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Regulatory Networks of the Phytohormone Abscisic Acid

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Structurally similar to retinoic acid (RA), the phytohormone abscisic acid (ABA) controls many developmental and physiological processes via complicated signaling networks that are composed of receptors, secondary messengers, protein kinase/phosphatase cascades, transcription factors, and chromatin-remodeling factors. In addition, ABA signaling is further modulated by mRNA maturation and stability, microRNA (miRNA) levels, nuclear speckling, and protein degradation.

Vitamins and Hormones, Volume 72

0083-6729/05 $35.00
DOI: 10.1016/S0083-6729(05)72007-0

This chapter highlights the identified regulators of ABA signaling and reports their homologues in dicotyledonous and monocotyledonous plants.

I. INTRODUCTION

Abscisic acid plays a variety of roles in plant development, bud and seed dormancy, germination, cell division and movement, leaf senescence and abscission, and cellular response to environmental stresses (Leung and Giraudat, 1998; Rohde *et al.*, 2000; Zhu, 2002). It is ubiquitous in lower and higher plants and has also been found in algae (Hirsch *et al.*, 1989), fungi (Yamamoto *et al.*, 2000), and even mammalian brain tissue (Le Page-Degivry *et al.*, 1986). Abscisic acid and RA are similar in several aspects: (1) the structure of ABA, a 15-carbon sesquiterpenoid carboxylic acid, is very similar to RA (Fig. 1); (2) both ABA and RA are synthesized ultimately from β-carotene (provitamine A); and (3) only certain geometric isomers

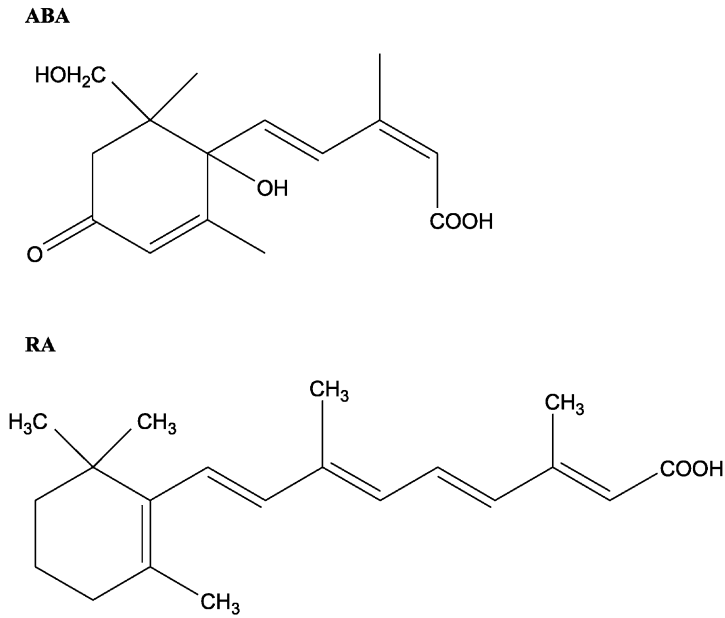

FIGURE 1. Abscisic acid (ABA) is structurally similar to retinoic acid (RA). Shown here are the geometric isomers of biologically active ABA (C2-*cis*, C4-*trans* isomer) and RA (all *trans*). Another geometric isomer of RA, 9-*cis* RA is also biologically active.

are biologically active. Retinoic acid is active in two forms: all *trans* RA and 9-*cis* RA. For ABA, the C2-*cis*, C4-*trans* isomer, but not the C2-*trans*, C4-*trans* isomer, is biologically active (Milborrow, 1978). However, the mechanisms of cellular response to RA and ABA are quite different. Retinoic acid is perceived by an intracellular receptor that belongs to the nuclear receptor superfamily. The RA receptor (RAR) forms a heterodimer with a common nuclear receptor monomer, RXR, that is located exclusively in the nucleus. In the absence of ligand, the heterodimer represses transcription of promoters that contain the cognate RA response elements by directing histone deacetylation at nearby nucleosomes. Binding of RA to RAR results in a dramatic conformational change of RAR that can still heterodimerize with RXR. However, in the ligand-bound conformation, the heterodimeric nuclear receptors direct hyperacetylation of histones in nearby nucleosomes to reverse the effects of the ligand-free heterodimer. The ligand-binding domain of nuclear receptors also binds mediators and stimulates the assembly of transcriptional pre-initiation complexes (Lodish *et al.*, 2004). In contrast, the response of plant cells to ABA involves a signal network containing receptors, secondary messengers, protein kinases and phosphatases, chromatin-remodeling proteins, transcriptional regulators, RNA-binding proteins, and protein degradation complexes (Chinnusamy *et al.*, 2004; Fan *et al.*, 2004; Finkelstein and Rock, 2001; Hare *et al.*, 2003; Himmelbach *et al.*, 2003; Kuhn and Schroeder, 2003; Lovegrove and Hooley, 2000; Ritchie *et al.*, 2002; Rock, 2000; Schroeder *et al.*, 2001).

Aleurone cells, suspension cells, protoplasts, and mutants/transgenic plants of several species have been used to address the complicated ABA-signaling networks for guard cell movement and other aspects of stress responses, seed germination, and growth of vegetative tissues. However, it is believed that ABA-signaling networks are conserved among higher plant species; information derived from several plant species has been used to compile a network map of ABA signaling (Finkelstein and Rock, 2001; Himmelbach *et al.*, 2003). Assessment of the universality of ABA-signaling mechanisms is greatly facilitated by the availability of the genome sequences and full-length cDNA sequences of *Arabidopsis* (Seki *et al.*, 2002b; The *Arabidopsis* Initiative, 2000) and rice (Goff *et al.*, 2002; Kikuchi *et al.*, 2003; Yu *et al.*, 2002). In this review, we summarize the advances in ABA-signaling research and report the closest (lowest *E*-value) rice homologues of known ABA-signaling regulators. We identified these homologues by BLAST searching against a comprehensive rice peptide database that contains the sequences downloaded from NCBI (http://www.ncbi.nlm.nih.gov) and TIGR (http://www.tigr.org/) and those deduced from the longest open reading frame (ORF) of rice full-length cDNA sequences (Kikuchi *et al.*, 2003).

II. SIGNALING PATHWAYS

A. RECEPTORS

The site and nature of ABA perception were addressed in barley aleurone cells and guard cells of several plant species. Externally applied but not microinjected, ABA could repress gibberellin (GA)-induced α-amylase expression in aleurone protoplasts, suggesting an extracellular perception of ABA (Gilroy and Jones, 1994). This notion is supported by two studies using ABA-protein conjugates that cannot enter the cell, yet are able to regulate ion channel activity (Jeannette *et al.*, 1999) and gene expression (Jeannette *et al.*, 1999; Schultz and Quatrano, 1997). It is also supported by a study in *Commelina* guard cells (Anderson *et al.*, 1994). In contrast, introduction of ABA into the cytoplasm by microinjection (Schwartz *et al.*, 1994) or a patch-clamp electrode (Allan *et al.*, 1994) triggered or maintained stomatal closure arguing for intracellular perceiving sites. Other approaches taken to identify ABA receptors (Desikan *et al.*, 1999; Leyman *et al.*, 1999, 2000; Sutton *et al.*, 2000; Yamazaki *et al.*, 2003) have resulted in several leads. One promising receptor candidate is ABAP1 (Fig. 2) that is located in membrane fractions of ABA-treated barley aleurone cells. It is capable of specifically yet reversibly binding to ABA at a capacity of 0.8 mol of ABA mol^{-1} protein with a K_d of 2.8×10^{-8} M, and it is present in diverse monocotyledonous and dicotyledonous species (Razem *et al.*, 2004). Another candidate is GCR1, a putative G-protein–coupled receptor identified in *Arabidopsis* (Pandey and Assmann, 2004) that can directly interact with GPA1, the α-subunit of G-proteins. The *Arabidopsis gcr1* knockout mutant is more sensitive to ABA and more tolerant to drought stress due to reduced rates of water loss. These data suggest that GCR1 may function as a negative regulator of ABA signaling (Pandey and Assmann, 2004). The closest rice homologues of ABAP1, GPA1, and GCR1 are shown in Table I.

