The Plant Cell, S15–S45, Supplement 2002, www.plantcell.org © 2002 American Society of Plant Biologists

Abscisic Acid Signaling in Seeds and Seedlings

Ruth R. Finkelstein,[a,1] Srinivas S. L. Gampala,[b] and Christopher D. Rock[b]

[a] Department of Molecular, Cellular, and Developmental Biology, University of California at Santa Barbara, Santa Barbara, California 93106

[b] Department of Biology, Hong Kong University of Science and Technology, Kowloon, Hong Kong, China

INTRODUCTION

Abscisic acid (ABA) regulates many agronomically important aspects of plant development, including the synthesis of seed storage proteins and lipids, the promotion of seed desiccation tolerance and dormancy, and the inhibition of the phase transitions from embryonic to germinative growth and from vegetative to reproductive growth (reviewed by Leung and Giraudat, 1998; Rock, 2000; Rohde et al., 2000b). In addition, ABA mediates some aspects of physiological responses to environmental stresses such as drought- or osmotica-induced stomatal closure, the induction of tolerance of water, salt, hypoxic, and cold stress, and wound or pathogen response (Leung and Giraudat, 1998; Rock, 2000; Shinozaki and Yamaguchi-Shinozaki, 2000). A traditional distinction among these responses has been that of speed: the stomatal responses are relatively fast, occurring within minutes and involving changes in the activity of various signaling molecules and ion channels, whereas the rest are slower and require changes in gene expression. However, these sets of responses clearly require the action of common signaling elements, because several individual mutants (e.g., the Arabidopsis ABA-insensitive *abi1* and *abi2* mutants and the ABA-hypersensitive *era1* mutant) affect subsets of both types of responses. Furthermore, cell biological studies have implicated common classes of secondary messengers or components of phosphorylation cascades in both fast and slow responses to ABA.

Despite the existence of these common elements in signaling, some of them highly pleiotropic, none of the genetically defined elements are required for all responses to ABA. One possible explanation for this is that a mutation producing a loss of all ABA responses would be lethal, such that a recessive mutation might be recovered as a heterozygote but could not be characterized physiologically. Alternatively, there is substantial evidence for multiple redundant ABA perception and signaling mechanisms, such that no element would be absolutely required for all responses. Furthermore, in addition to the well-characterized antagonisms between ABA and gibberellic acid (GA), cytokinins, or auxins, recent studies have demonstrated interactions between signaling by ABA and ethylene, brassinosteroid, light, or sugars (reviewed by Rock, 2000; Finkelstein and Rock, 2001; Gazzarrini and McCourt, 2001; Finkelstein and Gibson, 2002). Thus, it may be more accurate to think of some of the shared signaling elements as "nodes" or even "free agents" interacting with a variety of components in a complex signaling web than as "common threads" in a shared linear signaling pathway.

Regulatory factors that control ABA response have been identified by genetic, biochemical, and pharmacological/cell biological approaches (reviewed by Rock, 2000; Finkelstein and Rock, 2002). Genetic screens used to date have been based on aberrant growth or gene expression responses to ABA. Recently, "reverse-genetics" studies have been used to test a specific gene's functional role; these studies use either screening of large mutagenized populations (McCallum et al., 2000; Parinov and Sundaresan, 2000) or a transgenic approach to overexpress or disrupt a target gene. Biochemical studies have identified a variety of gene promoter elements, kinases, kinase inhibitors, phosphatases, phospholipases, and transcription factors correlated with ABA response. Cell biological studies have tested the roles of candidate secondary messengers and signaling intermediates in regulating cellular responses to ABA.

The availability of the Arabidopsis genome sequence (Arabidopsis Genome Initiative, 2000), the public availability of the rice genome sequence (Sasaki and Burr, 2000; Barry, 2001), and the existence of many expressed sequence tag projects in a variety of species provide a fourth approach to identify potential regulatory factors by computerized screens for homologs of other known regulators. This approach requires functional testing by reverse genetics or other approaches, which is complicated by the fact that >60% of the genes in Arabidopsis belong to multigene families. Functional redundancy within these families can mask the effects of loss-of-function alleles isolated by traditional forward genetic strategies or sequence-based screens for insertional mutations. As a result, ABA mutant or engineered phenotypes may be tissue specific and subtle.

[1] To whom correspondence should be addressed. E-mail finkelst@lifesci.ucsb.edu; fax 805-893-4724.

Article, publication date, and citation information can be found at www.plantcell.org/cgi/doi/10.1105/tpc.010441.

ABA PERCEPTION

After arrival at its site of action, the first step in ABA response is expected to be some kind of recognition event. Several lines of indirect evidence suggest that there are multiple receptor types: the stereospecificity of ABA analog activities varies among ABA responses, and there appear to be both intracellular and extracellular sites of ABA action. However, no ABA receptors have been identified to date.

Intracellular or Extracellular Perception?

The site of action has been investigated by testing cellular responses to ABA introduced via microinjection, applied externally at various pH values to modulate uptake, or as a protein conjugate to prevent uptake. Initial evidence for an intracellular site of ABA action in stomatal regulation was provided by three observations: (1) the extent to which ABA inhibited stomatal opening and promoted stomatal closure was proportional to radioactive ABA uptake; (2) microinjection of ABA into the cytoplasm of guard cells triggered stomatal closure; and (3) application of ABA to the cytosol of guard cell protoplasts via a patch-clamp electrode inhibited inward K^+ currents, an effect sufficient to prevent stomatal opening (Schwartz et al., 1994). Similarly, Allan et al. (1994) showed that stomatal closure followed the intracellular release of microinjected "caged" ABA after photolysis. Recent studies of ABA effects on Ca^{2+} flux across the guard cell plasma membrane showed that ABA led rapidly to Ca^{2+} channel activation when added to the cytosolic side of the membrane in inside-out patches, but the activation showed a significant delay when ABA was added in the cell-attached configuration (Hamilton et al., 2000). These studies imply recognition on the cytoplasmic side of the plasma membrane and a close physical association between the presumed ABA receptor and the Ca^{2+} channel.

In other studies with guard cells, microinjected ABA had no effect on stomatal opening, yet extracellular application of ABA was nearly twice as effective at inhibiting stomatal opening at pH 6.15, at which the fully protonated form can readily cross the guard cell plasma membrane, than at pH 8.0, at which the mostly anionic ABA is unable to cross the membrane as an anion (Anderson et al., 1994). MacRobbie (1995) also observed a correlation between high external pH and attenuation of ABA-induced ion efflux. However, the observation that substantial ABA effects were induced even at high external pH led to the conclusion that intracellular ABA alone did not suffice to inhibit stomatal opening.

More direct evidence of extracellular perception was provided by the observation that externally applied, but not microinjected, ABA could repress GA-induced α-amylase expression in barley aleurone protoplasts (Gilroy and Jones, 1994). Extracellular ABA perception also was observed in two studies using ABA-protein conjugates that cannot enter the cell, but that are biologically active, to induce ion channel activity (Jeannette et al., 1999) and gene expression (Schultz and Quatrano, 1997; Jeannette et al., 1999).

Together, these results are consistent with the existence of both extracellular and intracellular ABA receptors. However, other interpretations are possible, for example, direct ABA action on plasma and tonoplast membranes (or ion channels) from the cytoplasmic side, higher affinity of an ABA receptor for the protonated form, or pH-dependent pathways.

The Search for Receptors

The use of ABA analogs in germination and gene expression bioassays has suggested the existence of multiple ABA receptors with different structural requirements for activity in different response pathways (Walker-Simmons et al., 1997; Kim et al., 1999). Furthermore, the rapid regulation of plasma membrane and tonoplast ion channel activities (Assmann and Shimazaki, 1999) might reflect direct interactions between ABA and transport proteins or other metabolic factors (e.g., via allosteric sites for ABA binding). Given the lack of concrete leads, the search for ABA receptors should include both intracellular and extracellular compartments and nonproteinaceous molecules. It is critically important for any receptor studies to use ABA analogs to correlate the specificity of interaction with the degree of biological activity.

In contrast to studies of ethylene and cytokinin signaling, a genetic approach has failed to identify any putative ABA receptor(s), to date. Consequently, the greatest progress has been made using biochemical and cell biological approaches. However, with the exception of an unconfirmed report (Hornberg and Weiler, 1984), no ABA receptors have been described. Although ABA binding proteins (Hocking et al., 1978; Pedron et al., 1998; Zhang et al., 1999) and carrier-mediated uptake of ABA (Astle and Rubery, 1983; Bianco-Colomas et al., 1991; Perras et al., 1994; Windsor et al., 1994) have been reported, there is no evidence to link these proteins to the physiological effects of ABA.

To further confound the situation, ABA has direct effects on membrane fluidity and thermal behavior (Parasassi et al., 1990; Burner et al., 1993; Shripathi et al., 1997), raising the possibility that ABA activity does not require interaction with a receptor. Indeed, it is entirely plausible that ABA may be analogous to lipophilic vitamins such as α-tocopherol (vitamin E) or vitamin K, low-molecular-weight compounds that are required in animals for fertility and blood clotting, respectively. Vitamin E can modulate transcription, yet its molecular mechanism of action is not known (Carlberg, 1999). On the other hand, the similarities between ABA in plants and retinoic acid in animals cannot be ignored. Both are synthesized ultimately from β-carotene (also known as pro-

vitamin A) by oxidative cleavage catalyzed by an evolutionarily conserved enzyme (Gu et al., 1997; Schwartz et al., 1997). If perception mechanisms also are conserved, ABA might bind to an intracellular receptor capable of acting as a transcription factor.

Screening of cDNA expression libraries with novel polyclonal antisera against ABA-related antigens has been one approach used to identify a putative ABA receptor. Liu et al. (1999) described a novel barley cDNA that is ABA inducible in embryos (*aba45*) and whose product binds a polyclonal antiserum raised against an anti-ABA monoclonal antibody. In theory, this means that the polyclonal antibodies may have epitopes (anti-idiotypic) that mimic the structure of ABA and therefore could bind to ABA binding proteins, including an ABA receptor. Interestingly, two Arabidopsis homologs of *aba45* were found in a yeast two-hybrid screen using the Arabidopsis formin-like protein AFH1, which itself is a membrane-localized protein that might be involved in the organization of the actin cytoskeleton (Banno and Chua, 2000). However, there is no evidence (e.g., specific and saturable binding of ABA to the gene product) to indicate that the *aba45*-like gene encodes an ABA receptor or that it interacts with the cytoskeleton.

Further circumstantial evidence for an intracellular ABA receptor comes from the results reported by Zheng et al. (1998), who screened a maize cDNA expression library with anti-ABA binding protein antibodies and identified a clone with 60% homology with nucleic acid binding proteins. The affinity-purified ABA binding complex used to raise these antibodies contained rRNA, suggesting a direct effect of ABA in regulating translation. Recently, several ABA response loci were identified that encode either double-stranded RNA binding proteins (Lu and Fedoroff, 2000) or putative RNA processing proteins (Hugouvieux et al., 2001; Xiong et al. 2001a). However, there is no evidence of ABA binding for these gene products, so their regulatory effects are likely to be indirect.

Given the substantial evidence for an extracellular perception site, the plasma membrane is an obvious place to look for an ABA receptor. A surface plasmon resonance biosensor was used in conjunction with flow cytometry of protoplasts to provide indirect, correlative in vitro evidence for an ABA receptor complex that interacts with a cell surface glycoprotein (Desikan et al., 1999). JIM19 is one of a panel of monoclonal antibodies generated previously against pea guard cell protoplasts that can modulate ABA responses in barley aleurone and rice protoplasts (Wang et al., 1995; Desikan et al., 1999). Using surface plasmon resonance biosensor technology, Desikan et al. (1999) observed specific binding of plasma membranes to JIM19, and the binding was antagonized significantly by ABA but not by the ABA catabolite phaseic acid. The in vitro interactions of plasma membranes, JIM19, and ABA correlated with the biological activities of JIM19, ABA, and phaseic acid on the activation of a green fluorescent protein reporter construct driven by the ABA-inducible early Met-labeled late-embryogenesis-abundant gene (*Em*) promoter, assayed by flow cytometry of transformed protoplasts. Together, these data suggest that JIM19 interacts with a functional complex involved in ABA signaling. However, phaseic acid can substitute for ABA in regulating other responses (Walker-Simmons et al., 1997), suggesting that additional ABA receptors are likely to exist.

