any ABA receptors, a chemical genetics approach succeeded by using an agonist of ABA, pyrabactin. Pyrabactin was earlier isolated from a chemical library as a seed germination inhibitor (Zhao et al. 2007). In addition to their shared seed germination

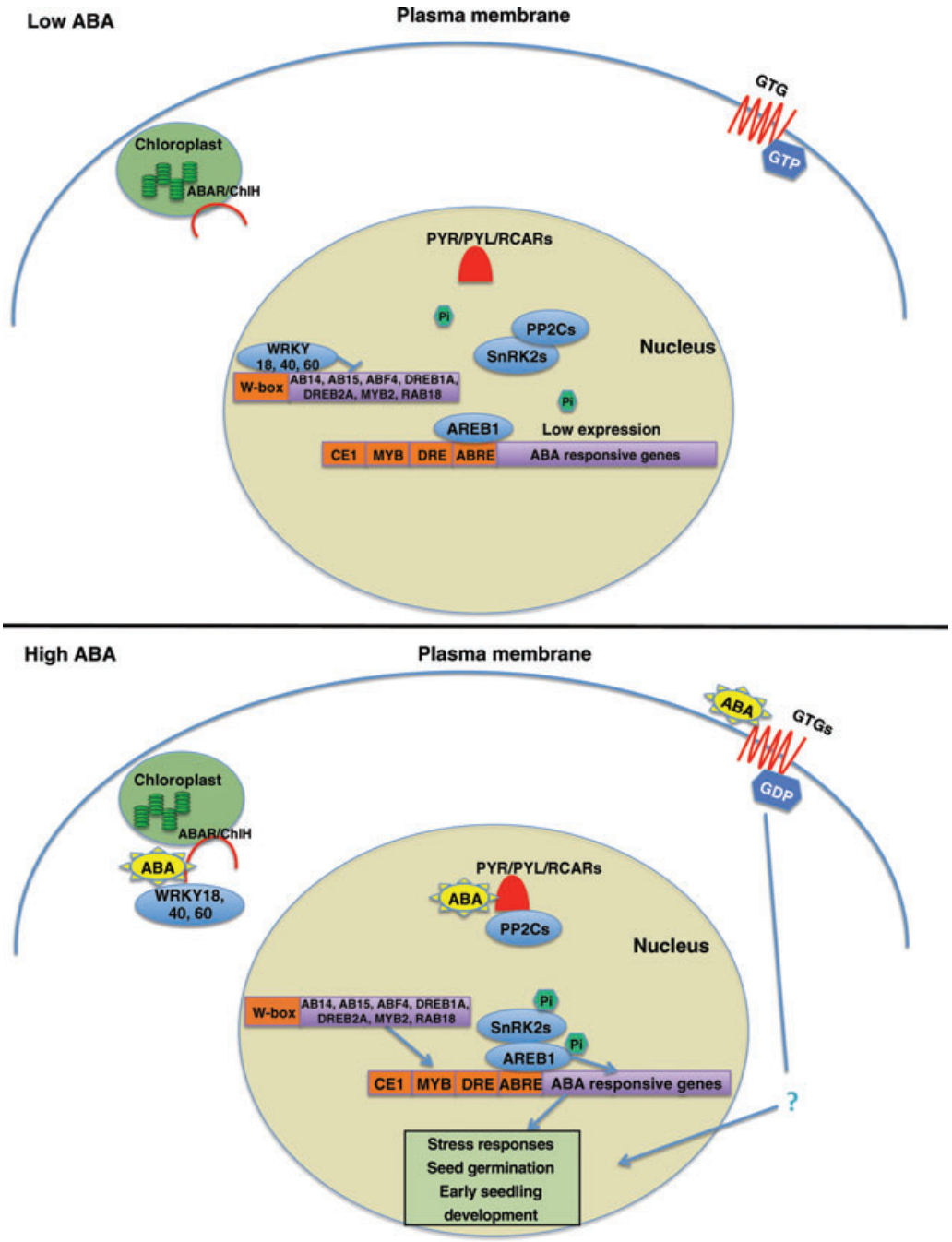

Figure 1. Abscisic acid (ABA) receptors-mediated signaling pathways in the regulation of transcriptional responses.