B. G-PROTEINS

As mentioned previously, heterotrimeric G-proteins are involved in the transduction of ABA signal in *Arabidopsis* (Pandey and Assmann, 2004; Wang *et al.*, 2001). In cereal aleurone cells, the activation of a plasma-membrane–bound ABA-inducible phospholipase D (PLD) is essential for ABA response (Ritchie and Gilroy, 2000). This process is GTP-dependent; addition of GTPγS transiently stimulates PLD in an ABA-independent manner, whereas treatment with GDPβS or pertussis toxin blocks the PLD activation by ABA. These data suggest the involvement of G-protein activity in the ABA response of barley (Ritchie and Gilroy, 2000). Monomeric G-proteins also regulate ABA responses (Lemichez *et al.*, 2001; Yang, 2002). ROP10, a plasma-membrane–associated small GTPase, appears to negatively

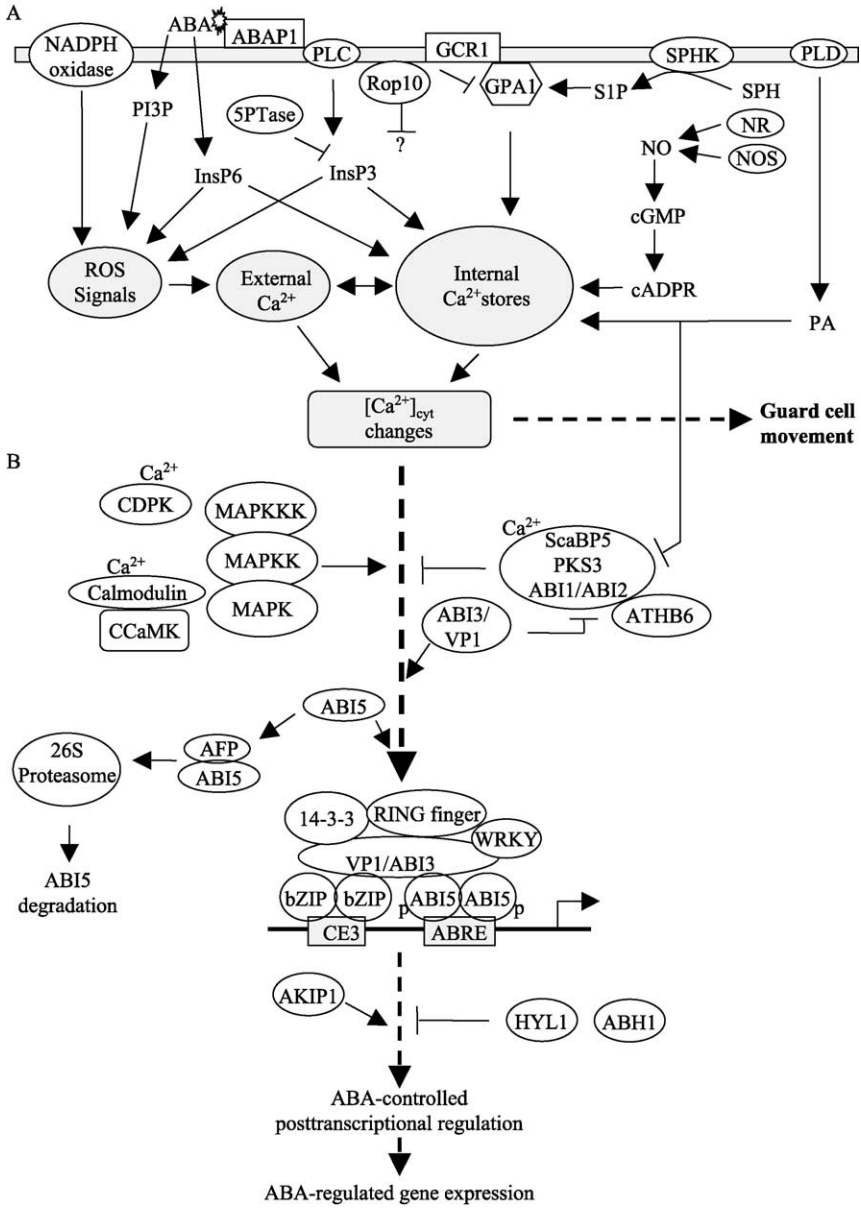

FIGURE 2. An integrated schematic diagram of abscisic acid (ABA)-signaling networks. The model is not comprehensive and does not address tissue specificity. Most relationships in (A) are derived from studies in guard cells while those in (B) are derived from studies in seeds or seedlings. Abscisic acid signaling is perceived by ABAP1 and GCR1 that interacts with GPA1 and functions as a negative regulator. Abscisic acid-induced sphingosine kinase (SPHK) converts sphingosine (SPH) into sphingosine-1-phosphate (S1P), which promotes ABA

regulate ABA responses in seed germination and seedling growth of *Arabidopsis* (Yang, 2002). The recruitment of ROP10 to the plasma membrane requires a functional farnesylation site. However, farnesylation of ROP10 appears to be independent of *ERA1*, that encodes the β-subunit of farnesyl transferase (Cutler *et al.*, 1996) because ROP10 localization is only weakly affected in the *era1* mutant (Zheng *et al.*, 2002). The closest rice homologues of this group of proteins are shown in Table I.