Further indirect evidence of a plasma membrane–localized receptor came from in vitro assays of ABA-stimulated phospholipase D (PLD) activity in plasma membrane–enriched fractions from barley aleurone protoplasts (Ritchie and Gilroy, 2000). In vitro activation of PLD by ABA was similar in kinetics and degree to that measured in vivo and was mediated by G protein. These results suggest, but do not prove, the existence of an ABA receptor system and elements (e.g., glycoproteins) at the plasma membrane linked via G proteins to PLD activation.

Another way to identify a receptor is to reconstitute an ABA signaling pathway in a heterologous system (e.g., *Xenopus* oocytes). Sutton et al. (2000) microinjected oocytes with cell-specific (mesophyll versus guard cells) *Vicia faba* mRNA pools and then assayed their ability to modulate K^+ currents with ABA treatments. They found that expression of mesophyll cell RNA produced an ABA-regulated outward K^+ current in oocytes comparable to that observed in mesophyll cells, but only guard cell mRNA conferred ABA regulation of a coexpressed guard cell–specific K^+ inward-rectifying channel. The authors concluded that distinct receptor types and/or signal transduction pathways function in the ABA regulation of K^+ channels in mesophyll versus guard cells. The ability to reconstitute ABA perception pathways in oocytes could be useful in characterizing ABA response mechanisms, for example, by expression cloning of rate-limiting or autonomously functioning components. However, this approach might not work if multiple components must be coexpressed to reconstitute the response mechanism.

Another study using the oocyte system found that expression of mRNA from drought-stressed tobacco led to ABA activation of the endogenous Ca^{2+}-dependent Cl^- current (Leyman et al., 1999). Assays with subpools of transcripts from a cDNA library led to cloning of a syntaxin-like protein, Nt-SYR1, whose enrichment correlated with an increase in the ABA-evoked current. Paradoxically, expression of Nt-SYR1 alone was sufficient for ABA-independent modulation of the Cl^- current in oocytes. The relevance of Nt-SYR1 to ABA signaling in plants was demonstrated by the correlated disruption of syntaxin function and ABA-regulated ion fluxes when exposed to either *Clostridium botulinum* type C toxin (an inhibitor of syntaxin-dependent vesicle trafficking) or a soluble fragment of the SYR1 protein (expected to exert a dominant-negative effect by competing for protein–protein interactions). Biochemical analysis indicated that Nt-Syr1 is located primarily at the plasma membrane in roots and to lesser extents in stems, leaves, and flowers. Expression of the protein in leaves is enhanced transiently by ABA and stress signals (Leyman et al., 2000). However, there is no

evidence that Nt-Syr1 binds ABA, and the ABA-independent effect of the purified transcript in the oocyte system suggests that its effect on ABA signaling is indirect. Some likely contribution(s) of syntaxin-like genes to stomatal response might be the regulation of membrane vesicle trafficking and the resulting change in surface area and channel composition of guard cell membranes, direct binding and regulation of ion channel activities (as described in neurons), and scaffolding of a receptor complex (reviewed by Blatt, 2000).

It is hoped that proteomics approaches, combined with biochemical, genetic, and cell biological studies, will finally identify the enigmatic ABA receptor(s).

IDENTIFICATION OF SIGNALING INTERMEDIATES

Genetic Approach

Dozens of mutants with defects in ABA response have been isolated to date, the vast majority of them in Arabidopsis. The genetic screens and selections that have been used include production of seeds exhibiting vivipary or defective germination (Robertson, 1955; Sturaro et al., 1996); increased transpiration rates resulting in decreased leaf temperatures (Raskin and Ladyman, 1988); ABA-independent desiccation tolerance of cell cultures (Furini et al., 1997); altered embryonic development (Meinke et al., 1994); loss or gain of sensitivity to ABA at germination (Koornneef et al., 1984; Finkelstein, 1994; Cutler et al., 1996), seedling growth (Lopez-Molina and Chua, 2000), or root growth (Himmelbach et al., 1998); incorrect expression of reporter genes (Ishitani et al., 1997; Foster and Chua, 1999; Delseny et al., 2001); and screens for suppressors or enhancers of GA-deficient nongerminating lines or *ABA-INSENSITIVE* (*ABI*) lines (Steber et al., 1998; Beaudoin et al., 2000; Ghassemian et al., 2000) (Table 1). The loci that have been cloned to date include those encoding a variety of transcription factors, RNA binding proteins, protein kinases and phosphatases, an enzyme of phosphoinositide metabolism, and a subunit of farnesyl transferase.

Additional mutants with defects in responses to multiple signals, including ABA, have been isolated via non-ABA-based screens such as salt-resistant germination (Quesada et al., 2000), sugar-resistant seedling growth or gene expression (Arenas-Huertero et al., 2000; Huijser et al., 2000; Laby et al., 2000; Rook et al., 2001), or defects in auxin, brassinosteroid, or ethylene response (Wilson et al., 1990; Alonso et al., 1999; Ephritikhine et al., 1999) (Table 1). The fact that only some of the hormone response loci appear to regulate multiple signaling pathways suggests that interactions among these pathways are relatively specific. Possible mechanisms of interactions are discussed in many recent reviews (McCourt, 1999; Sheen et al., 1999; Gibson, 2000; Coruzzi and Zhou, 2001; Gazzarrini and McCourt, 2001).

Biochemical and Cell Biological Approach

Transcriptional Regulation

A major avenue of biochemical studies of ABA signaling began with the identification of ABA-regulated genes (reviewed by Busk and Pages, 1998; Rock, 2000). In most vegetative tissues, these are genes involved in response to abiotic stresses that result in cellular dehydration (Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 2000; Xiong et al., 2002). In maturing seed, ABA-regulated genes include those required for the synthesis of storage reserves and the acquisition of desiccation tolerance (Thomas, 1993; Rock, 2000). Overall, ABA-regulated genes range from relatively high-abundance transcripts, which are required for adaptation to stress or reserve synthesis, to low-abundance transcripts, which encode signaling components. Although studies of ABA-regulated genes initially focused on working backward to regulators of the high-abundance transcripts by sequential identification of *cis*-acting regulatory regions and the transcription factors that specifically recognize these DNA sequences, recent studies have focused on the physiological roles of ABA-regulated kinases, lipases, etc. These studies have been conducted in a variety of species, but heterologous expression studies and identification of orthologs of the regulatory genes have shown that the ABA signaling mechanisms are highly conserved.

Four main groups of *cis*-acting sequences are known to be required or sufficient for ABA inducibility: the G-box elements designated ABA response elements (ABREs) and the functionally equivalent CE3 (coupling element)-like sequences; the RY/Sph elements; and recognition sequences for MYB- and MYC-class transcription factors (Table 2) (reviewed by Busk and Pages, 1998; Rock, 2000). The corresponding *trans*-acting factors were identified initially by ligand binding screens of cDNA expression libraries (Guiltinan et al., 1990; Oeda et al., 1991); more recent efforts have used one-hybrid screens in yeast with the *cis*-acting sequence of interest to control reporter gene expression (Kim et al., 1997; Choi et al., 2000; Uno et al., 2000). These studies have shown that the ABREs and RY elements are bound by proteins containing basic Leu zipper domains (bZIPs) and B3 domain proteins, respectively.

There are 81 predicted bZIP factor genes in Arabidopsis (Riechmann et al., 2000), many of which probably are not involved in ABA response. Only one bZIP subfamily has been linked genetically to ABA response: that composed of ABI5 and its homologs, the ABRE binding factors (ABFs and AREBs) (Choi et al., 2000; Uno et al., 2000), and AtDPBFs (*Arabidopsis thaliana Dc3* promoter binding factors) (T. Thomas, personal communication). Homologs of these genes have been characterized in sunflower and rice (Kim et al., 1997; Kim and Thomas, 1998; Hobo et al., 1999), in which they are correlated with ABA-, seed- or stress-induced gene expression. However, studies of bZIPs from other species have shown

that in vitro binding of ABREs need not reflect action in ABA signaling in vivo (Guiltinan et al., 1990; Izawa et al., 1993; Nantel and Quatrano, 1996). Conversely, although first identified in connection with light-regulated gene expression, the bZIP GBF3 is ABA inducible and may participate in ABA regulation (Lu et al., 1996). Reverse genetic studies for each of the bZIPs should identify those family members responsible for responding to specific signals in specific tissues.

The Arabidopsis genome encodes 43 members of the B3 domain family, but only 14 of them are within the ABI3/VP1-related subfamily (Riechmann et al., 2000). This subfamily also includes FUS3 (Luerssen et al., 1998) and LEC2 (Stone et al., 2001), members of the leafy-cotyledon class of regulators that control embryo maturation. Some of the more distantly related B3 domain family members are in the auxin response factor subfamily and participate in auxin regulation of gene expression (reviewed by Ulmasov et al., 1999).

Members of both the MYB and MYC transcription factor families are expressed in response to abiotic stress (Urao et al., 1993; Abe et al., 1997). Although the *MYB* gene superfamily in Arabidopsis is composed of 190 genes (Riechmann et al., 2000), there is only a single *MYC* gene with the canonical b-HLH-ZIP domain structure, but this shares extensive homology with 139 bHLH factor genes. Both drought and ABA induce the expression of *AtMyc1* and three specific *MYB* family members (Abe et al., 1997). The MYB/MYC response system is somewhat slower than the bZIP-ABRE system, reflecting the need for de novo synthesis of MYB and MYC proteins. It has been suggested that the MYB/MYC system regulates slow adaptive responses to dehydration stress (Shinozaki and Yamaguchi-Shinozaki, 2000). ABA or abiotic stress also induces the expression of some members of the homeodomain Leu zipper (HD-Zip) family of transcription factors (*ATHB6*, *ATHB7*, and *ATHB12*), but their roles in ABA response are not known (Söderman et al., 1996, 1999; Lee and Chun, 1998).

Although hundreds of ABA-regulated genes have been identified to date, many of them homologs from a broad range of species, these are likely to represent a somewhat anecdotal sampling of the full spectrum of ABA-responsive genes. Preliminary transcriptional profiling studies in Arabidopsis have shown that as many as 8 to 10% of the genes on a partial genome chip are either induced or repressed by 12 hr of exposure to ABA at a single developmental stage (T. Thomas, personal communication). Assuming a similar abundance of ABA-regulated genes in the rest of the genome leads to an estimation of at least 2000 ABA-responsive genes in Arabidopsis alone. The advent of genome-wide transcriptional profiling, coupled with the public availability of the complete genome sequence for Arabidopsis, should facilitate the identification of unknown ABA-responsive genes, the correlation of target genes with specific regulatory factors, and the rapid identification of candidate *cis*-acting sequences of coordinately regulated genes. Such studies may lead to new classes of regulators or new targets or functions for known regulators. In combination with the modular nature of promoters, the existence of at least four classes of regulators, most with dozens of family members, provides an enormous potential for redundancy and combinatorial controls in ABA-regulated gene expression.

Early Signaling Intermediates

Assays with single cells provide relatively simple biological systems for testing the roles of candidate secondary messengers and signaling intermediates in regulating cellular responses. The best-characterized single cell systems for analyzing ABA responses are inhibition of stomatal opening and stomatal closing resulting from ionic and osmotically driven turgor and shape changes in guard cells, and transient gene expression assays in protoplasts or microinjected tissues. Although the initial studies have been performed on diverse species, most of the signaling mechanisms are conserved and can be dissected further by mutant analyses with Arabidopsis or inclusion of Arabidopsis genes in heterologous assay systems.