Under low cellular ABA concentrations: (1) GTG is present as GTP-bound, inactive form; (2) ABAR/ChlH cannot sequester WRKYs, therefore WRKYs bind to the W-box of many key ABA-signaling transcription factors, such as ABI4, ABI5, ABF4, DREB1A, DREB2A, MYB2 and RAB18, and inhibit their expression; and (3) PYR1/PYL/RCAR cannot bind PP2C, hence PP2C binds, de-phosphorylates and deactivates SnRK2s. Under high ABA concentrations (e.g. triggered by environmental stimuli): (1) GDP-bound form of GTG binds ABA and regulates the expression of ABA-responsive genes via an unknown mechanism; (2) ABA-bound ABAR/ChlH sequesters WRKYs to the cytosol, thus allowing the expression of ABA-signaling transcription factors which, through binding to different cis-elements, such as CE1, MYB, DRE and ABRE, activate the expression of ABA-responsive genes; and (3) ABA-bound PYR/PYL/RCARs bind and deactivate the phosphatase activity of PP2C. Consequently, SnRK2 can be activated via autophosphorylation, which in turn phosphorylates AREB1, which recognizes

inhibition property, pyrabactin and ABA also induced highly correlated transcriptional response (r = 0.98) in seeds. In contrast, the transcriptional response in seedlings for ABA and pyrabactin was poorly correlated and few ABA-responsive genes significantly respond to pyrabactin. Having noticed that pyrabactin is a selective agonist of ABA, Park et al. (2009) used this compound to identify ABA signaling components. In a screen for pyrabactin-resistant mutants, Park et al. (2009) isolated 12 PYRABACTIN RESISTANCE 1 (*Pyr1*) mutant alleles in *Arabidopsis*. Mapping results revealed that PYR1 belongs to the START/Bet v I superfamily and possesses the conserved hydrophobic ligand-binding pocket. The *Arabidopsis* genome contains 13 *PYR1*-like genes (named *Pyl1*-*Pyl13*). Although *pyr1* single mutants displayed normal response to ABA, *pyr1*;*pyl1*;*pyl4* triple and *pyr1*; *pyl1*; *pyl2*; *pyl4* quadruple mutants displayed strong hyposensitivity to ABA with respect to ABA-regulated seed germination, root growth, transcription reprogramming and SnRK2 (Sucrose non-fermentation kinase subfamily 2) kinase activity. The *pyr1*; *pyl1*; *pyl2*; *pyl4* quadruple mutant also displayed ABA-hyposensitivity in ABA-regulated stomatal closure (Nishimura et al. 2010). Using PYR1 as bait in a yeast-two-hybrid screen in the presence of pyrabactin, HAB1 (hypersensitive to ABA 1), a member of the group A subfamily of plant PP2Cs, which has long been known to play a central role in ABA signaling (Leube et al. 1998; Rodriguez 1998; Rodriguez et al. 1998), was isolated as a physical interacting partner of PYR1. Two other members of the same group of PP2Cs also physically interacted with PYR1 in yeast two hybrid assay in the presence of pyrabactin or ABA (Park et al. 2009). Furthermore, RCAR/PYR/PYL proteins were also isolated in a Y2H screening using HAB1 as bait (Santiago et al. 2009b) and in an liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay of *in vivo* ABI1-complex (Nishimura et al. 2010). Further analysis confirmed that ABA binds PYR1 and the ABA-bound PYR1 then binds to PP2C and inhibits its activity, supporting the view that PYR/PYLs function as ABA receptors through inhibiting the phosphatase activity of PP2C.