C. SECONDARY MESSENGERS

The primary intracellular messenger of ABA responses is Ca^{2+} which also mediates the signaling of other hormones. However, the specificity of Ca^{2+} signaling is thought to be determined by the magnitude, timing, spatial distribution, and frequency of its change. Abscisic acid activates the vacuolar H^+ATPase (Barkla *et al.*, 1999) and regulates the influx of Ca^{2+} across the plasma membrane through ABA-activated channels (Hamilton *et al.*, 2000; Schroeder and Hagiwara, 1990). In addition, the concentration of Ca^{2+} in cytosol ($[Ca^{2+}]_{cyt}$) is further modulated by other secondary messengers including inositol 1,4,5 triphosphate (InsP3), phosphatidic acid (PA), myo-inositol hexakisphosphate (InsP6), sphingosine-1-phosphate (S1P), hydrogen peroxide (H_2O_2), nitric oxide (NO), cyclic ADP ribose (cADPR), and cyclic guanosine monophosphate (cGMP) (Himmelbach *et al.*, 2003; Leckie *et al.*, 1998; Wu *et al.*, 1997). Abscisic acid enhances the activities of phospholipase C (PLC), PLD, and ADPR cyclase to produce InsP3 (Sanchez and Chua, 2001), PA (Ritchie and Gilroy, 2000), and cADPR (Sanchez *et al.*, 2004), respectively. Overexpression of inositol 5-phosphatase, an enzyme that hydrolyzes InsP3 and inositol 1,3,4,5-tetrakisphosphate, results in

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responses by mobilizing internal Ca^{2+} stores. In addition, other secondary messengers including cyclic ADP ribose (cADPR), inositol 1,4,5 triphosphate (InsP3), myo-inositol hexakisphosphate (InsP6), phosphatidic acid (PA), and NO, control $[Ca^{2+}]_{cyt}$ by releasing Ca^{2+} from internal storages. Reactive oxygen species (ROS), which is produced by NADPH-oxidase or promoted by secondary messengers (PI3P, InsP6, and InsP3), enhances $[Ca^{2+}]_{cyt}$ by activating Ca^{2+} channels on the plasma membrane. $[Ca^{2+}]_{cyt}$ changes ultimately control guard cell movement and gene expression. Ca^{2+} signaling is negatively regulated by a protein complex including protein phosphatases (ABI1 or ABI2), a protein kinase (CIPK15/PKS3), a Ca^{2+}-binding protein (CBL/ScaBP5), and a homeodomain leucine zipper protein (ATHB6). In turn, ABI1/ABI2 is repressed by both the secondary messenger (PA) and ABI3/VP1. This pathway is positively regulated by a MAP kinase cascade, calcium-dependent protein kinases (CDPKs), and probably Ca^{2+}-calmodulin-dependent protein kinases II (CCaMKs). Abscisic acid-response promoter complex is composed of an ACGT-box and a coupling element. ABI3/VP1 and 14-3-3 increase the binding affinity of ABI5 to ABRE and CE elements, forming a transcriptional complex that likely includes a ring zinc finger and WRKY proteins. ABI5 binding protein (AFP) promotes the degradation of ABI5 by 26S proteasomes. Three RNA-binding proteins, one functioning as a positive regulator (AKIP1) and two as negative regulators (HYL1 and ABH1) modulate ABA signaling at the posttranscriptional level.

TABLE I. Genes Involved in Early ABA Signaling Events and Production of Secondary Messenger

Gene[a]	Accession[b]	Length[c]	Gene product	Mutation	Material	Response[d]	Reference	Rice homologue accession[e]	Length[f]	*E*-value[g]
HvABAP1	AAF97846	472	ABA-binding protein		Aleurone cells	Induced	Razem *et al.* (2004)	**AAT72462**	738	0
AtERA1	NP_198844	482	Farnesyl transferase, beta-subunit	*era1, wiggum*	Seed/ guard cells	Oversensitive	Cutler *et al.* (1996); Pei *et al.* (1998)	5147.m00126	478	e−132
AtGCR1	AAN15633	326	G protein-coupled receptors	*gcr1-3*, *gcr1-4*	Root/ guard cells	Oversensitive	Pandey and Assmann (2004)	6498.m00149	321	e−120
AtGPA1	AAA32805	383	Heterotrimeric GTP-binding (G) protein	*gpa1*	Guard cells	Insensitive	Wang *et al.* (2001)	4351.m00157	390	e−166
At5PTase1	AAD10828	590	Inositol 5-phosphatase	Ecotypic expression	Guard cells	Insensitive	Burnette *et al.* (2003)	4384.m00157	676	e−152
z*AtFRY1*	AAC49263	353	Inositol polyphosphate-1-phosphatase	*fry1*, *hos2*	Seed/ seedling	Oversensitive	Xiong *et al.* (2001a)	7124.m00171	441	e−126
AtIP5PII	NP_849402	613	Ins(1,4,5)P_3 5-phosphatase	Ecotypic expression	Seed/ seedling	Insensitive	Sanchez and Chua (2001)	4384.m00157	676	e−124

(*Continues*)

TABLE I. (*Continued*)

Gene[a]	Accession[b]	Length[c]	Gene product	Mutation	Material	Response[d]	Reference	Rice homologue accession[e]	Length[f]	*E*-value[g]
AtrbohD	NP_199602	921	NADPH oxidase catalytic subunit genes	*atrbohD/F*	Seed/ guard cells/ root	Insensitive	Kwak *et al.* (2003)	7173.m00220	941	0
AtrbohF	NP_564821	944	NADPH oxidase catalytic subunit genes	*atrbohD/F*	Seed/ guard cells/ root	Insensitive	Kwak *et al.* (2003)	**NP_916447**	943	0
AtNIA1	CAA31786	393	Nitrate reductase	*nia1/nia2*	Guard cells	Insensitive	Desikan *et al.* (2002)	4982.m00153	681	e−153
AtNIA2	AAK56261	917	Nitrate reductase	*nia1/nia2*	Guard cells	Insensitive	Desikan *et al.* (2002)	**BAD09558**	916	0
AtNOS1	AAU95423	561	Nitric oxide synthase	*Atnos1*	Seedling/ guard cells	Insensitive	Quo *et al.* (2003)	2463.m00107	547	0
AtPLC1	BAA07547	561	Phospholipase C1	Antisense inhibition	Seed/ seedling	Insensitive	Sanchez and Chua (2001)	**AAS90683**	598	e−153

AtPLDa1	Q38882	810	Phospholipase D	*PLDalpha1*	Guard cells	Insensitive	Zhang *et al.* (2004a)	**BAA11136**	812	0
AtROP2	Q38919	195	Rho-type small GTPase	Dominant negative	Seed	Oversensitive	Li *et al.* (2001)	**AAF28764**	197	e−101
AtROP10	Q9SU67	208	Rop subfamily of Rho GTPases	*rop10*	Seed/ root	Oversensitive	Zheng *et al.* (2002)	3857.m00131	215	e−100
AtSYP61	AAK40222	245	SNARE superfamily of proteins. SNAP receptor	*osm1*	Guard cells	Insensitive	Zhu *et al.* (2002)	**NP_914267**	270	5.00 e−74
AtSphK	BAB07787	763	Sphingosine kinase	SphKs inhibitor	Guard cells	Insensitive	Coursol *et al.* (2003)	903.m00132	757	0

Peptide sequences of the genes listed in the first column were used to search against the comprehensive rice peptide database for rice homologues.

[a]Gene names with the abbreviated names of the species: *At*, *Arabidopsis thaliana*; *Cp*, *Craterostigma plantagineum*; *Hv*, *Hordeum vulgare*; *Lt*, *Larrea tridentata*; *Os*, *Oryza sativa*; *Pv*, *Phaseolus vulgaris*; *Ta*, *Triticum aestivum*; *Vf*, *Vicia faba*; *Zm*, *Zea mays*.

[b]Genbank accession numbers for the peptide sequences.

[c]Lengths of the peptide sequences.

[d]Phenotypes of the mutations or expression of the reporter genes driven by the ABA-responsive promoters.

[e]Accession numbers.

[f]Lengths of the homologous rice peptide sequences.