G Proteins

Biochemical and pharmacological studies have shown that G proteins, phospholipases, protein kinases, and protein phosphatases participate in early events in ABA signaling (reviewed by Rock, 2000; Assmann, 2002; Yang, 2002). Because these classes of proteins function in a wide range of signaling events, a critical question regards how specificity is conferred. For those classes of regulators represented by large gene families, individual family members may perform specialized functions, but this must be confirmed by functional tests. However, Arabidopsis has only one or two isoforms of each G protein subunit, 1 order of magnitude fewer than are present in animal genomes. Recent studies show that loss of function for the single Gα gene (*GPA1*) disrupts aspects of both auxin and ABA signaling (Ullah et al., 2001; Wang et al., 2001), indicating that this component is not a likely source of response specificity.

The other major class of GTPase molecular switches in plants are the monomeric Rops (Rho/rac-related GTPases from plants, a plant-specific branch of the RAS superfamily), whose Arabidopsis members also have been known as Aracs and AtRacs (Yang, 2002). This subfamily of GTPases is composed of 11 members in Arabidopsis, representing four distinct groups, whose physiological and developmental roles are being analyzed in loss-of-function lines and gain-of-function (constitutively active and dominant-negative) transgenic lines. These studies have shown that *Rop9* and *Rop10* appear to act redundantly in negatively regulating ABA effects on seed germination and seedling growth, such that double mutants resemble *era1* mutants (Yang, 2002). Both of these Rops contain farnesylation motifs and display *ERA1*-dependent localization to the plasma membrane,

Table 1. Mutants Defective in ABA Synthesis or Response

Species	Mutation	Selection/Screen	Phenotype	Alleles or Orthologs	Gene product	References
Arabidopsis	*aba1*	Suppressors of non-germinating GA-deficient lines	ABA deficient; wilty; decreased stress or ABA induction of gene expression; sugar-resistant seedling growth	*los6* *npq2*	Zeaxanthin epoxidase	Koornneef et al., 1982; Ishitani et al., 1997; Niyogi et al. 1998; Xiong et al., 2001b
	aba2	Reduced dormancy	ABA deficient; wilty; decreased stress or ABA induction of gene expression; sugar-resistant seedling growth	*gin1* *isi4* *sis4*		Leon-Kloosterziel et al., 1996; Laby et al., 2000; Rook et al., 2001
	aba3	Reduced dormancy	ABA deficient; wilty; decreased stress or ABA induction of gene expression; freezing sensitive; sugar-resistant seedling growth	*frs1* *los5*	Aldehyde oxidase Moco	Leon-Kloosterziel et al., 1996; Ishitani et al., 1997; Llorente et al., 2000; Rook et al., 2001; Xiong et al., 2001c
	aao3	Wilty phenotype	ABA-deficient leaves; wilty, but near-normal seed dormancy	Tomato *sitiens?*	Aldehyde oxidase	Seo et al., 2000
	abi1-1	ABA-resistant germination	Nondormant seed; pleiotropic defects in vegetative ABA response		Protein phosphatase 2C	Koornneef et al., 1984; Leung et al., 1994; Meyer et al., 1994
	abi2-1	ABA-resistant germination	Similar to *abi1-1*		Protein phosphatase 2C	Koornneef et al., 1984; Leung et al., 1997; Rodriguez et al., 1998
	abi3	ABA-resistant germination	Pleiotropic defects in seed maturation; vegetative effects on plastid differentiation	Cereal *VP1*	B3 domain transcription factor	Koornneef et al., 1984; Giraudat et al., 1992
	abi4	ABA-resistant germination	Sugar- and salt-resistant germination and seedling growth	*gin6* *isi3* *san5* *sis5* *sun6*	APETALA2 domain transcription factor	Finkelstein, 1994; Finkelstein et al., 1998; Arenas-Huertero et al., 2000; Huijser et al., 2000; Laby et al., 2000; Rook et al., 2001
	abi5	ABA-resistant germination	Slightly sugar-resistant germination and seedling growth	*AtDPBF1*	bZIP domain transcription factor	Finkelstein, 1994; Finkelstein and Lynch, 2000a; Lopez-Molina and Chua, 2000
	abi8	ABA-resistant germination	Stunted growth, defective stomatal regulation; male sterile			Finkelstein and Lynch, 1997
	abh1	ABA-hypersensitive germination and guard cell response	Pleiotropic; also enhanced drought tolerance		mRNA CAP binding protein	Hugouvieux et al., 2001
	era1	Enhanced response to ABA at germination	Enhanced stomatal response/ drought tolerance, meristem defect	*wiggum*	Farnesyl transferase, β-subunit	Cutler et al., 1996
	era3	Enhanced response to ABA at germination	Allelic to *ein2*; ethylene insensitive	*ein2*	Membrane-bound metal sensor?	Alonso et al., 1999; Ghassemian et al., 2000
	ctr1	Enhance ABA resistance of *abi1-1*	Reduced dormancy, constitutive triple response to ethylene		Protein kinase (Raf family)	Kieber et al., 1993; Beaudoin et al., 2000
	gca1 *gca2*	ABA-resistant root growth	Pleiotropic effects on growth, stomatal regulation, and germination			Himmelbach et al., 1998; Pei et al., 2000
	gca3-gca8	ABA-resistant root growth				Himmelbach et al., 1998
	ade1	Deregulation of KIN2::LUC expression	No growth phenotype			Foster and Chua, 1999
	hlq *sbr*	Aberrant patterns of Dc3::GUS expression	Pleiotropic, seedling lethal			Rock, 2000; Subramanian et al., 2002

Continued

Table 1. (continued).

Species	Mutation	Selection/Screen	Phenotype	Alleles or Orthologs	Gene product	References
	hos1	Hypersensitive to osmotic stress induction of RD29::LUC	Hypersensitive to ABA-induced gene expression		RING finger protein	(Ishitani et al., 1997; Lee et al., 2001
	hos2 *hos5*	Hypersensitive to osmotic stress induction of RD29::LUC	Hypersensitive to ABA-induced gene expression			Ishitani et al., 1997; Xiong et al., 1999
	sad1	Supersensitive to ABA and drought induction of RD29::LUC	Reduced ABA biosynthesis, hypersensitive to ABA inhibition of germination and root growth		U6-related Sm-like small ribonucleoprotein	Xiong et al., 2001a
	cla1	Chloroplasts altered	ABA deficient		1-Deoxy-D-xylulose-5-phosphate synthase	Estévez et al., 2001
	hyl1	Hyponastic leaves	Hypersensitive to ABA		Double-stranded RNA binding protein	Lu and Fedoroff, 2000
	axr2	Auxin-resistant root growth	Resistant to ABA and ethylene; dominant negative		IAA7 transcription regulation	Wilson et al., 1990; Nagpal et al., 2000
	bri1	Brassinosteroid insensitive	ABA hypersensitive		Ser/Thr protein kinase	Li and Chory, 1997; Steber and McCourt, 2001
	det2	De-etiolated	ABA hypersensitive		Steroid reductase	Steber and McCourt, 2001
	fry1	Constitutive expression of RD29::LUC	ABA hypersensitive		Inositol polyphosphate-1-phosphatase	Xiong et al., 2001b
	jar1 *jin4*	Jasmonic acid resistant	Hypersensitive to ABA-inhibition of germination			Staswick et al., 1992; Berger et al., 1996
	lec1	Leafy cotyledons, seed lethal	Slightly ABA-resistant germination		CCAAT-box binding, HAP3 homolog	Meinke et al., 1994; Parcy et al., 1997; Lotan et al., 1998
	los1	Low sensitivity to osmotic stress induction of RD29::LUC	Low sensitivity to ABA induction of gene expression			
	prl1	Hypersensitivity to Glc and Suc	Hypersensitive to ABA (also to cytokinin, ethylene, and auxin)		Nuclear WD40 domain protein	Németh et al., 1998; Bhalerao et al., 1999
	sax1	Hypersensitive to auxin	ABA hypersensitive, BR deficient			Ephritikhine et al., 1999
	uvs66	UV light sensitivity	Hypersensitive to ABA inhibition of root growth			Albinsky et al., 1999
Resurrection plant	*cdt-1*	ABA-independent desiccation tolerance	Constitutive ABA response in callus cultures		Regulatory RNA or small peptide	Furini et al., 1997
Barley	*cool*	Decreased leaf temperature	ABA insensitivity in guard cells			Raskin and Ladyman, 1988
Maize	*vp1*	Viviparous	ABA-insensitive seeds	ABI3	B3-domain transcription factor	Robertson, 1955; McCarty et al., 1991
	vp2-vp14	Viviparous	ABA deficient			Robertson, 1955; Neill et al., 1986; Schwartz et al., 1997
	rea	Defective germination	Red embryonic axis caused by anthocyanins; ABA-resistant germination; occasional vivipary			Sturaro et al., 1996

Table 2. Transcriptional Regulators Implicated in ABA Signaling

Binding Site/Factor Class	Genus	Factor	References
ABA response elements (ABREs)/bZIPs	*Arabidopsis*	ABI5/AtDPBF1	Choi et al., 2000; Finkelstein and Lynch, 2000a; Lopez-Molina and Chua, 2000; Uno et al., 2000
		AtDPBF2	
		AtDPBF3/AREB3	
		AtDPBF4	
		AtDPBF5/ABF3	
		ABF1	
		ABF2/AREB1	
		ABF4/AREB2	
		GBF3	Lu et al., 1996
	Helianthus	DPBF1, -2 and -3	Kim et al., 1997; Kim and Thomas, 1998
	Oryza	TRAB1	Hobo et al., 1999
	Phaseolus	PvZIP6	AF369792[a]
		ROM2 (repressor)	Chern et al., 1996
	Triticum	EmBP-1	Guiltinan et al., 1990
RY/Sph elements/B3 domain proteins	*Arabidopsis*	ABI3	Giraudat et al., 1992
	Avena	AfVP1	Jones et al., 1997
	Craterostigma	CpVP1	Chandler and Bartels, 1997
	Daucus	C-ABI3	Shiota et al., 1998
	Phaseolus	PvALF	Bobb et al., 1995
	Populus	PtABI3	Rohde et al., 1998
	Oryza	OsVP1	Hattori et al., 1994
	Triticum	TaVP1	Bailey et al., 1999
	Zea mays	VP1	McCarty et al., 1991
MYB	*Arabidopsis*	AtMYB2	Abe et al., 1997
MYC	*Arabidopsis*	AtMYC	Abe et al., 1997
Unknown/HD-Zip	*Arabidopsis*	ATHB6	Söderman et al., 1996; Lee and Chun, 1998; Söderman et al., 1999
		ATHB7	
		ATHB12	
Unknown/AP2	*Arabidopsis*	ABI4	Finkelstein et al., 1998; Söderman et al., 2000

[a] Member of the ABI5 homologous subfamily, but no direct evidence for a role in ABA signaling; isolated from ethylene-treated leaf abscission zones of bean.

suggesting that they might act downstream of *ERA1* in a signaling pathway.

Transgenic studies also have shown that *Rop6*/*AtRac1* can inhibit ABA effects on the actin cytoskeleton in guard cells (Lemichez et al., 2001), whereas *Rop2* negatively regulates seed dormancy and inhibition of germination by ABA (Li et al., 2001). However, manipulation of Rop2 activity also disrupted a wide variety of developmental processes as well as responses to auxins and brassinolides. Although interpretation of the transgenic phenotypes is complicated by the possibilities that the transgenes act ectopically or disrupt closely related family members, it appears that several of the Rops inhibit various aspects of ABA response. Furthermore, *ERA1* is allelic to *WIGGUM*, which, along with CLAVATA as part of a Rop- and KAPP protein phosphatase–containing complex, controls floral meristem proliferation (Trotochaud et al., 1999; Ziegelhoffer et al., 2000). The Rops may be important nodes in a complex regulatory network that mediates hormonal and environmental control of growth and development. However, the pleiotropic defects of the transgenic lines suggest that Rop response specificity may not be explained simply by specialization of function among the monomeric G proteins but also may depend on interactions with specific activators and targets.