In an independent study, Ma et al. (2009) isolated another member of the PYR/PYL family, PYL9, as one of the ABI2 (also a member of the A group PP2Cs) interacting proteins, and named it the Regulatory Component of ABA Receptor 1 (RCAR1) (Ma et al. 2009). In earlier studies, Ma et al. (2009) noticed that micro-molar level of ABA leads to about 20% reduction of ABI1 phosphatase activity, but no ABA-bound ABI1 was detected (Leube et al. 1998). In the presence of RCAR1, the ABA almost completely blocked the phosphatase activity of ABI2 at nano-molar level (Ma et al. 2009). In addition, transient overexpression of RCAR1 enhanced the ABA response of the expression of some ABA marker genes and stable RCAR1 overexpression transgenic lines displayed ABA-hypersensitivity with regard to ABA-regulated inhibition of seed germination, inhibition of root elongation and stomatal closure. Binding assays indicate that ABA binds RCAR1 with a ratio of one ABA molecule per RCAR1 molecule (Ma et al. 2009).

Structure of PYR/PYL/RCAR and the functional implication

While genetic, biochemistry and physiological studies advocate for PYR1/PYL/RCARs as ABA receptor, solving its crystal structure could provide invaluable information for elucidating its molecular mechanism of action. Recently, much progress has been made in deciphering the crystal structures of PYR1/RCAR11 and PYL9/RCAR1 in apo form, (+)-ABA-bound form and ABA/PYR/PP2C ternary complex (Melcher et al. 2009; Miyazono et al. 2009; Nishimura et al. 2009; Santiago et al. 2009a; Yin et al. 2009). These studies further confirmed that PYR/PYL/RCARs are ABA receptors and elucidated the biochemical actions of PYR and PP2Cs in ABA signal perception and transduction.

Via structural, biochemical and point mutation studies, it was established that PYR/PYL/RCARs consist of homodimers with one subunit binding ABA. The binding of ABA was suggested to dissociate the dimer. The ABA binding cavity of PYR/PYL/RCARs is flanked by the gate and latch loops, which, upon binding of an ABA molecule, fold over and enclose it inside (Melcher et al. 2009; Miyazono et al. 2009; Nishimura et al. 2009; Santiago et al. 2009a; Yin et al. 2009). The ABA-binding

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ABRE elements of ABA responsive genes, resulting in the activation of their expression. The transcriptome reprogramming triggered by ABA signaling will eventually cause physiological and developmental readouts, such as stress responses, seed germination and early seedling development. ABA, abscisic acid; GTGs (GPCR type G-proteins); ChlH (Chloroplast Mg^{2+} chelatase H-subunit)/ABAR (ABA receptor); PYR (pyrabactin resistant)/PYL (pyrabactin resistant-like)/RCARs (regulatory component of ABA receptor); PP2Cs (protein phosphatase 2C proteins); SnRK2s (sucrose non-fermentation kinase 2 proteins); AREB1 (ABRE binding protein 1); ABI4 (ABA insensitive 4); ABI5 (ABA insensitive 5); ABF4 (ABRE binding factor 4); DREB1A (DRE-binding protein 1A); DREB2A (DRE-binding protein 2A); RAB18 (responsive to ABA 18); WRKY (WRKYGQK-containing transcription factor); MYB2 (v-myb myeloblastosis viral oncogene homolog 2); W-box (WRKY-binding cis-elements); MYB (MYB transcription factor-binding cis-elements); DRE (drought responsive cis-element); ABRE (ABA responsive cis-elements); CE1 (coupling element 1, can be bound by ABI4); Pi (phosphate). GCR2 is not included in these modes due to its contradictory role as an ABA receptor.