[g]*E*-values of the blast analyses. Accession numbers in bold represent sequences from NCBI; those in regular font represent sequences from TIGR; and those in italic represent sequences from translated full-length cDNAs.

hyposensitivity of guard cells to ABA (Burnette *et al.*, 2003). InsP6 promotes the releases of Ca^{2+} from endomembrane compartments such as the vacuole (Lemtiri-Chlieh *et al.*, 2003). Sphingosine-1-phosphate, which is converted from the long-chain amine alcohol (sphingosine) by ABA-induced activation of sphingosine kinase (Coursol *et al.*, 2003), acts at trimeric G-protein GPA1 (Coursol *et al.*, 2003) and its receptor GCR1 (Pandey and Assmann, 2004) to mobilize calcium (Ng *et al.*, 2001). Reactive oxygen species (ROS), such as H_2O_2 produced by a membrane-bound NADPH-oxidase (Kwak *et al.*, 2003), and NO resulting from the activities of nitrate reductase (Desikan *et al.*, 2002) and a glycine decarboxylase complex (Chandok *et al.*, 2003) also serve as secondary messengers in ABA signaling. Mutations in the *Arabidopsis* nitrate reductase apoprotein genes, *NIA1* and *NIA2* (Desikan *et al.*, 2002), or the NO synthase gene, *AtNOS1* (Guo *et al.*, 2003), diminish NO synthesis and impair stomatal closure in response to ABA, although stomatal opening is still inhibited by ABA. Cyclic ADP ribose and cGMP are required for the induction of ABA response by NO, suggesting that NO acts upstream of these two secondary messengers (Desikan *et al.*, 2002). It was shown that a new inositol phosphate, phosphatidylinositol 3-phophate (PI3P), might act upstream of ROS in ABA signaling because treatments with phosphatidylinositol 3-kinase inhibitors impair ABA-induced stomatal closure in *Vicia faba* (Jung *et al.*, 2002), and inhibition can be partially rescued by applying H_2O_2 (Park *et al.*, 2003). These messengers control $[Ca^{2+}]_{cyt}$ by releasing Ca^{2+} from the internal storage sites (such as vacuoles and the ER), producing Ca^{2+} oscillations (Allen *et al.*, 2001) that serve as a primary regulator of ABA signaling to control the movement of guard cells for the closing and opening of stomata (Fan *et al.*, 2004).

The calcium oscillations regulated by these secondary messengers also control ABA-regulated gene expressions in other cell types (Chen *et al.*, 1997; Sheen, 1996; Wu *et al.*, 1997). Indeed, inactivation of an inositol polyphosphate 1-phosphatase that is capable of dephosphorylating InsP3 results in oversensitivity to ABA in seed germination and postembryonic development (Xiong *et al.*, 2001b). Double mutation of the NADPH-oxidase catalytic subunit genes *AtrbohD* and *AtrbohF*, impairs ABA-induced ROS production and increases in $[Ca^{2+}]_{cyt}$, thereby interfering with ABA-induced stomatal closing and ABA-inhibition of seed germination and root elongation (Kwak *et al.*, 2003). The closest rice homologues of the enzymes producing these secondary messengers are shown in Table I.

D. PHOSPHATASES AND KINASES

Mutation studies suggest that several *Arabidopsis* protein phosphatases 2C, such as ABI1 and ABI2, function as negative regulators of ABA signaling (Himmelbach *et al.*, 2003; Ianzano *et al.*, 2004; Leonhardt *et al.*, 2004b; Merlot *et al.*, 2001). Electrophysiological studies indicate that *abi1-1* and

abi2-1 mutations disrupt ABA activation of calcium channels (Murata *et al.*, 2001) and reduce ABA-induced cytosolic calcium increases in guard cells (Allen *et al.*, 1999), suggesting these two phosphatases act upstream of $[Ca^{2+}]_{cyt}$. However, other studies suggest they act downstream of cADPR (Sanchez *et al.*, 2004; Wu *et al.*, 2003) and NO (Desikan *et al.*, 2002). The activities of protein phosphatases are modulated by secondary messengers (PA and Ca^{2+}) and protein kinases. Phosphatidic acid binds to and inhibits ABI1 activity (Zhang *et al.*, 2004a). ABI2 and ABI1 physically interact with PKS3 (or its homologue CIPK3), a Ser/Thr protein kinase. This kinase is also associated with the calcineurin B-like Ca^{2+} binding protein, SCaBP5 (or its homologue CBL), forming a complex that negatively controls ABA sensitivity (Guo *et al.*, 2002; Kim *et al.*, 2003). Another calcium sensor (CBL9) functions as a negative regulator of ABA signaling and biosynthesis (Pandey *et al.*, 2004). In contrast, the protein phosphatase 2A encoded by *RCN1* functions as a positive regulator of ABA signaling (Kwak *et al.*, 2002).

Protein kinases also can function as positive regulators of ABA signaling. Calcium-dependent protein kinases (CDPKs) contain a protein kinase domain and a carboxyl-terminal calmodulin-like structure that directly binds calcium (Cheng *et al.*, 2002). Two *Arabidopsis* CDPKs (AtCPK10 and AtCPK30) activate an ABA-inducible barley promoter in the absence of the hormone (Cheng *et al.*, 2002). Abscisic acid and H_2O_2 activate the *Arabidopsis* mitogen-activated protein kinase, ANP1, which initiates a phosphorylation cascade involving two mitogen-activated protein kinases (MAPK), AtMPK3, and AtMPK6 (Kovtun *et al.*, 2000). Overexpression of *AtMAPK3* increases ABA sensitivity while inhibition of MAPK activity by inhibitor PD98059 decreases ABA sensitivity (Lu *et al.*, 2002). Sucrose nonfermenting1-related protein kinases function as activators of ABA signaling in rice (Kobayashi *et al.*, 2004) and wheat (Johnson *et al.*, 2002). There are several other protein kinase genes whose expressions are induced or whose protein activities are activated by ABA. However, it is unknown how they are involved in ABA responses (Finkelstein and Rock, 2001). The closest homologues of these regulators in rice are shown in Table II.

E. TRANSCRIPTIONAL REGULATION

1. *Cis*-Acting Elements

The ABA-response elements include those with an ACGT-core (G-box/ABRE, /ACGT-box), a CGT-core (CE3-like) or a GCC-core (Motif I-like), Sph/RY sequences (CATGCA(TG)), DRE (CCGA(C/G)), MYC and MYB binding sites (ACACGCATGTG and YAAC(G/T)G, respectively), and coupling elements. Most of these elements are defined in transient expression systems, including protoplasts, suspension cells, and aleurone cells (Rock, 2000; Shen and Ho, 1997). The cereal aleurone layers are composed of