Secondary Messengers

Inositol triphosphate (IP_3), which is produced by phospholipase C (PLC) activity, acts as a secondary messenger in ABA signaling, regulating stomatal function and gene expression (Gilroy et al., 1990). Expression of only one of the six Arabidopsis PLC genes, *AtPLC1*, is induced by ABA (Hirayama et al., 1995). Studies of antisense and overexpression lines have shown that *AtPLC1* is necessary, but not sufficient, for ABA effects on germination, growth, and vegetative gene expression (Sanchez and Chua, 2001). More highly phosphorylated inositides also have been shown to act as signals in animal cells (reviewed by Shears, 1998), and IP_6 appears to function in the ABA inhibition of stomatal opening (Lemtiri-Chlieh et al., 2000). The recent discovery that a defect in phosphoinositide metabolism results in hypersensitivity to ABA and

abiotic stresses further emphasizes the role of phosphoinositides as secondary messengers (Xiong et al., 2001b).

Phosphatidic acid (PA), which is produced by the action of phospholipase D (PLD), also mediates ABA regulation of stomatal aperture (Jacob et al., 1999) and gene expression (Ritchie and Gilroy, 1998; Gampala et al., 2001). ABA stimulation of PLD activity in microsomes derived from barley aleurone plasma membranes is initiated by ABA perception at the plasma membrane and mediated by G protein activity (Ritchie and Gilroy, 2000). The Arabidopsis genome contains 11 predicted PLD genes grouped into five subfamilies. Although many of these homologs are induced by a variety of stresses (Wang et al., 2000) and show different tissue distributions and subcellular localizations (Fan et al., 1999), ABA promotes the expression and increased activity of only the most prevalent PLD, PLDα. Antisense suppression of PLDα slows abscisic acid– and ethylene-promoted senescence of detached Arabidopsis leaves, providing functional evidence of a role for this family member in ABA signaling (Fan et al., 1997). In contrast, AtPLDδ expression and activity are induced by dehydration but not by ABA (Katagiri et al., 2001).

Pharmacological and Ca^{2+}-imaging studies have shown that stomatal closure is induced by exposure to external Ca^{2+} or elicitors of Ca^{2+} release, and ABA-induced closing involves both Ca^{2+}-dependent and Ca^{2+}-independent mechanisms (reviewed by Schroeder et al., 2001). However, recent studies suggest that the Ca^{2+}-independent signaling elements do not represent a completely separate Ca^{2+}-independent ABA signaling pathway (Webb et al., 2001). Auxin-induced stomatal opening also is correlated with cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) increases, indicating that the detailed characteristics of the Ca^{2+} oscillations, amplitudes, and localizations (the "Ca^{2+} signature") and the cellular interpretation of the $[Ca^{2+}]_{cyt}$ change are critical determinants of response specificity.

$[Ca^{2+}]_{cyt}$ increases can result from the release of Ca^{2+} from intracellular stores and/or influx through plasma membrane channels. Ca^{2+} release from intracellular stores can be induced by IP_3 (Gilroy et al., 1990) or cyclic ADP-Rib (Leckie et al., 1998); inhibiting the production or action of either signal only partially blocks ABA-induced $[Ca^{2+}]_{cyt}$ increases (MacRobbie, 2000), indicating that both contribute to the increases but that neither is sufficient for full response. Another calcium-mobilizing molecule, sphingosine-1-phosphate, also appears to contribute to drought-induced ABA signaling leading to stomatal closure (Ng et al., 2001). $[Ca^{2+}]_{cyt}$ increases also may be self-amplifying by promoting further Ca^{2+} release from the vacuoles (McAinsh et al., 1995). Transient Ca^{2+} influx currents are induced by hyperpolarization activation of Ca^{2+}-permeable plasma membrane channels (Grabov and Blatt, 1998a).

Recent studies have shown that ABA sensitizes these channels, apparently by enhancing the production of reactive oxygen species (ROS; e.g., H_2O_2) that can serve as secondary messengers leading to channel activation (Pei et al., 2000; Zhang et al., 2001). ROS production is a common Rop-dependent response to several stresses leading to stomatal closure, including drought and pathogen attack (Lee et al., 1999), and the ROS-dependent pathway of response may be shared by multiple stresses (Yang, 2002). Consistent with this view, constitutive activation of an H_2O_2-activated mitogen-activated protein kinase cascade confers enhanced tolerance of abiotic stresses (Kovtun et al., 2000). Finally, although ABA signaling can result in sustained steady state $[Ca^{2+}]_{cyt}$ increases, various signals that affect stomatal aperture induce $[Ca^{2+}]_{cyt}$ oscillations with distinct periodicity, and responses in ABA mutants can be "rescued" by imposing the correct periodicity with exchanges of external buffer solutions (Allen et al., 2000, 2001).

In addition to the Ca^{2+}-mediated changes, ABA-induced stomatal closing depends in part on cytosolic alkalization (reviewed by Grabov and Blatt, 1998b). The pH stimulation of stomatal closure can occur in isolated membrane patches and appears to function in part by increasing the number of K^+_{out} channels available for activation (Miedema and Assmann, 1996). In addition, increased external pH decreases the activity of K^+_{in} channels (Hedrich et al., 1995) and increases the activity of GORK, a guard cell–localized K^+_{out} channel (Ache et al., 2000). Because ABI1 protein phosphatase 2C is activated by increasing pH (Leube et al., 1998) and inactivates ABA responses (Gosti et al., 1999; Merlot et al., 2001), the activation also may be a feedback mechanism for ABA desensitization.

Transient gene expression assays monitor responses to ABA during a period of several hours rather than minutes to hours. These studies have made use of microinjection to introduce potential signals or inhibitors into intact tissues. Similar to guard cell signaling, Ca^{2+}, IP_3, PA, and cyclic ADP-Rib can act as secondary messengers for the ABA induction of gene expression (Gilroy and Jones, 1992; Heimovaara-Dijkstra et al., 1995; Wu et al., 1997; Ritchie and Gilroy, 1998; Ghelis et al., 2000b; Webb et al., 2001). Surprisingly, even S-type anion channel activity is required for ABA-induced gene expression (Ghelis et al., 2000a). Bombardment and electroporation of genes encoding signaling components also have been used to test these genes' functions in intact tissues and protoplasts, respectively. Such studies have been used to analyze interactions among transcription factors and their dependence on specific secondary messengers or phosphorylation states (Hagenbeek et al., 2000; Uno et al., 2000; Gampala et al., 2001).

Phosphorylation Cascades

Many kinases have been implicated in ABA signaling affecting stomatal regulation and/or gene expression (Table 3). Some of these show ABA-inducible expression (Hwang and Goodman, 1995; Hong et al., 1997; Lee et al., 1998; Mikami et al., 1998; Gómez-Cadenas et al., 1999; Piao et al., 1999), whereas others are expressed constitutively but are activated by ABA (Li and Assmann, 1996; Burnett et al., 2000).

Table 3. Genes Implicated in ABA Signaling by Reverse Genetics or Expression Pattern

Locus/Gene	Engineered Effect	Phenotype	Response to ABA	Gene Product	References
AAPK (from *Vicia faba*)	Dominant negative	No ABA-induced stomatal closure; no ABA activation of plasma membrane anion channels	Activated	Ser/Thr protein kinase	Li and Assmann, 1996; Li et al., 2000
AMBP kinase (from *Pisum sativum*)			Activation; correlated with stomatal closure & dehydrin expression	ABA-activated myelin basic protein kinase	Burnett et al., 2000
ARSK1			Induced by ABA or NaCl	Root specific ser/thr kinase	Hwang and Goodman, 1995
ATCDPK1 *ATCDPK1a*	Constitutively active mutants expressed transiently in maize leaf protoplasts	Constitutive activation of an ABA responsive reporter gene (HVA1-LUC)		Calcium-dependent protein kinases	Sheen, 1996
AtIP5PII	Overexpression	ABA-insensitive germination, growth and gene expression		Inositol polyphosphate 5-phosphatase II	Sanchez and Chua, 2001
AtPLC1	Antisense suppression	ABA-insensitive germination, growth and gene expression		Phosphoinositide specific phospholipase C	Sanchez and Chua, 2001
AtPP2C	Wild-type and mutant proteins expressed transiently in maize leaf protoplasts; antisense suppression	Over-expression blocked ABA-inducible transcription; null mutation had little effect; dominant interfering mutant strongly repressed ABA responses. Antisense conferred increased sensitivity to ABA-induced cold-tolerance and growth inhibition		Protein phosphatase 2C	Sheen, 1998; Tahtiharju and Palva, 2001
AtRac1/Rop6	Dominant-positive and dominant-negative	Dominant positive blocked ABA-mediated effects on actin cytoskeleton and stomatal closure; dominant negative induced closure in absence of ABA		Small GTPase	Lemichez et al., 2001
			Repressed; correlated with cell division activity	Cyclin-dependent kinase	Hemerly et al., 1993
GPA1	T-DNA insertion	Increased leaf transpiration; no ABA inhibition of guard cell K^+_{in} channels and pH-independent ABA-activation of anion channels		G protein α-subunit	Wang et al., 2001
			Induced by ABA and NaCl; may function in stress response	GSK3/shaggy-like protein kinase	Piao et al., 1999
			ABA effect unknown; induced by osmotic stress	His kinase osmosensor	Urao et al., 1999

Continued

Table 3. (continued).

Locus/Gene	Engineered effect	Phenotype	Response to ABA	Gene product	References
ICK1			Induced; may suppress cell division	Inhibitor of cyclin-dependent kinase	Wang et al., 1998
MAPKKK			ABA effect unknown; induced by abiotic stresses	Mitogen-activated protein kinase kinase kinase	Mizoguchi et al., 1996
PIP5K			Induced by ABA and abiotic stresses	Phosphatidylinositol-4-phosphate 5-kinase	Mikami et al., 1998
PKABA1	Constitutive expression	Suppression of GA-inducible gene expression in aleurone; small effect on the ABA induction of a LEA gene	Induced	Ser/Thr-protein kinase (from barley)	Gómez-Cadenas et al., 1999
PLDα	Antisense	Decreased ABA and ethylene promotion of senescence		Phospholipase Dα	Fan et al., 1997
ROP2	Dominant negative constitutive	Altered sensitivity to germination inhibition by ABA		Rho-type small GTPase	Li et al., 200
RPK1			Induced by ABA and abiotic stresses; abiotic stress-induction not ABA-dependent	Receptor-like protein kinase	Hong et al., 1997
			Unknown; induced by abiotic stresses	Ribosomal S6 kinase-like	Mizoguchi et al., 1996
WAPK (from tobacco)			Induced	Wounding-induced protein kinase	Lee et al., 1998

Functional evidence of kinase participation in any response initially relied on pharmacological inhibitors; recent studies have made use of dominant-negative alleles to assay the roles of specific kinases (Sheen, 1996; Li et al., 2000). For example, *AAPK* encodes a guard cell–specific ABA-activated Ser/Thr protein kinase that was suspected of functioning in the ABA regulation of stomatal aperture (Li et al., 2000). Expression of recombinant AAPK defective in ATP binding inhibits ABA-induced stomatal closure by preventing the activation of anion channels, but it does not affect the ABA inhibition of stomatal opening, thereby demonstrating its role in a segment of the ABA signaling chain that is specific for stomatal closing. Presumably, identification of its substrate will help identify additional members of this signaling pathway.

In addition, some members of the protein phosphatase 2C family have been shown to have pleiotropic negative effects on ABA signaling (Sheen, 1998; Gosti et al., 1999; Merlot et al., 2001). It is intriguing to speculate that these may act to reverse the phosphorylation catalyzed by kinases such as AAPK, but none of their specific substrates have been identified. Similarly, both ABA-induced gene expression and stomatal closure can be altered by okadaic acid, an inhibitor of PP1/PP2A phosphatases, but whether okadaic acid stimulates or inhibits ABA response varies among species (Kuo et al., 1996; Grabov et al., 1997; Pei et al., 1997; Wu et al., 1997). In Arabidopsis, okadaic acid partially inhibits ABA activation of S-type anion channels and stomatal closure (Pei et al., 1997). Recently, a PP2B (calcineurin-like) Ca^{2+} binding protein, AtCBL1, was found to be induced by drought (Kudla et al., 1999); this protein could participate in response to $[Ca^{2+}]_{cyt}$ changes. Although a variety of potential Ca^{2+} sensors correlated with stress response have been identified, relatively few also are ABA responsive (Takahashi et al., 2000).