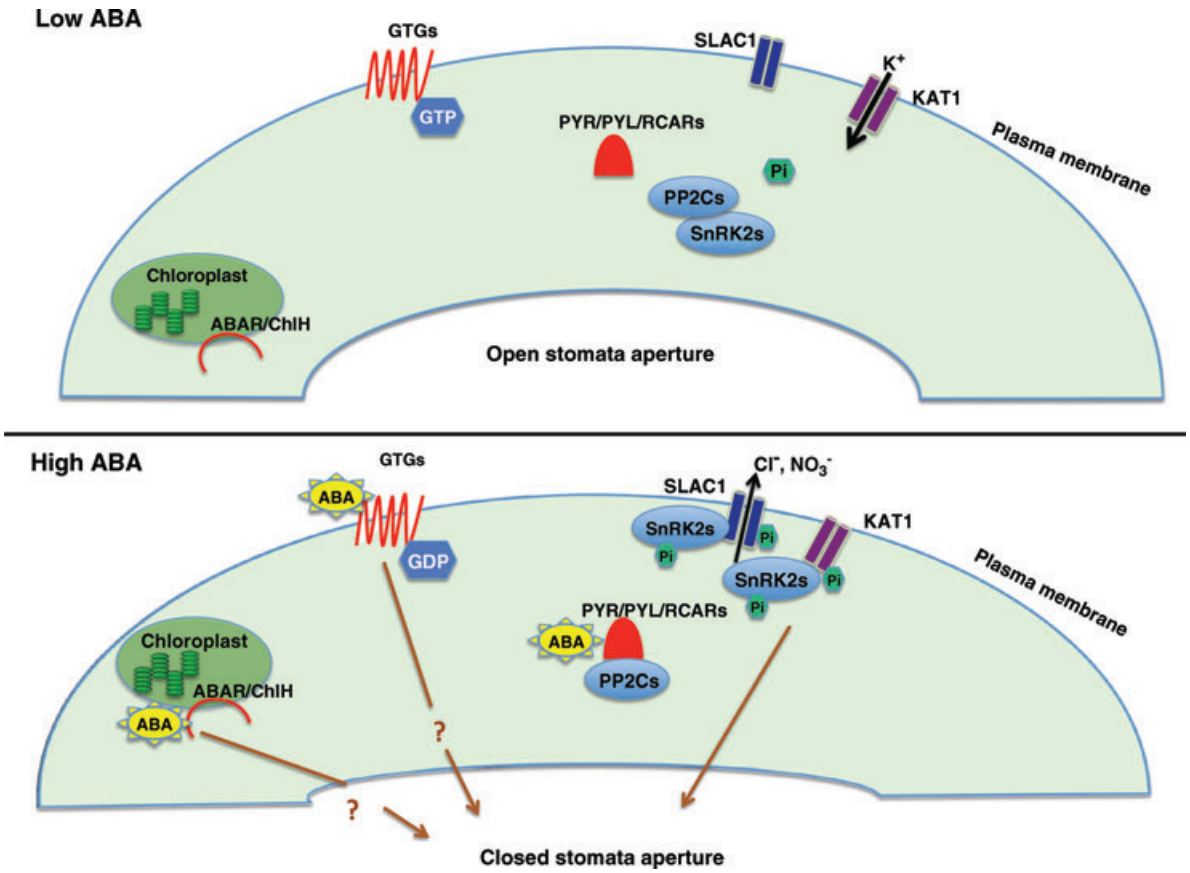

Figure 2. Abscisic acid (ABA)-receptors-mediated signaling pathways in the regulation of stomatal closure.