TABLE II. Kinases and Phosphatases Involved in ABA Signaling

Gene[a]	Accession[b]	Length[c]	Gene product	Mutation	Material	Response[d]	Reference	Rice homologue accession[e]	Length[f]	*E*-value[g]
AtCDPK1	BAA04829	493	Calcium-dependent protein kinases	Ecotypic expression	Leaf protoplast	Upregulation	Sheen (1996)	**AAT81734**	574	0
AtCDPK1a	EAA19816	251	Calcium-dependent protein kinases	Ecotypic expression	Leaf protoplast	Upregulation	Sheen (1996)	8220.m00104	328	4.00 e−33
AtPKS3	AAK26842	421	Protein kinase	RNA interference	Seed/ seedling/ guard cells	Oversensitive	Quo *et al.* (2002)	4351.m00164	456	e−151
AtCIPK3	NP_850095	382	Ser/Thr protein kinase	*cipk3*	Seed	Oversensitive	Kim *et al.* (2003)	6635.m00186	454	e−173
HvPKABA1	BAB61736	342	Ser/Thr protein kinase	Over-expression	Aleurone cells	Suppression	Gómez-Cadenas *et al.* (1999)	8364.m00150	342	e−179
AtSCaBP5	AAC26008	213	Ca(2+) binding protein	RNA interference	Seed/ seedling/ guard cells	Oversensitive	Quo *et al.* (2003)	6848.m00127	225	2.00 e−74

AtCBL9	AAL10301	213	Calcineurin B-like proteins	*cbl9*	Seed/ seedling	Oversensitive	Pandey *et al.* (2004)	6848.m00127	225	5.00 e−74
AtRCN1	AAC49255	588	Protein phosphatases 2A	*rcn1*	Seed/ guard cells	Insensitive	Kwak *et al.* (2002)	5660.m00128	587	0
AtABI1	NP_194338	434	Protein phosphatase 2C	*abi1-1*	Seedling/ guard cells	Insensitive	Leung *et al.* (1994); Wu *et al.* (2003)	**XP_463364**	467	e−109
AtABI2	O04719	423	Protein phosphatase 2C	*abi2-1*	Guard cells	Insensitive	Finkelstein (1993)	**XP_463364**	467	e−112
AtP2C-HA	AAG51849	511	Protein phosphatase 2C	*atp2c-ha*	Seed/ guard cells	Oversensitive	Leonhardt *et al.* (2004a,b)	**XP_463364**	467	e−115
AtPP2CA	BAA07287	399	Protein phosphatases 2C	Antisense inhibition	Seed/ seedling	Oversensitive	Tahtiharju and Palva (2001)	3955.m00148	416	3.00e−97

Peptide sequences of the genes listed in the first column were used to search against the comprehensive rice peptide database for rice homologues.

[a]Gene names with the abbreviated names of the species: *At*, *Arabidopsis thaliana*; *Cp*, *Craterostigma plantagineum*; *Hv*, *Hordeum vulgare*; *Lt*, *Larrea tridentata*; *Os*, *Oryza sativa*; *Pv*, *Phaseolus vulgaris*; *Ta*, *Triticum aestivum*; *Vf*, *Vicia faba*; *Zm*, *Zea mays*.

[b]Genbank accession numbers for the peptide sequences.

[c]Lengths of the peptide sequences.

[d]Phenotypes of the mutations or expression of the reporter genes driven by the ABA-responsive promoters.

[e]Accession numbers.

[f]Lengths of the homologous rice peptide sequences.

[g]*E*-values of the blast analyses. Accession numbers in bold represent sequences from NCBI; those in regular font represent sequences from TIGR; and those in italic represent sequences from translated full-length cDNAs.

uniform, synchronized, and highly differentiated cells that can be easily prepared in large quantity within a short period of time (Bethke *et al.*, 1997). None of the *cis*-acting elements described earlier can function alone (Hobo *et al.*, 1999a; Narusaka *et al.*, 2003). Instead, they form ABA-response promoter complexes called ABRC (Shen and Ho, 1995, 1997, 1998; Shen *et al.*, 1996, 2001, 2004). For two barley genes, each ABRC consists of an ACGT core containing element (ACGT-box) and a coupling element (CE1 or CE3), forming two different ABRCs called ABRC1 and ABRC3 (Fig. 2). These two promoter complexes are different in the sequences of the coupling elements, the orientation constraints of the coupling elements, and the distances between an ACGT-box and a CE (Shen *et al.*, 2004). Extensive deletion and point mutation analyses suggest that the ACGT element requires the sequence 5′-ACGTGGC-3′ and the elements CE1 and CE3 require the sequences CCACC and GCGTGTC, respectively. It is suggested that the ACGT-box and CE3 are functionally equivalent because the OsTRAB1/ABI5 binds to both the ACGT-box and CE3 element *in vitro* (Hobo *et al.*, 1999a). However, data indicate that the coupling between an ACGT-box and a CE, or between two ACGT-boxes is essential for a high level of ABA induction; two copies of CE3 are much less active (Shen *et al.*, 2004). Furthermore, a partially purified nuclear extract from barley embryos has specific binding activity for the ACGT-box present in ABRC3. It recognizes the wild-type version of the ABRC3 and two copies of the ACGT-box but possesses low affinity for two copies of the coupling element CE3, suggesting that it is likely a bZIP protein that is different from ABI5 binds to the CE3 element *in vivo* (Casaretto and Ho, 2003; Shen *et al.*, 2004). An ACGT-box can form other types of ABRCs by coupling with elements such as DRE (Narusaka *et al.*, 2003).

2. *Trans*-Acting Factors

Several transcription factors have been well documented to mediate ABA signaling. The ABI5-type bZIP proteins from *Arabidopsis*, sunflower, wheat, barley, and rice bind as dimers to the ACGT-box or CE3 to activate the promoters (Finkelstein and Rock, 2001). ABI5 is upregulated by ABA through an increase in the transcript level as well as the stability of the protein. AP2-type proteins from maize and barley, ZmABI4, HvDRF1, ZmDBF1, ZmDBF2, DREB1s/CBFs, and DREB2s (Narusaka *et al.*, 2003), interact with CE1 or its related C-rich motifs including DRE (Himmelbach *et al.*, 2003; Xue and Loveridge, 2004). AtMYC2 and AtMYB2 bind to MYC and MYB recognition sites, respectively, and function as activators of ABA signaling (Abe *et al.*, 2003). Abscisic acid-inducible NAC activator proteins were also found to interact with the MYC site (Fujita *et al.*, 2004; Tran *et al.*, 2004).

The activities of ABI5 and its orthologues/homologues (Table III) are modified by some kinases (Johnson *et al.*, 2002; Kagaya *et al.*, 2002; Lu

et al., 2002), VP1 (Casaretto and Ho, 2003; Suzuki *et al.*, 2003), FUS3, and LEC1 (Finkelstein and Rock, 2001). Phosphorylation in the nucleus of preexisting AtABI5/OsTRAB1/TaABF is found to be the nearly terminal event of ABA response (Johnson *et al.*, 2002; Kagaya *et al.*, 2002; Lopez-Molina *et al.*, 2001). VP1 has a coactivation/repression domain at the N-terminus and three basic domains (B1, B2, and B3) at the C-terminus. The N-terminal domain is necessary for activating the ABA pathway and repressing the gibberellin (GA) pathway (Suzuki *et al.*, 2003). The C-terminal B3 domain is shown to bind specifically to the Sph1/RY element, although the full-length VP1 does not bind to DNA (Suzuki *et al.*, 1997). The B1 and B2 domains are likely to be involved in nuclear localization and interaction with ABI5, WRKY, 14-3-3, ring (C3HC3-type) zinc finger proteins, and RNA polymerase II subunit RPB5 to potentiate ABA-inducible gene expression (Hobo *et al.*, 1999b; Jones *et al.*, 2000; Kurup *et al.*, 2000; Nakamura *et al.*, 2001; Schultz *et al.*, 1998; Zou *et al.*, 2004).