ABA SIGNALING IN SEEDS AND SEEDLINGS

Phase Transitions: The Decisions to Divide or Enlarge, Arrest or Germinate

Seed maturation begins when developing embryos cease cell division and start growing by cell enlargement as they begin to accumulate storage reserves. This transition is

correlated with an increase in seed ABA content, consistent with the fact that ABA can induce the expression of a cyclin-dependent kinase inhibitor (ICK1) (Wang et al., 1998) that would lead to cell cycle arrest at the G1/S transition. Studies with ABA-deficient mutants of tomato showed that mature seed had a significantly higher proportion of G2 cells (4C DNA) than other genotypes, providing further support for the idea that endogenous ABA is required for arrest in G1 (Liu et al., 1994).

During seed maturation in many species, there are two peaks of ABA accumulation. In some species (e.g., *Brassica napus*), these peaks are correlated with low germinability of isolated embryos (Finkelstein et al., 1985), whereas in others (e.g., maize), there is no correlation (Rivin and Grudt, 1991). Genetic studies in Arabidopsis demonstrated that the first ABA peak is maternally derived and immediately precedes the maturation phase (Karssen et al., 1983). This is important, in conjunction with the leafy-cotyledon *FUS3* and *LEC* genes, for preventing premature germination at the end of the cell division phase of embryogenesis (Raz et al., 2001). However, although this early ABA peak is reduced threefold in *fus3* mutants, only the double mutants combining *fus3* with ABA deficiency are highly viviparous (Nambara et al., 2000); no ABA-insensitive (*abi*) or even digenic ABA-deficient (*aba3* and *aao3*) (Seo et al., 2000) Arabidopsis lines are viviparous. In contrast, maize mutants with single defects in either ABA response (*vp1*) or synthesis (other *vp* mutants) are viviparous (Robertson, 1955; Robichaud et al., 1980). Although some combinations of *abi* and leafy-cotyledon mutations also lead to vivipary, there is no good correlation between the degrees of vivipary and seed sensitivity to ABA (e.g., compare *abi4 lec1* and *abi5 lec1*) (Table 4). These results further accentuate the distinction between the mechanisms controlling vivipary and the ABA sensitivity of germination.

Another maize mutant, *rea* (*red embryonic axis*), has a phenotype that combines aspects of the Arabidopsis leafy-cotyledon and ABA-insensitive classes (Sturaro et al., 1996). These mutants exhibit occasional vivipary and ABA-resistant germination despite normal ABA levels, mildly impaired ABA induction of gene expression, and anthocyanin accumulation in embryonic tissue. Furthermore, in cereals such as barley, sorghum, and wheat, some cultivars are prone to "preharvest sprouting" (PHS) when maturation occurs under moist conditions. Genetic analyses of PHS have used quantitative trait locus (QTL) mapping to identify regions correlated with control of vivipary and compared expression of candidate genes (e.g., *VP1*) in cultivars with different propensities for PHS. Although wheat and rice *VP1* orthologs show no linkage to QTLs associated with PHS (Anderson et al., 1993; Bailey et al., 1999), vivipary is correlated with a high frequency of incorrect splicing of the wheat *VP1* homeologs (homologs of *VP1* derived from each of the three wheat genomes) (Holdsworth et al., 2001). Furthermore, a sorghum *VP1* ortholog is linked to such a QTL (Lijavetzky et al., 2000) and shows altered transcript accumulation in a PHS-susceptible line (Carrari et al., 2001). The differences in frequency of vivipary among species might partly reflect the relative humidities in the maturing fruit. However, the lack of linkage between wheat *Vp1* and PHS traits, and the fact that some Arabidopsis double mutants exhibit vivipary even at low humidity, suggest that Arabidopsis and wheat may have redundant control mechanisms suppressing vivipary that are lacking or less redundant in species such as maize.

The second peak of ABA accumulation in wild-type Arabidopsis seed depends on synthesis in the embryo itself (Karssen et al., 1983). Although the embryonic ABA accumulates to only one-third the level accumulated at 10 days after pollination, it is essential for the induction of dormancy, which is maintained despite a substantial (approximately sixfold) decrease in ABA by seed maturity. The ABA content of a wild-type mature dry seed is only 1.4-fold that of the peak ABA level in a nondormant ABA-deficient mutant, suggesting that endogenous ABA is not the only signal for dormancy maintenance in mature seed. However, comparisons

Table 4. Comparison of Vivipary and ABA Sensitivity in *abi*, *fus3*, and *lec1* Monogenic and Digenic Mutants

Genotype	Percent Vivipary[a]	Percent Germination on ABA[b]	
		3 μM	100 μM
Wild type (Wasilewskija)	0	0	0
abi4	0	70 (56–88)	0
abi5	0	39 (18–60)	0
lec1	3.5 (0–7)	32 (0–55)	0
abi4, *lec1*	0.6 (0–1.4)	100	97 (94–100)
abi5, *lec1*	12 (2–32)	49 (39–58)	4 (3–4)
fus3	0	5 (0–16)	0
abi4, *fus3*	19 (9–34)	100	96 (88–100)
abi5, *fus3*	18 (5–28)	100	74 (53–94)

Percentages shown are means of multiple assay values; ranges are presented in parentheses.

[a] The presence of germinated seeds in mature dry siliques was scored in at least four batches of 60 to 400 seed; high variability partly reflects the dependence on RH.

[b] Germination of seeds excised from siliques in late embryogenesis, just before desiccation, was scored after 3 days of incubation at 4°C followed by 7 days at 22°C in continuous light on minimal mineral salt medium supplemented with the indicated concentrations of ABA; variability partly reflects differences in viabilities among excised seed lots.

of dormant versus nondormant seed of sunflower (Le Page-Degivry and Garello, 1992), barley (Wang et al., 1995), and *Nicotiana plumbaginifolia* (Grappin et al., 2000) have demonstrated that dormancy is correlated with de novo synthesis of ABA during imbibition.

Despite the strong evidence for a fundamental role of ABA in regulating dormancy, this is a complex trait controlled by many factors, including only a subset of the known Arabidopsis ABA response loci (i.e., *ABI1*, *ABI2*, *ABI3*, and *ERA1*). Consistent with a conserved role for ABI3/VP1 in regulating dormancy, expression levels for wheat VP1 have been found to correlate with the degree of dormancy in different cultivars (Nakamura and Toyama, 2001). In addition, several reduced-dormancy Arabidopsis mutants (e.g., *rdo1*, *rdo2*, and *dag1*) have been identified that have wild-type ABA levels and sensitivity to ABA (Leon-Kloosterziel et al., 1996; Papi et al., 2000). However, the genetic interactions among the *rdo* and *abi3* mutants were interpreted as evidence that *RDO2* acts in a pathway mediating ABA-induced dormancy, whereas *RDO1* acts in a distinct pathway. Most defective-testa mutants also have decreased dormancy (Debeaujon et al., 2000), and it has been suggested that GAs are required to overcome the germination constraints imposed by both the seed coat and ABA-related embryo dormancy (Debeaujon and Koornneef, 2000). Additional screens in a variety of species have used mapping studies to identify QTLs that correlate with dormancy (reviewed by Foley, 2001).

Although many of the mutants described above have pleiotropic effects on growth, the hyperdormant mutant *comatose* (*cts*) has a seed-specific defect in GA response (Russell et al., 2000). The *aba*, *abi3*, *fus3*, and *lec1* mutations all are epistatic to *cts*, indicating that *CTS* is required to break the dormancy imposed by the action of these other loci, but its function is not necessary in the absence of dormancy. Contrasting results were obtained in studies of the control of vivipary by the balance between GA and ABA in developing maize seed. Seed defective in both GA and ABA synthesis were not viviparous, indicating that precocious germination requires promotion by GA synthesized early in development, even in the absence of inhibition by ABA (White et al., 2000). However, *vp1* mutants are viviparous regardless of their GA content, leading White and colleagues (2000) to suggest that *VP1* acts downstream of both ABA and GA in controlling the seed maturation-germination transition. Thus, although vivipary and dormancy both affect the decision of whether or not to germinate, the timing of the decision and some of the relevant regulatory factors appear to differ both within and among species.

Identities, Roles, and Interactions of Known Regulators Controlling Seed Maturation

Aspects of maturation such as reserve accumulation and late-embryogenesis-abundant (LEA) gene expression are controlled largely by the coordinated action of transcription factors. Promoter sequences for storage protein and LEA genes contain elements essential to confer hormone responsiveness and stage and tissue specificity (reviewed by Rock and Quatrano, 1995; Busk and Pages, 1998). Although many DNA binding factors that interact with these promoters have been identified by biochemical or yeast one-hybrid approaches, the functional roles of most of them still must be tested by reverse genetic analyses. To date, only six classes of transcription factors have been demonstrated by genetic analyses to be essential for some ABA- or seed-specific gene expression: ABI3/VP1, ABI4, ABI5, LEC1, LEC2, and FUS3.

ABI3 and *VP1* are orthologous genes from Arabidopsis and maize, respectively, and encode transcription factors of the B3 domain family (McCarty et al., 1991; Giraudat et al., 1992). Additional orthologs include the rice *OsVP1* (Hattori et al., 1994), wheat *TaVP1* (Bailey et al., 1999), oat *AfVP1* (Jones et al., 1997), carrot *C-ABI3* (Shiota et al., 1998), bean *PvALF* (Bobb et al., 1995), resurrection plant *CpVP1* (Chandler and Bartels, 1997), and poplar *PtABI3* (Rohde et al., 1998) genes (Table 2). *ABI3*/*VP1* and their orthologs contain four conserved domains: an acidic activation domain and three basic domains (B1, B2, and B3) (Figure 1). The *LEC2* and *FUS3* genes also have been cloned and found to encode members of the B3 domain family (Luerssen et al., 1998; Stone et al., 2001). The Arabidopsis, maize, rice, bean, and resurrection plant VP1 orthologs all have been shown to activate the transcription of ABA-inducible promoters in vivo. Furthermore, the conserved B3 domain of VP1 binds in vitro to the conserved RY element present in many seed-specific promoters, including those of the C1 and Em genes (Suzuki et al., 1997).

Similarly, FUS3 binds the RY element in vitro and can transactivate genes containing this element in their promoters (Reidt et al., 2000). However, the B3 domain is not essential for ABA-regulated gene expression in the seed (Carson et al., 1997). Mutational studies have shown that the B2 domain is required for the regulation of *Em* and *2S* albumin genes (Bies-Etheve et al., 1999) and for interaction with an ABRE (Ezcurra et al., 2000) and the ABRE binding protein EmBP-1 (Hill et al., 1996). However, full-length VP1 does not bind DNA specifically in vitro, suggesting that it interacts with other proteins that mediate DNA binding (Suzuki et al., 1997). Consistent with this hypothesis, mutational analyses of VP1/ABI3-responsive promoters have shown that the ABREs present in the Em1a and Em1b elements are sufficient but not necessary for VP1 transactivation (Vasil et al., 1995). VP1 also acts as a transcriptional repressor of some genes expressed during germination (Hoecker et al., 1995); the VP1 repressor function is distinct from the activation domain. Furthermore, repression is not cell autonomous and requires embryo-specific factors other than ABA and VP1 (Hoecker et al., 1999).

ABI4 and ABI5 also contain presumed DNA binding and protein interaction domains. ABI4 is related most closely to

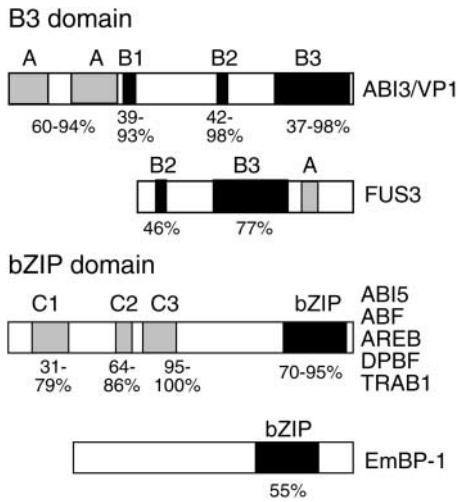

Figure 1. Domain Structure of B3 and bZIP Domain Transcription Factors Affecting ABA Response.

