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.
This chapter highlights the identified regulators of ABA signaling and reports their homologues in dicotyledonous and monocotyledonous plants. © 2005 Elsevier Inc.

1. 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](image_url)

**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 \textit{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 \textit{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 \textit{et al.}, 2004; Fan \textit{et al.}, 2004; Finkelstein and Rock, 2001; Hare \textit{et al.}, 2003; Himmelbach \textit{et al.}, 2003; Kuhn and Schroeder, 2003; Lovegrove and Hooley, 2000; Ritchie \textit{et al.}, 2002; Rock, 2000; Schroeder \textit{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 \textit{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 \textit{Arabidopsis} (Seki \textit{et al.}, 2002b; The \textit{Arabidopsis} Initiative, 2000) and rice (Goff \textit{et al.}, 2002; Kikuchi \textit{et al.}, 2003; Yu \textit{et al.}, 2002). In this review, we summarize the advances in ABA-signaling research and report the closest (lowest \textit{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 \textit{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 z-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 $2.8 \times 10^{-8}$ 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 $\alpha$-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 GTP$\gamma$S transiently stimulates PLD in an ABA-independent manner, whereas treatment with GDP$\beta$S 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 (SIP), hydrogen peroxide (H$_2$O$_2$), nitric oxide (NO), cyclic ADP ribose (cADPR), and cyclic guanosine monophosphate (cGMP) (Himmelbach et al., 2003; Leckie et al., 1998; Wu et al., 1997). ABA is also a secondary messenger that hydrolyzes InsP3 and inositol 1,3,4,5-tetrakisphosphate, results in 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 storage. 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). ABA is also a secondary messenger that hydrolyzes InsP3 and inositol 1,3,4,5-tetrakisphosphate, results in
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<th>Mutation</th>
<th>Material</th>
<th>Response</th>
<th>Reference</th>
<th>Rice homologue accession</th>
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<td><em>HvABAP1</em></td>
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<td>472</td>
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<td>482</td>
<td>Farnesyl transferase, beta-subunit</td>
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<td>Oversensitive</td>
<td>Cutler et al. (1996); Pei et al. (1998)</td>
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<td><em>AtGCR1</em></td>
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<td>fry1, hos2</td>
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<td>Ins(1,4,5)P3 5-phosphatase</td>
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<td>Nitrate reductase</td>
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<td>Guard cells</td>
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<td>Desikan et al. (2002)</td>
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<td>Nitric oxide synthase</td>
<td>Atnos1</td>
<td>Seedling/guard cells</td>
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<td>Quo et al. (2003)</td>
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<td>Rho-type small GTPase</td>
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Peptide sequences of the genes listed in the first column were used to search against the comprehensive rice peptide database for rice homologues.

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.

Genbank accession numbers for the peptide sequences.

Lengths of the peptide sequences.

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

Accession numbers.

Lengths of the homologous rice peptide sequences.

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$_2$O$_2$ 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-phosphate (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$_2$O$_2$ (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 ArabohD and ArabohF, 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 $[Ca^{2+}]_{\text{cyt}}$. 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
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Peptide sequences of the genes listed in the first column were used to search against the comprehensive rice peptide database for rice homologues. For 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*.

| a | Genebank accession numbers for the peptide sequences. |
| b | Lengths of the peptide sequences. |
| c | Phenotypes of the mutations or expression of the reporter genes driven by the ABA-responsive promoters. |
| d | Accession numbers. |
| e | Lengths of the homologous rice peptide sequences. |
| f | 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/CFBs, 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-terminal 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,
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* 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*.

* Genbank accession numbers for the peptide sequences.

* Lengths of the peptide sequences.

* Phenotypes of the mutations or expression of the reporter genes driven by the ABA-responsive promoters.

* Accession numbers.

* Lengths of homologous rice peptide sequences.

* 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.
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eAccession numbers.

fLengths of homologous rice peptide sequences.

gE-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 $\alpha$-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 $[\text{Ca}^{2+}]_{\text{cyt}}$ 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.

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