The activity/assembly of the transcription complex for ABA signaling appears to be modulated by at least four classes of transcriptional repressors. The first class of repressors are bZIP proteins that negatively regulate ABA-induced gene expression by sequestering bZIP activators or competing with bZIP activators for binding to the ACGT-box. For example, two rice bZIP proteins (OsZIP-2a and OsZIP-2b) do not bind to ABRE by themselves. However, they heterodimerize via the leucine zipper with EmBP-1 (Table IV) and prevent it from binding to the ACGT-box (Nantel and Quatrano, 1996). In contrast, ROM2 binds to the ACGT-box but functions as a repressor (Chern *et al.*, 1996). The second class of repressors are protein phosphatases. In addition to ABI1 and ABI2 protein phosphatases 2C described earlier, the C-terminal domain phosphatase-like protein, AtCPL3, also functions as a repressor of ABA signaling. AtCPL3 specifically downregulates ABA-responsive gene expression possibly by contacting and dephosphorylating the carboxyl-terminal domain (CTD) of the RNA polymerase II, thereby blocking transcription initiation (Koiwa *et al.*, 2002). The third class of repressors are homeodomain proteins that bind to the *cis*-acting element, CAATTATTA; ATHB6 physically interacts with ABI1 and acts downstream of ABI-1 in mediating ABA signaling (Himmelbach *et al.*, 2002). The fourth class of repressors are WRKY proteins; of the 77 published *OsWRKY* genes (Zhang *et al.*, 2004b) at least two function as repressors of ABA signaling in aleurone cells (Z. Xie and Q. Shen, unpublished data).

Inactivation of repressors hence is essential for ABA signaling. Indeed, prior to becoming part of the transcription complex, VP1/ABI3 appears to play two additional roles: to inactivate ABI1 and ABI2 protein phosphatases (Suzuki *et al.*, 2003) and to modify the chromatin structure (Li *et al.*, 1999). Two other B3 proteins, LEC2 and FUS3, might participate in the chromatin-remodeling process (Luerssen *et al.*, 1998; Stone *et al.*, 2001). In addition,

TABLE III. Transcriptional Regulators Involved in ABA Signaling

Gene[a]	Accession[b]	Length[c]	Gene product	Mutation	Material	Response[d]	Binding site	Reference	Rice homologue accession[e]	Length[f]	*E*-value[g]
AtABI4	AAF18736	328	AP2 domain protein	*abi4*, *gin6*, *isi3*, *san5*, *sis5*, *sun6*	Seed/ seedling	Insensitive		Finkelstein *et al.* (1998)	3353.m00161	318	2.00e−23
ZmABI4	AAM95247	248	AP2 domain protein	Over expression in abi4	Seed	Insensitive	CE1	Niu *et al.* (2002)	3353.m00161	318	1.00e−23
AtABI3	NP_189108	720	B3 domain protein	*abi3*	Seed	Insensitive	ABRE, RY/ G-box	Ezcurra *et al.* (2000)	**BAA04066**	728	3.00e−95
AtFUS3	AAC35246	310	B3 domain protein	*ML1:: FUS3-GFP*	Seed	Oversensitive		Gazzarrini *et al.* (2004)	2880.m00122	728	2.00e−31
OsVP1	BAA04066	728	B3 domain protein	Over expression	Protoplast	Upregulation	RY/Sph	Hattori *et al.* (1995)			
HvVP1	AAO06117	394	B3 domain protein	Over expression	Aleurone cells	Upregulation		Casaretto and Ho (2003)	2880.m00122	728	e−133
PvALF	T10864	700	B3 domain protein	Over expression	Cotyledon	Upregulation	RY/Sph	Bobb *et al.* (1997)	2880.m00122	728	2.00e−84
ZmVP1	CAA04889	449	B3 domain protein	*vp1*	Seed	Insensitive	ABRE	McCarty *et al.* (1991); Schultz *et al.* (1998)	2880.m00121	704	e−138

AtMYC2	Q39204	623	bHLH	*atmyc2*	Seed	Insensitive	MYC site	Abe *et al.* (2003)	749.m00139	688	e−146
AtIMB1	AAO22056	386	Bromodomain proteins	*imb1*	Seedling	Oversensitive		Duque and Chua (2003)	3028.m00190	344	5.00e−87
AtABF3	BAD43614	454	bZIP protein	Over expression	Seed/ seedling	Oversensitive	ABRE	Choi *et al.* (2000); Kang *et al.* (2002)	**BAD17130**	357	1.00e−64
AtABF4	AAF27182	431	bZIP protein	Over expression	Seed/ seedling	Oversensitive	ABRE	Choi *et al.* (2000); Kang *et al.* (2002)	3486.m00122	644	1.00e−50
AtABI5	AAD21438	442	bZip protein	*abi5*	Seed	Insensitive	ABRE	Carles *et al.* (2002); Finkelstein and Lynch (2000)	5149.m00135	388	4.00e−61
HvABI5	AAO06115	353	bZip protein	Over expression	Aleurone cells	Upregulation	ABRE, CE3	Casaretto and Ho (2003)	**BAD38293**	376	e−142
OsTRAB1	XP_482899	318	bZip protein	Over expression	Protoplast	Upregulation	ABRE	Hobo *et al.* (1999a)			

(*Continues*)

TABLE III. (*Continued*)

Gene[a]	Accession[b]	Length[c]	Gene product	Mutation	Material	Response[d]	Binding site	Reference	Rice homologue accession[e]	Length[f]	*E*-value[g]
OsZip-1a	AAC49556	390	bZip protein	Over expression	Protoplast	Heterodimerized, upregulation	ABRE	Nantel and Quatrano (1996)			
OsZip-2a	AAC49557	124	bZip protein	Over expression	Protoplast		ABRE	Nantel and Quatrano (1996)			
PvROM2	T10985	424	bZip protein	Over expression	Cotyledon	Downregulation	ABRE	Chern *et al.* (1996)	*AK065440*	608	4.00e−60
TaEmBP-1	P25032	354	bZip protein	Over expression	Protoplast	Upregulation	ABRE	Guiltinan *et al.* (1990); Hill *et al.* (1996)	**BAC83673**	423	3.00e−73
AtCPL1	NP_193898	995	C-terminal domain phosphatase-like	*cpl1*, *fry2*	Seed/ seedling	Insensitive(seed)/ oversensitive (seedling)		Koiwa *et al.* (2002); Xiong *et al.* (2002)	**BAD25346**	940	0
AtCPL3	NP_180912	1190	C-terminal domain phosphatase-like	*cpl3*	Seedling	Upregulation		Koiwa *et al.* (2002)	7174.m00156	1272	0