Percentages shown beneath the conserved domains in B3 domain family proteins indicate the range of similarity to maize VP1 among orthologs from Arabidopsis, rice, oat, carrot, bean, resurrection plant, and poplar and with the related FUS3 protein. Comparisons among the bZIP domain family represent percentage of similarity to ABI5 within the conserved domains among homologous genes in Arabidopsis, sunflower, and rice. The EmBP-1 protein used for comparison was from wheat.

the drought response element binding (DREB)/CBF subfamily of the APETALA2 (AP2) domain family, but the similarity is confined to the AP2 domain. The target sequence for ABI4 binding is unknown at present; DRE *cis* elements are not present in a variety of ABI4-regulated genes (R.R. Finkelstein, unpublished observations). In contrast, *ABI5* was identified independently by homology with a sunflower gene isolated via a yeast one-hybrid screen using the ABA-responsive Dc3 promoter as "bait" (T. Thomas, personal communication). In both sunflower and Arabidopsis, ABI5 and its ortholog are members of a small gene subfamily, whose members are designated (At)DPBFs (*Arabidopsis thaliana* Dc3 promoter binding factors) (Kim et al., 1997; Kim and Thomas, 1998; T. Thomas, personal communication); *ABI5* corresponds to *AtDPBF1*. A rice homolog of ABI5, TRAB1, was identified by a two-hybrid screen using the basic domains of OsVP1 as bait and shown to interact with ABREs in vitro and to activate ABA-inducible transcription in rice protoplasts (Hobo et al., 1999).

Additional Arabidopsis ABI5 family members have been correlated with ABA- or stress-induced gene expression in seedlings and designated AREBs or ABFs (Choi et al., 2000; Uno et al., 2000); all share three conserved charged domains in their N-terminal halves as well as the bZIP domain at their C termini (Figure 1). In vitro studies with the sunflower DPBFs have demonstrated that this subfamily binds to G-box elements required for ABA regulation, the ABREs (Kim et al., 1997). The ABI5/DPBF/ABF/AREB subfamily members differ from the other bZIP proteins in that they tolerate variability in the ACGT core element essential to the ABRE G-box. These bZIP factors have been shown to form heterodimers in some combinations, including with ABI5 (Kim et al., 1997; T. Thomas, personal communication). This finding suggests that they are likely to participate in the regulation of many of the same target genes, thereby providing functional redundancy that could explain the weakly ABA-resistant phenotype of *abi5* null mutants (Finkelstein, 1994; Finkelstein and Lynch, 2000a; Lopez-Molina and Chua, 2000) as well as the failure to find mutants with defects in any of the other family members. Analyses of transcript accumulation in *abi5* mutants indicate that, like ABI3, ABI5 can either activate or repress gene expression, but ABI5 and ABI3 may have either synergistic or antagonistic effects on gene expression, depending on the gene (Finkelstein and Lynch, 2000a; Delseny et al., 2001).

The *LEC1* gene encodes a homolog of the HAP3 subunit of CCAAT binding factors (Lotan et al., 1998), a family composed of 10 genes in Arabidopsis. Although CCAAT boxes are common features of promoters transcribed by RNA polymerase II, their binding factors often show tissue- or stage-specific expression, such that specific sets of genes are activated by different heterodimers or homodimers (Lekstrom-Himes and Xanthopoulos, 1998). Although *lec1* mutations have very limited effects on ABA sensitivity (Meinke et al., 1994; West et al., 1994), *LEC1* appears to potentiate the ABA response by genetic interactions with *ABI3*, *ABI4*, and *ABI5* (Parcy et al., 1997; I. Brocard and R.R. Finkelstein, unpublished observations). In the case of ABI3, LEC1 promotes protein accumulation (Parcy et al., 1997).

Of the known factors that regulate gene expression during mid to late embryogenesis, some regulate ABA response (e.g., ABI3/VP1, ABI4, and ABI5) and others primarily regulate the transition from embryogenesis to germinative growth (e.g., LEC1, LEC2, and FUS) (reviewed by Holdsworth et al., 1999, 2001). However, this is not a clean distinction, because ABI3/VP1 also represses the phase transition to germination. Post-transcriptional control also has been demonstrated for the accumulation of some ABA-inducible proteins (Bies et al., 1998), but the specific regulators involved have not been identified. Some of the recently identified loci encoding putative RNA processing or binding proteins (e.g., *ABH1*, *HYL1*, and *SAD1*) (Lu and Fedoroff, 2000; Hugouvieux et al., 2001; Xiong et al., 2001a) might contribute to this level of regulation.

Mutations in the *ABI3*, *ABI4*, and *ABI5* loci have similar qualitative effects on seed development and ABA sensitivity, but null mutations in *ABI3* are more severe than those in *ABI4* or *ABI5* (Parcy et al., 1994; Finkelstein et al., 1998; Finkelstein and Lynch, 2000a). These loci also display similar genetic interactions: digenic mutants combining the leaky *abi3-1* alleles with severe mutations in either *ABI4* or *ABI5* produce seed that are only slightly more resistant

to ABA than their monogenic parents, whereas mutations at any of these three loci greatly enhance the ABA resistance of *abi1-1* mutants (Finkelstein and Somerville, 1990; Finkelstein, 1994). Consistent with the similarities in their phenotypes and genetic interactions, recent studies show extensive cross-regulation of expression among *ABI3*, *ABI4*, and *ABI5* (Söderman et al., 2000). Furthermore, ectopic expression of any of these ABI transcription factors results in ABA hypersensitivity of vegetative tissues, which is partly dependent on increased ABI5 expression (Parcy et al., 1994; Söderman et al., 2000; Lopez-Molina et al., 2001).

Together, these results suggest that these three transcription factors participate in combinatorial control of gene expression, possibly by forming a regulatory complex mediating seed-specific and/or ABA-inducible expression. Consistent with this, two-hybrid analyses in yeast and transient reporter activation assays in rice protoplasts have demonstrated direct and synergistic interactions between ABI3 (or its monocot ortholog VP1) and ABI5 (or its rice homolog TRAB1) (Hobo et al., 1999; Nakamura et al., 2001; Gampala et al., 2002). These studies have shown that ABI3 and ABI5 interact directly via the B1 domain of ABI3 and two of the conserved charged domains in ABI5 (Nakamura et al., 2001), suggesting that ABI5 binding to ABRE elements may tether ABI3 to target promoters and facilitate its interaction with RY elements and transcription complexes. Yeast two-hybrid screens using only the B2 and B3 domains of ABI3 or oat VP1 as bait identified interactions with several presumed transcription factors. The interacting proteins included a CONSTANS-related factor, the RPB5 subunit of RNA polymerase II, and a homolog of the human C1 protein involved in cell cycle control (Jones et al., 2000; Kurup et al., 2000), but not ABI5 or its homologs.

Synergistic effects of ABI3 and heat shock factors also have been shown to be important for the ABI3-dependent expression of small heat shock proteins (Rojas et al., 1999). In addition, other bZIP proteins may be linked indirectly to ABI3 via interactions with a 14-3-3 protein, as described for connections among the proteins EmBP1, Vp1, and GF14 (Schultz et al., 1998). Such interactions may either promote or inhibit DNA binding (Nantel and Quatrano, 1996), and it has been suggested that PvALF (the bean ortholog of ABI3/VP1) may trigger chromatin remodeling to permit ABA-mediated gene activation (Li et al., 1999). Despite the observed genetic interactions, ABI4 does not appear to interact physically with either ABI3 or ABI5 (Nakamura et al., 2001); two-hybrid screens are in progress to identify potential cellular contacts for ABI4 (R.R. Finkelstein and T. Lynch, unpublished results).

The ectopic expression studies were interpreted initially to mean that the seed specificity of embryonic gene expression was attributable to the seed-specific expression of key regulators (Parcy et al., 1994), as had been observed for the maize *VP1* gene (McCarty et al., 1991). However, most of the Arabidopsis regulators are expressed and functional in other processes during vegetative growth (Finkelstein et al., 1998; Arenas-Huertero et al., 2000; Finkelstein and Lynch, 2000a; Huijser et al., 2000; Laby et al., 2000; Lopez-Molina and Chua, 2000; Rohde et al., 2000b; Lopez-Molina et al., 2001). In fact, seed specificity may be conferred by genes such as *PICKLE* (*PKL*) that repress embryogenesis-promoting regulators such as *LEC1* (Ogas et al., 1997, 1999) after germination. It is not known whether *PKL* interacts with any of the ABA response loci or how repression by this constitutively expressed gene is limited to postgerminative events, but the *pkl* phenotype is far more sensitive to modulation of GA levels than ABA levels (J. Ogas, personal communication).

Null alleles of *ABI3* (*abi3-3* and *abi3-4*) (Giraudat et al., 1992; Nambara et al., 1992) or double mutants combining the weak *abi3-1* allele with ABA deficiency (the *aba1-1* mutant) (Koornneef et al., 1989) have more severe defects in seed maturation than any of the other *abi* mutants. These plants produce "green seed" that fail to lose chlorophyll, accumulate storage proteins, or attain desiccation tolerance and have a high degree of denatured protein (Koornneef et al., 1989; Ooms et al., 1993; Wolkers et al., 1998). Although applied ABA does not induce dormancy in ABA synthesis or ABA response mutants (Karssen et al., 1983), it reverses the *aba1* and *abi3-1* effects on desiccation tolerance and seed protein accumulation (Meurs et al., 1992). In this respect, exogenous ABA functions similarly to maternal ABA, which is required for progression into the maturation phase of embryogenesis (Raz et al., 2001).

Collectively, these results suggest that *ABI3* is required for processes in seed development that can respond to high endogenous ABA but do not require it. However, the severe reduction of free ABA by seed-specific expression of anti-ABA antibodies results in a green-seed phenotype in transgenic tobacco (Phillips et al., 1997), indicating that some endogenous ABA is essential for seed maturation. In contrast to the results with *abi3* mutants, the dominant-negative *abi1-1* and *abi2-1* alleles do not affect storage reserve synthesis or desiccation tolerance, regardless of whether ABA biosynthesis is altered by the presence of the *aba1-1* allele (Koornneef et al., 1989; Finkelstein and Somerville, 1990).

Even more severe defects are observed with some combinations of mutations in ABA biosynthetic loci (*ABI1*, *ABI3*, *ABI4*, or *ABI5*) with those in *FUS3* or *LEC1*; these plants produce highly pigmented seed that fail to accumulate storage reserves or attain desiccation tolerance and that sometimes are viviparous (Keith et al., 1994; Meinke et al., 1994; Parcy et al., 1997; Nambara et al., 2000; I. Brocard and R.R. Finkelstein, unpublished observation). Germination assays with exogenous ABA show that the double mutants are 10-fold to >30-fold less sensitive to ABA than their monogenic *abi* parents, even though the *lec1* and *fus3* mutations have little or no effect, respectively, on ABA sensitivity. Thus, the FUS3 and LEC1 gene products appear to potentiate ABI-dependent ABA sensitivity. Although *fus3* and *lec1* seed accumulate normal amounts of ABI3 protein, its accumulation was decreased significantly in the double mutants (Parcy et al., 1997).

These results show that most of the embryonic regulators are transcription factors with complex patterns of cross-regulation and some direct interactions. Consequently, ectopic expression of any of these factors can move some aspects of "seed-specific" gene expression to another developmental stage or tissue. The effects of ABA and some key regulators at mid and late embryogenesis are summarized in Figure 2. Note that the regulators are arranged in different combinations that affect various processes, not in a regulatory hierarchy.