Under low cellular ABA concentrations: none of the ABA receptors-signaling pathways is activated; the non-phosphorylated SLAC1 channel is closed while the non-phosphorylated KAT1 channel is open, and the stomata stay open. Under high cellular ABA concentrations: (1) both GTG and ABAR/ChlH can mediate ABA signaling and lead to stomatal closure through unknown mechanisms; and (2) sequestration and deactivation of PP2Cs by ABA-bound PYR/PYL/RCAR leads to the activation of SnRK2, which can then phosphorylate/activate SLAC1 and phosphorylate/deactivate KAT1, resulting in a change of ion flux and cellular osmotic pressure leading to stomatal closure. GTGs (GPCR type G-proteins); ChlH (Mg^{2+} chelatase H-subunit)/ABAR (ABA receptor); PYR (pyrabactin resistant)/PYL (pyrabactin resistant like)/RCARs (regulatory component of ABA receptor); PP2Cs (protein phosphatase 2C proteins); SnRK2s (sucrose non-fermentation kinase 2 proteins); SLAC1 (slow anion channel 1); KAT1 (inward rectifying K^+ channel 1); Pi (phosphate). GCR2 is not included in these modes due to its contradictory role as an ABA receptor.

induces conformational changes of PYR/PYL/RCARs, which creates a new surface for PP2Cs to interact with. The interacting interface between ABA-bound PYR/PYL/RCARs and PP2Cs is located at the enzymatic active site of the PP2Cs, and therefore the interaction inhibits the phosphatase activity of PP2Cs by blocking the access of their substrates to the catalytic center. In addition, structural studies provided some answers to the mechanism of how pyrabactin acts as a selective agonist of ABA. When binding to its preferred PYR/PYL/RCAR members such as PYR1, pyrabactin can trigger a functional conformation change of PYR1 similar to that of ABA and allows PYR1 to bind and deactivate PP2Cs. When pyrabactin binds to unfavorable PYR/PYL/RCAR members such as PYL2, the conformation change was not complete and the interaction between PYR/PYL/RACA with PP2C could not happen (Hao et al. 2010; Peterson et al. 2010).

A central ABA signaling complex

The plant AMPK (AMP-activated Protein Kinase)/Snf1 (Sucrose-non-fermentation 1)-related kinases subfamily 2 (SnRK2) has long been recognized as a positive regulator of ABA signaling (Mustilli et al. 2002; Nakashima et al. 2009). The SnRK2 subfamily contains 10 members in *Arabidopsis*, and

triple mutant of the three *SnRK2s* that were most dramatically activated by ABA (Boudsocq et al. 2004, 2007) essentially lost response to ABA and were able to germinate on growth medium containing 50 μM ABA (Fujii and Zhu 2009). Recent studies discovered that the A group PP2Cs inhibit the activity of SnRK2.6 (OST1) by dephosphorylating SnRK2.6 at Serine 175 (Umezawa et al. 2009; Vlad et al. 2009). The addition of PYR1 and ABA can overcome the inhibitory effect of PP2Cs on SnRK2.6 activity. Therefore, the regulation of the activity of SnRK2s by PP2Cs and the regulation of the activity of PP2Cs by ABA-bound PYR/PYL/RCARs have emerged as a central mechanism of PYR/PYL/RCARs-mediated ABA signaling (**Figure 1**).

It is proposed that under normal growth conditions where cellular ABA levels are low, PP2Cs bind to and dephosphorylate SnRK2s to keep the SnRK2s in an inactive state. When plants are subjected to stress conditions, elevated cellular ABA binds PYR/PYL/RCARs, which in turn bind and deactivate phosphatase activity of PP2Cs. The SnRK2s are then released from PP2Cs and self-activated via auto-phosphorylation. The activated SnRK2s are able to phosphorylate different downstream targets to trigger various ABA-induced physiological responses. Two classes of SnRK2 downstream targets have been identified to date. The first class comprises the members of the b-ZIP transcription factors that can bind ABA-responsive elements (ABRE) in the promoter of ABA responsive genes, including *AREB1* (*ABRE binding protein 1*), *AREB2*, *AREB3* and *ABI5* (Uno et al. 2000; Fujita et al. 2005; Furihata et al. 2006; Fujii et al. 2009; Yoshida et al. 2010). SnRK2s can phosphorylate AREB1 and the phosphorylated AREB1 can then activate the expression of ABA responsive genes (Furihata et al. 2006; Fujii et al. 2009) (**Figure 1**). The second class of the SnRK2s targets includes two ion channels, the SLAC1 (slow anion channel 1) and the KAT (inward rectifying K^+ channel), both of which are known to mediate ABA-regulated stomatal closure (Pilot et al. 2001; Vahisalu et al. 2008). The SnRK2s phosphorylation activates SLAC1 and deactivates KAT, and consequently leads to the depolarization of plasma membrane and activation of the outward K^+ channel. The leaking of anions and K^+ causes a loss of turgor in guard cells and hence stomatal closure (Mori et al. 2000; Geiger et al. 2009; Lee et al. 2009) (**Figure 2**).