AtLEC1	NP_173616	238	HAP3 subunit of CCAAT protein	*lec1*	Seed	Insensitive	CCAAT	Brocard-Gifford *et al.* (2003)	2534.m00206	254	4.00e−43
AtHB5	P46667	312	Homeodomain protein	Ecotypic expression	Seed/ seedling	Oversensitive	AT-rich	Johannesson *et al.* (2001, 2003)	7458.m00131	269	4.00e−40
AtHB6	AAD41726	291	Homeodomain protein	Over expression	Seed/ guard cells	Insensitive	AT-rich	Himmelbach *et al.* (2002)	**BAD22271**	277	1.00e−39
AtMYB2	BAA03534	273	MYB protein	Overexpression	Seed	Oversensitive	MYB site	Abe *et al.* (2003)	8346.m00103	329	4.00e−62
AtRD26	NP_849457	164	NAC protein	Over expression	Seedling	Oversensitive		Fujita *et al.* (2004)	2885.m00175	303	1.00e−05
LtWRKY21	AY792618	314	WRKY protein	Over expression	Seed	Upregulation		Zou *et al.* (2004)	**DAA05093**	374	2.00e−52
AtMARD1	AAK92226	263	Zinc-finger protein	*mard1*	Seed	Insensitive		He and Gan (2004)	*AK066202*	429	3.00e−29

[a]Gene names with the abbreviated names of the species: *At*, *Arabidopsis thaliana*; *Cp*, *Craterostigma plantagineum*; *Hv*, *Hordeum vulgare*; *Lt*, *Larrea tridentata*; *Os*, *Oryza sativa*; *Pv*, *Phaseolus vulgaris*; *Ta*, *Triticum aestivum*; *Vf*, *Vicia faba*; *Zm*, *Zea mays*.

[b]Genbank accession numbers for the peptide sequences.

[c]Lengths of the peptide sequences.

[d]Phenotypes of the mutations or expression of the reporter genes driven by the ABA-responsive promoters.

[e]Accession numbers.

[f]Lengths of homologous rice peptide sequences.

[g]*E*-values of the BLAST analyses. Accession numbers in bold represent sequences from NCBI; those in regular font represent sequences from TIGR; and those in italic represent sequences from translated full-length cDNAs.

TABLE IV. Genes Modulating ABA Responses at Posttranscriptional Regulation

Gene[a]	Accession[b]	Length[c]	Gene product	Mutation	Material	Response[d]	Reference	Rice homologue accession[e]	Length[f]	*E*-value[g]
AtAFP	AAF67775	335	ABI five binding protein	*afp-1*, *afp-2*	Seed/ seedling	Oversensitive	Lopez-Molina *et al.* (2003)	6877.m00115	307	9.00e−41
AtHYL1	AAG49890	419	Double-stranded RNA binding protein	*hyl1*	Seed/ root	Oversensitive	Lu and Fedoroff (2000)	*AK103543*	577	5.00e−58
AtABH1	NP_565356	848	mRNA CAP binding protein	*abh1*	Seed/ guard cells	Oversensitive	Hugouvieux *et al.* (2001)	**AAG54079**	910	0
AtSAD1	AAK61592	88	U6-related Sm-like small ribonucleoprotein	*sad1*	Seed/ root	Oversensitive	Xiong *et al.* (2001b)	AK059190	218	2.00e−40

AtPRL1	NP_193325	486	WD40 domain protein	*prl1*	Seedling	Oversensitive	Nemeth *et al.* (1998)	6133.m00106	472	0
AtOST1	CAC87047	362	Ser/Thr protein kinase	*ost1-1*, *ost1-2*, *srk2e*	Guard cells	Insensitive	Mustilli *et al.* (2002); Yoshida *et al.* (2002)	8370.m00178	362	e−176
VfAAPK	AAF27340	349	Ser/Thr protein kinase	Dominant negative	Guard cells	Insensitive	Li *et al.* (2002)	8370.m00178	362	e−161
VfAKIP1	AAM73765	515	RNA-binding protein		Guard cells		Li *et al.* (2002)	2729.m00136	490	e−100

[a]Gene names with the abbreviated names of the species: *At*, *Arabidopsis thaliana*; *Cp*, *Craterostigma plantagineum*; *Hv*, *Hordeum vulgare*; *Lt*, *Larrea tridentata*; *Os*, *Oryza sativa*; *Pv*, *Phaseolus vulgaris*; *Ta*, *Triticum aestivum*; *Vf*, *Vicia faba*; *Zm*, *Zea mays*.

[b]Genbank accession numbers for the peptide sequences.

[c]Lengths of the peptide sequences.

[d]Phenotypes of the mutations or expression of the reporter genes driven by the ABA-responsive promoters.

[e]Accession numbers.

[f]Lengths of homologous rice peptide sequences.

[g]*E*-values of the BLAST analyses. Accession numbers in bold represent sequences from NCBI; those in regular font represent sequences from TIGR; and those in italic represent sequences from translated full-length cDNAs.

LEC1 encodes a transcription factor homologous to CCAAT box-binding factor HAP3 subunit (Lotan *et al.*, 1998). Transcription of *LEC1*, *LEC2*, and *FUS3* genes is repressed by *PKL* that encodes a CHD3-chromatin-remodeling factor. Hence, ABI3, FUS3, LEC2, and PKL might work together to control the remodeling of chromatin structure prior to the binding of transcriptional activators such as ABI4 and ABI5 to promoters. Although *ABI3*, *LEC1*, and *FUS3* all interact with *ABI4* and *ABI5* genetically (Brocard-Gifford *et al.*, 2003), only ABI3/VP1 has been shown to directly interact with ABI5/OsTRAB1 (Hobo *et al.*, 1999b; Nakamura *et al.*, 2001). Furthermore, only *VP1* and *LEC1*, but not *LEC2* and *FUS3*, have been implicated in ABA signaling.

Although VP1 binds to the Sph/RY element (Suzuki *et al.*, 1997) to activate the *C1* promoter in the absence of ABA (Kao *et al.*, 1996), VP1 also can enhance the transcription of the ABRC-containing promoters that lacks an Sph/RY element. This has been well demonstrated by over-expression studies in barley aleurone cells (Shen *et al.*, 1996) and rice protoplasts (Gampala *et al.*, 2002; Hobo *et al.*, 1999b) and by double-stranded RNA interference experiments in barley aleurone cells (Casaretto and Ho, 2003). As many as 70 VP1-dependent ABA-activated genes have been found in a transcriptional profiling study with transgenic *Arabidopsis* carrying 35S promoter::VP1 in an *abi3* null mutant background (Suzuki *et al.*, 2003). However, VP1 does not always function as an agonist of ABA responses. In fact, 49 *Arabidopsis* ABA-inducible genes are repressed by VP1 and nine ABA-repressed genes are enhanced by VP1 (Suzuki *et al.*, 2003). The closest homologues of these regulators in rice are shown in Table III.

F. POSTTRANSCRIPTIONAL REGULATION

Abscisic acid regulation is also exerted at the posttranscriptional level (Fig. 2, Table IV). Abscisic acid induces the expression of several RNA-binding proteins, including: (1) the maize glycine-rich protein MA16 that preferentially interacts with uridine-rich and guanosine-rich RNA fragments (Freire and Pages, 1995); (2) AtABH1 and AtCBP20 that form a dimeric *Arabidopsis* mRNA cap-binding complex (Hugouvieux *et al.*, 2002); (3) AtSAD1 that is similar to multifunctional Sm-like small nuclear ribonucleoproteins; and (4) the dsRNA-binding protein HYL1, mutations in which lead to enhanced levels of ABI5 and MAPK (Lu and Fedoroff, 2000). Except for MA16, whose function remains unknown, these RNA-binding proteins function as negative regulators of ABA signaling. Another RNA-binding protein, AKIP1, is a substrate of the protein kinase AAPK. Phosphorylated AKIP1 interacts with the mRNA that encodes a dehydrin, a protein implicated in cell protection under stress conditions (Li *et al.*, 2002).