Having identified a series of transcriptional activators and their target genes, it should be possible to dissect the signaling pathway involved in the ABA activation of these transcription factors. Of the three ABI transcription factors, only ABI5 and some of its family members show significant ABA inducibility of their transcripts. In the case of ABI5, protein accumulation is enhanced further by ABA-induced phosphorylation and the resulting stabilization of the protein, at least during the early phases of germination (Lopez-Molina et al., 2001). To evaluate the contributions of presumed earlier components of the signaling pathways, as well as the interactions among these transcription factors, we assayed ABA-responsive promoter activity in a rice embryonic protoplast transient expression system. Activation of Em–β-glucuronidase (GUS) expression by five different effectors was compared in protoplasts treated with or without 100 μM ABA (Figure 3A). These studies show that VP1 and most of the ABI5/ABF bZIPs tested are sufficient for Em-GUS activation and enhanced ABA response, but the contributions and ABA dependence of these effectors vary widely.

It is interesting that EmBP1, a DNA binding protein found to bind the ABREs of the Em promoter, does not contribute to the activation of this fusion even though it appears to be present in a complex that includes VP1 (Schultz et al., 1998). However, maize VP1 and Arabidopsis ABI5, like rice OsVP1 and TRAB1, act synergistically to activate Em-GUS expression (Figure 3B), demonstrating that this interaction involves components conserved between monocots and dicots. Furthermore, ABA enhances this synergism. Additional studies have shown that La^{3+}, which is hypothesized to promote Ca^{2+} release, acts synergistically with ABA and VP1 to activate ABA-responsive promoters (Gampala et al., 2001, 2002). In contrast, an inhibitor of PLD (1-butanol) antagonizes ABA-inducible expression but does not fully block ABA/VP1 induction (Figure 3C). The observation that 1-butanol or *abi1-1* is less effective at blocking ABA/VP1 responses than the response to ABA alone suggests that VP1 signaling may depend in part on PLD- and ABI1-independent mechanism(s) (Gampala et al., 2001).

Germination of Mature Seed

Although endogenous ABA is essential for the induction of dormancy and the germination of mature Arabidopsis seed can be suppressed by as little as 3 μM exogenous ABA, the role of the low level of endogenous ABA remaining in seed at this stage is not clear. In fact, if suppression of germination requires de novo ABA synthesis, as described for dormant seed, the residual ABA produced during embryogenesis may be irrelevant. However, in other species, ABA is high at maturity, and increased germinability is correlated with removal of the endogenous ABA. Low concentrations of sugar (either Glc or Suc at 30 to 90 mM) or peptone can overcome exogenous ABA inhibition of radicle emergence,

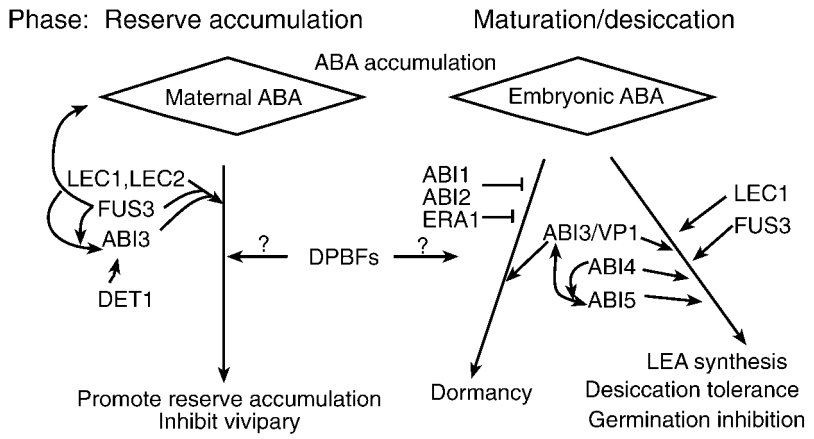

Figure 2. Scheme of Signaling Pathways in Seed Development.

Arrows represent promotion of processes or expression of the regulators. Bars represent inhibitors of the indicated processes. The positions of loci do not imply the order of gene action.

even at concentrations up to 100 μM ABA, but greening and subsequent seedling growth still are blocked (Garciarrubio et al., 1997; Finkelstein and Lynch, 2000b). This antagonism is light dependent (Finkelstein and Lynch, 2000b), possibly because GA synthesis increases (Toyomasu et al., 1998) or ABA levels decrease (Toyomasu et al., 1994) in light.

It has been suggested that exogenous sugar permits germination by overcoming a nutritional deficiency (Garciarrubio et al., 1997) caused by the inhibition of reserve mobilization by exogenous ABA. However, the optimal concentration of Glc for promotion of seed germination is too low to be consistent with a purely nutritional effect (Finkelstein and Lynch, 2000b). In addition, recent studies of isocitrate lyase–deficient mutants have shown that the glyoxylate cycle is not essential for Arabidopsis germination, and postgerminative growth can be supported by either photosynthesis or exogenous sugar in the absence of a functional glyoxylate cycle (Eastmond et al., 2000). Furthermore, mature Arabidopsis seed apparently differ from other oilseeds in that they mobilize much of their lipid reserves in the presence of ABA and no sugar, despite showing no visible signs of germination (Pritchard and Graham, 2001).

Other studies show that wild-type seed incubated on low Suc and ABA for up to 5 days after stratification, such that germination is arrested after radicle emergence (Finkelstein and Lynch, 2000b), accumulate ABI5 protein (Lopez-Molina et al., 2001). ABI5 accumulation is correlated strongly with the maintenance of desiccation tolerance in these arrested seedlings. ABA, the induced ABI5 gene product, and potentially other interacting factors may be required to delay or prevent escape from phase II of germination (and the accompanying loss of desiccation tolerance) under conditions of insufficient moisture to support seedling growth.

ABA effects on germination also are antagonized by gibberellins, ethylene, and brassinosteroids (BRs). Consistent with this fact, ABA-deficient mutations were isolated initially as suppressors of nongermination caused by GA deficiency (Koornneef et al., 1982), and the GA response mutant *sleepy* (*sly*) was isolated as a suppressor of *abi1-1* (Steber et al., 1998). Recent studies show that nongermination of *sly* is rescued by BR, whereas BR-deficient or BR-insensitive lines are hypersensitive to ABA inhibition of germination (Steber and McCourt, 2001). Furthermore, new alleles of ethylene response genes such as *ctr1* and *ein2* also have been identified in screens for suppressors and enhancers of seed sensitivity to ABA, respectively, and it appears that even the single mutants have slightly altered ABA response (Beaudoin et al., 2000; Ghassemian et al., 2000). Comparison of monogenic and digenic phenotypes show that the *ctr1* and *abi1-1* mutations synergistically enhance ABA-resistant germination (Beaudoin et al., 2000). In contrast, *abi3* mutations appear epistatic to the ABA hypersensitivity conferred by *ein2*, whereas the *ein2* and *abi1-1* effects are additive.

Despite questions regarding the biological relevance of the exogenous ABA inhibition of germination, this is a convenient quantitative assay for ABA sensitivity and has been used to dissect the response mechanism. In addition to being regulated by all five cloned *ABI* loci (Koornneef et al., 1984; Finkelstein, 1994), the *ERA* loci (Cutler et al., 1996; Ghassemian et al., 2000) *CTR1* (Beaudoin et al., 2000; Cutler et al., 1996; Ghassemian et al., 2000), *SLY* (Steber et al., 1998), *DET2*, *BRI1* (Steber and McCourt, 2001), *ABH1* (Hugouvieux et al., 2001), and Rops2, Rops9, and Rops10 (Yang, 2002), ABA response requires AtPLC1 activity and can be retarded by high-level expression of an inositol 1,4,5-trisphosphate 5 phosphatase (Sanchez and Chua, 2001). Transgenic lines that eliminated the ABA-induced increase in IP_3 levels displayed reduced ABA sensitivity in assays of germination, seedling growth, and gene expression. However, ectopic expression of PLC or antisense suppression of Ins(1,4,5)$P_3$5 phosphatase gene expression did not increase IP_3 levels or ABA response in the absence of added ABA, indicating that PLC1 expression is necessary but not sufficient for ABA response. In contrast, the defects in phosphoinositide catabolism in *fry1* mutants are sufficient to increase both IP_3 levels and ABA sensitivity (Xiong et al., 2001b).

Some of the best-characterized effects of ABA in germination are those associated with the antagonism of GA-induced reserve mobilization, especially starch mobilization in cereal grains (Lovegrove and Hooley, 2000). Starch mobilization is catalyzed by the activation of α-amylases, a family of enzymes subject to differential spatial, temporal, hormonal, and metabolic regulation affecting expression at multiple levels, including transcription, mRNA stability, and enzyme secretion into the starchy endosperm (Thomas and Rodriguez, 1994; Jacobsen, 1995). GA promotes the expression of responsive α-amylase genes by inducing the synthesis of a transcription factor, GAMyb, that specifically binds the GA response element of their promoters (Gubler et al., 1995). In addition to functioning in GA induction, the GA response element is required for downregulation by ABA (Skriver et al., 1991). Before seed maturity, the repressor activity of VP1 specifically inhibits the expression of GA-induced genes, resulting in very low GA sensitivity at this stage (Hoecker et al., 1995).

In aleurone of germinating mature barley seed, ABA induces the expression of a protein kinase, PKABA1, that represses the GA induction of GAMyb (Gómez-Cadenas et al., 1999, 2001). Inhibition by PKABA1 cannot be counteracted by the constitutive GA responsiveness conferred by the *slender* mutant, indicating that PKABA1 acts downstream of the step regulated by SLENDER. The *abi1-1* dominant-negative mutant also has no effect on the PKABA1 or ABA repression of α-amylase, and PKABA1 has little effect on ABA-induced gene expression, suggesting that ABA induction and repression are mediated by two separate signaling pathways (Shen et al., 2001). Early steps in this signaling pathway appear to involve G protein–mediated activation of PLD and signaling by PA and Ca^{2+} as secondary messengers. GA-induced α-amylase expression also can be inhibited in the scutellar epithelium by the combined effects of ABA and sugars (reviewed by Finkelstein and Gibson,

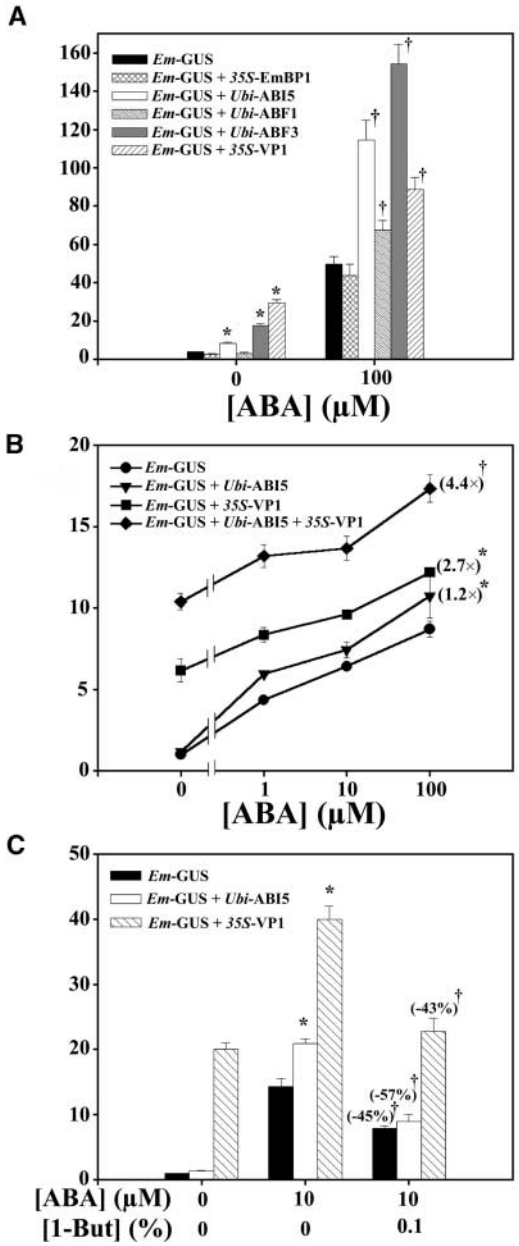

Figure 3. Regulation of ABA-Responsive Promoter Activity in a Rice Embryonic Protoplast Transient Expression System.