In *Arabidopsis*, there are 14 members in the PYR/PYL/RCAR family (Park et al. 2009), nine members in the A group of PP2C family (Schweighofer et al. 2004) and 10 members in the SnRK2 subfamily. While all members of PYR/PYL/RCAR family seem to be involved in ABA signaling (Park et al. 2009), six of the A group PP2Cs were identified as negative regulators of ABA signaling (Szostkiewicz et al. 2010), and five SnRK2s could be activated by ABA in an *Arabidopsis* leaf mesophyll protoplast system (Boudsocq et al. 2004, 2007). At least some of the specific interactions between individual SnRK2s and PP2Cs correlate with their expression pattern and their downstream role in ABA-induced physiological responses (Umezawa et al. 2009). Interestingly, while the interaction of HAB1 with PYR1 and PYL1 is dependent upon the presence of ABA, the interaction of HAB1 with PYL5, PYL6 or PYL8 is not (Park et al. 2009; Santiago et al. 2009b). Therefore, the combination of different interactions among different members of PYR, PP2C and SnRK2 offer a large capacity and complexity of ABA signaling.

Concluding Remarks and Future Directions

Although multiple ABA receptors have been identified, except for that of PYR/PYL/RCAR, most of their modes of action remain poorly understood. Therefore, identifying the chain of molecular events from ABA perception by these receptors to their physiological function is essential for better defining the molecular network of ABA signaling. Defining the crystal structure of these ABA receptors may help understand their molecular mechanisms of action. The second challenge is to understand the relationship among all of these ABA receptors. All ABA receptors identified so far are ubiquitously expressed and seem to participate in the regulation of every aspect of major known ABA-regulated physiological responses. However, because the acidic environment in the intercellular space favors the diffusion of ABA into the cell, there is no evidence so far about the biological meaning of so many receptors at different subcellular compartments, and about how and at which point the ABA signal perceived through different ABA receptors is converged. The third important question is how other numerous signaling molecules that have been demonstrated to be involved in ABA signaling pathway can be integrated into the established ABA signaling pathway derived from various ABA receptors. Furthermore, while the current models with ABA receptors help us understand a few aspects of ABA-regulated downstream events, such as transcriptional reprogramming and regulation of ion channels on guard cells, many other downstream events are yet to be elucidated. For example, abiotic stress and ABA have been long known to regulate protein translation (Bensen et al. 1988; Kawaguchi et al. 2003; Guo et al. 2011), RNA metabolism (Kuhn et al. 2008), and epigenetic modifications (Chinnusamy et al. 2008; Chinnusamy and Zhu 2009). How ABA perception is transduced to the regulation of these downstream events will be very interesting topics for future studies.

The study of structural and functional mode of the ABA/PYR/PP2C complex has profound influence not only in the understanding of ABA signaling, but also in molecular

engineering the ABA plant stress pathway (Liang et al. 2011). ABA is the major plant hormone mediating plant response to various stress conditions including drought. Understanding its functional mode *in vivo* and utilizing this knowledge to guide genetic engineering to produce drought-tolerant crops are therefore essential for helping to solve problems associated with the anticipated yield reduction from the global water crisis in coming years.

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