It is unknown how these RNA-binding proteins function in regulating ABA responses. However, homologues of ABH1, SAD1, and AKIP1 have

been reported to be components of RNA spliceosomes and exporting machinery. In addition, ABA enhances the partitioning of AKIP1 and HYL1 into subnuclear foci that are reminiscent of nuclear speckles (Han *et al.*, 2004; Li *et al.*, 2002). Finally, the levels of several miRNAs are reduced in the *hyl1* ABA hypersensitive mutant, suggesting that HYL1 protein is part of a nuclear macromolecular complex that is involved in miRNA-mediated gene regulation (Han *et al.*, 2004).

Protein degradation is also part of ABA signaling (Hare *et al.*, 2003). A nuclear-localized ABA-regulated protein AFP that physically interacts with ABI5 as shown by a yeast two-hybrid assay and co-immunoprecipitation, functions as a negative regulator of ABA signaling (Lopez-Molina *et al.*, 2003). Proteasome inhibitor studies show that ABI5 stability is regulated by ABA through ubiquitin-related events. Both AFP and ABI5 are co-localized in nuclear bodies that also contain COP1, a RING-finger–containing protein and WD40-repeat–containing protein that functions as a key repressor of seedling de-etiolation (Ang *et al.*, 1998). COP1 possesses autoubiquitination activity (E3) *in vitro* and can ubiquitinate, hence can likely promote the degradation of MYB-type transcription factors (Seo *et al.*, 2003). Although COP1 has not been shown to mediate ABA signaling, the mutation of another WD-40 protein, PRL1 (Table IV), results in oversensitivity to ABA (Nemeth *et al.*, 1998), suggesting that PRL1 is a repressor of ABA signaling. Phosphorylation of the ABI5 stabilizes the protein probably by blocking its AFP-promoted degradation by the 26S proteasome (Lopez-Molina *et al.*, 2003). These data suggest that AFP and PRL1 modulate ABA signaling by promoting degradation of transcriptional activators.

Data suggest that some *cis*-acting elements can be bound by both repressors and activators. Removing the repressors by a hormone-promoted and 26S-proteasome–mediated process facilitates binding of activators to the *cis*-acting elements, thereby enhancing transcription (Zhang *et al.*, 2004b). It remains to be determined whether AFP is also involved in the degradation of repressors of ABA signaling.

III. CROSS-TALK OF ABA AND GA

Increasing evidence suggests the connections of ABA, ethylene, sugar, and auxin synthesis and signaling (Fedoroff, 2002). However, the best-known interaction is the ABA and GA cross-talk in controlling seed germination. Abscisic acid downregulates many genes, especially those upregulated by GA. This effect is so drastic that ABA completely blocks GA-induced seed germination (Lovegrove and Hooley, 2000). In cereal aleurone tissue, GA induces and ABA suppresses the expression of α-amylases that are essential for the utilization of starch stored in the endosperm. The cross-talk of GA and ABA signaling is mediated by secondary messengers. For example,

application of PA to barley aleurone inhibits α-amylase production and induces an ABA-inducible amylase inhibitor and RAB (response to ABA) protein expression, mimicking the effect of ABA (Ritchie and Gilroy, 1998). The ABA inhibition also involves kinases. For example, although the ABA-induced protein kinase, PKABA1, has little activity on regulating the expression of ABA-inducible *HVA1* and *HVA22* genes, it almost completely suppresses the GA-induced expression of α-amylase and protease genes (Gómez-Cadenas *et al.*, 1999, 2001; Zentella *et al.*, 2002; Zhang *et al.*, 2004b). Because GA induction and ABA suppression of the α-amylase gene expression in barley aleurone cells appear to be dependent on the same set of *cis*-acting elements in the amylase promoter (Lanahan *et al.*, 1992), an intriguing question is at which site the ABA suppression on the GA-signaling pathway is exerted. Data indicate that PKABA1 acts upstream from the formation of functional GAMyb (a transcriptional activator of GA signaling) but downstream from the site of action of the Slender (a negative regulator of GA signaling) (Gómez-Cadenas *et al.*, 2001). However, there are more pathways mediating the suppression of GA signaling by PKABA1 because *PKABA1* RNA interference does not hamper the inhibitory effect of ABA on the expression of α-amylase (Zentella *et al.*, 2002). Indeed, two ABA-inducible OsWRKY proteins (Z. Xie and Q. Shen, unpublished data) also block GA signaling. Whether they represent components of the PKABA1-independent ABA-suppression pathway remains to be studied.

IV. CONCLUSIONS

Our understanding of ABA signaling has been dramatically improved in the past years with the studies in several dicotyledonous and monocotyledonous plants. Orthologues of a dozen reported ABA-signaling regulators have been found in these two great classes of angiosperms. In addition, for the 53 regulators that are reported only in dicotyledonous plants (mainly *Arabidopsis*), we have found their homologues in rice although the homology for 10 (19%) of these genes is quite low, with the *E*-values higher than e−50 (Tables I–IV). These data suggest that ABA-signaling networks might be highly conserved in several dicotyledonous and monocotyledonous plants. However, we should be cautious in reaching such a conclusion because of the following reasons: (1) the 10 ABA-signaling genes that share a low homology with those in rice, might be unique to dicotyledonous plants. (2) Conserved protein sequences do not necessarily mean conserved functions; experiments need to be carried out to study whether the proteins listed in the tables are truly the orthologues of the ABA-signaling regulators. (3) Even if they are indeed orthologues, their expression patterns upon ABA expression might be completely different, even reversed, as reported from the study of key regulator genes controlling photoperiodism in *Arabidopsis*

(a long-day plant) and rice (a short-day plant) (Hayama *et al.*, 2003). This question can be addressed in future by comparing the transcriptional profiling data of *Arabidopsis* (Duque and Chua, 2003; Leonhardt *et al.*, 2004b; Seki *et al.*, 2002a) and rice (Rabbani *et al.*, 2003; Yazaki *et al.*, 2004). (4) The signaling network might be regulated differently in different tissues. Example are certain mutations that only affect one or two aspects of ABA-regulated processes (Tables I–IV); ABA has opposite effects on $[Ca^{2+}]_{cyt}$ in the aleurone and guard cell (Ritchie *et al.*, 2002); and cGMP treatments elucidate ABA responses in guard cells but not aleurone cells (Penson *et al.*, 1996).

Now the challenge is to address the functions of the rapidly growing number of ABA-regulated genes and analyze their promoters experimentally after bioinformatics studies. Transgenic plants (over-expression and RNAi) and mutants (chemically-induced, T-DNA-induced, and transposon-induced) will continue to play important roles in helping to address gene functions. However, transient expression systems, especially the naturally synchronized aleurone cells, will remain extremely valuable for the dissection of promoter structures, definition of protein motifs, and determination of gene interactions.

ACKNOWLEDGMENTS

The authors are grateful to Drs. Andrew Andres, Frank van Breukelen, Jose Casaretto, Deborah Hoshizaki, and Chris Ross for helpful comments on this manuscript.

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