(A) Fold effect of transiently expressed bZIP transcription factors on the ABA activation of the wheat *Em* promoter. Rice protoplasts were transformed with *Em*-GUS and/or the effector construct *35S*-EmBP1, *Ubi*-ABI5, *Ubi*-ABF1, *Ubi*-ABF3, or *35S*-VP1 and treated with or without 100 μM ABA for 16 hr. Fold activation of *Em*-GUS was calculated relative to control (no ABA, no effector) and normalized to the non-ABA-inducible *Ubi*-LUC reporter, which was cotransformed as an internal control. Asterisks indicate values that are significantly different from the control ($P < 0.002$; two-sided Student's *t* test, equal variance assumed). Daggers indicate that treatments with effectors resulted in a significant difference in *Em*-GUS activity from ABA treatment alone ($P < 0.007$; two-sided Student's *t* test, equal variance assumed). "Dummy" DNA was transformed along with *Em*-GUS to balance the total amount of input plasmid DNA between various treatments. Comparisons were done between paired samples from parallel experiments and plotted to obtain the meta-analysis. Error bars represent ±SEM, with three or four replicates per sample.

(B) ABI5 interacts synergistically with ABA and VP1 to transactivate the wheat *Em* promoter. Protoplasts were transformed with either *Em*-GUS alone or in combination with *Ubi*-ABI5 and/or *35S*-VP1 constructs. Numbers in parentheses indicate average fold activation compared with ABA induction alone. Asterisks indicate values that are significantly different from the control ($P < 0.003$; two-sided paired Student's *t* test). The dagger indicates that ABI5/VP1 synergy is significantly different from activation by any of the effectors alone ($P < 1 \times 10^{-7}$; two-sided paired Student's *t* test). Error bars represent ±SEM, with three replicates per sample. (*Figure modified from Gampala et al. [2002]; reprinted with permission.*)

(C) 1-Butanol (1-But) antagonizes to the same extent the ABA induction of *Em*-GUS and ABI5 and VP1 synergy with ABA. Experimental treatments and fold induction calculations were as described in **(A)**. Numbers in parentheses indicate the relative percentage inhibition of *Em*-GUS expression compared with control samples (no 1-butanol added). Asterisks indicate that ABI5 and VP1 synergy with ABA is significantly different from ABA activation alone ($P < 0.01$; two-sided Student's *t* test, equal variance assumed). Daggers indicate significant difference from the no butanol treatment ($P < 0.008$; two-sided Student's *t* test, equal variance assumed). Values are averages (±SEM) of three replicate transformations.

In **(A)** to **(C)**, the *y* axis indicates fold induction in *Em*-GUS activity per unit of *Ubi*-LUC.

2002). In addition to antagonizing GA-induced gene expression, ABA dramatically slows the process of programmed cell death in aleurone cells, such that ABA-treated protoplasts remain viable roughly 30-fold longer than GA-treated cells (surviving for >6 months versus 5 to 8 days) (Fath et al., 2000). Similarly, ABA appears to delay the ethylene-induced programmed cell death that occurs at the end of the grain-filling period in cereal endosperm development (Young and Gallie, 2000), but the mechanisms for both of these effects on programmed cell death are unknown.

Interactions among some of the regulatory elements and hormonal and environmental signals that regulate germination are shown schematically in Figure 4. Additional regulators that specifically control dormancy (e.g., *RDO* and *DAG*) without altering ABA sensitivity are not included.

Seedling Growth

In contrast to the antagonistic effects of low sugar versus ABA on germination, high concentrations of sugars (>300 mM) can inhibit seedling growth in an ABA-dependent manner that cannot be explained simply by the osmotic effects of sugar (reviewed by Gibson, 2000). Consequently, numer-

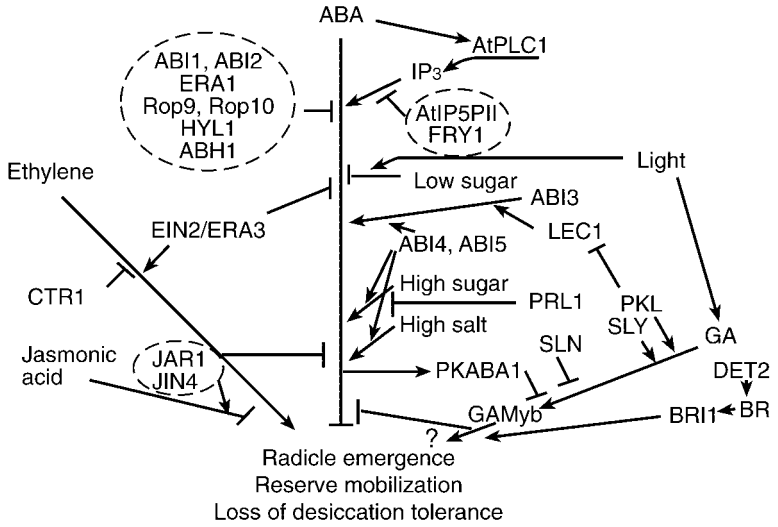

Figure 4. Scheme of Signaling Pathways That Interact with the ABA Regulation of Germination.

Arrows represent promotion of processes or expression of the regulators. Bars represent inhibitors of the indicated processes. The positions of loci do not imply the order of gene action.

ous screens for sugar-resistant seedling growth have identified new alleles of *ABI4* and two ABA biosynthetic loci (Arenas-Huertero et al., 2000; Huijser et al., 2000; Laby et al., 2000; Arroyo Becerra et al., 2001). In addition, such screens have revealed new alleles of *ctr1* (Gibson et al., 2001), which also enhances ABA-resistant germination (Beaudoin et al., 2000). Although these results suggest interactions between ABA, ethylene, and sugar signaling, they appear relatively specific because *abi1*, *abi2*, and *abi3* mutants show essentially normal sugar responses.

The observation that *abi4* or *aba* mutants can ameliorate the sugar hypersensitivity conferred by hexokinase overexpression (Arenas-Huertero et al., 2000) is consistent with models in which ABA and sugar could act in either parallel or intersecting pathways. However, high Glc induces both ABA synthesis and expression of *ABI4* and *ABI5*, suggesting that altered ABA levels or response may mediate some aspects of sugar signaling (Arroyo Becerra et al., 2001; I. Brocard and R.R. Finkelstein, unpublished data). Furthermore, we found that overexpression of *ABI3*, *ABI4*, or *ABI5* confers hypersensitivity to Glc, consistent with a role for these *ABI* genes in mediating the response to both ABA and sugar (Figure 5). It is noteworthy that Glc induces expression of these *ABI* genes (most strongly) when applied relatively early in germination/seedling growth, coinciding with the developmental window of sensitivities to the inhibitory effects of high sugar (Gibson et al., 2001) and the ABA induction of ABI5 accumulation (Lopez-Molina et al., 2001).

After germination, ABA and ethylene signaling display complex interactions. Although these hormones act antagonistically at germination, both inhibit root growth (Beaudoin et al., 2000; Ghassemian et al., 2000). Consistent with a model in which they act in the same or parallel pathways controlling root growth, either chemical or genetic disruption of ethylene signaling results in reduced sensitivity of root growth to inhibition by ABA. However, ethylene-overproducing mutants have decreased ABA sensitivity, and treatment with aminoethoxyvinylglycine to block ethylene synthesis results in increased ABA sensitivity, implying another antagonistic interaction. One suggested explanation for this apparent inconsistency is that ABA inhibits root growth by signaling through the *ETR1* response pathway, but it cannot use this pathway in the presence of ethylene (Ghassemian et al., 2000).

The inhibitory effects of ABA on growth have long been recognized as resulting from a combination of limited cell extensibility (Kutschera and Schopfer, 1986) and inhibited cell division attributable to arrest at the G1 phase of the cell cycle (Liu et al., 1994). A possible explanation for the effects of ABA on progression through the cell cycle has been provided by the discovery that ABA induces the expression of a cyclin-dependent protein kinase inhibitor that interacts with both Cdc2a and CycD3 and is correlated with decreased Cdc2-like histone H1 kinase activity (Wang et al., 1998). The participation of G proteins in the control of the cell cycle is suggested by the discovery that a null insertion mutant of the sole Gα gene in Arabidopsis confers reduced cell division to aerial tissues (Ullah et al., 2001) and blocks ABA inhibition of stomatal opening (Wang et al., 2001). However, in this case, the mutant phenotype indicates that G proteins mediate events that promote cell division, contrary to the observed effects of ABA. Thus, this G protein can function

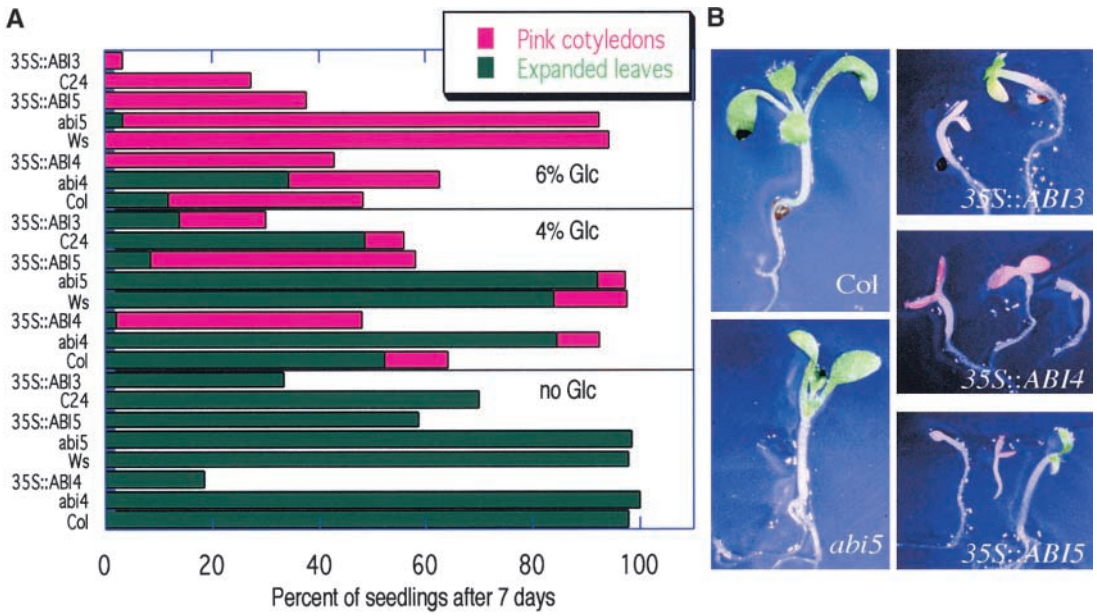

Figure 5. Sensitivity of Seedlings of Wild-Type, *abi*, and *ABI* Overexpression Lines to Glc.

(A) Growth and anthocyanin accumulation in seedlings exposed to Glc. Seed of the indicated genotypes were incubated on minimal mineral salt media supplemented with 0, 4, or 6% Glc for 7 days before scoring individuals with either expanded true leaves or pink cotyledons caused by anthocyanin accumulation. The low percentages of either characteristic observed for the *ABI* overexpression lines on all media, and even for the wild-type lines on high Glc, reflect the inhibition of both germination and seedling growth.

(B) Seedlings of the indicated genotypes after 7 days of growth on 4% Glc. The *35S*::*ABI3* line is isolate C7A19, described by Parcy et al. (1994); the *35S*::*ABI4* line is isolate 114, described by Söderman et al. (2000); the *35S*::*ABI5* transgene contains an *ABI5* cDNA, extending 23 bp 5′ to the initiating codon, fused downstream of the 35S promoter of *Cauliflower mosaic virus* in pGA643 (T. Lynch and R.R. Finkelstein, unpublished data).

Col, Columbia; Ws, Wassilewskija.

as either a promoter or an inhibitor of ABA response, depending on the response.

With regard to the potential mechanisms of ABA effects on cell cycle or extensibility, it is interesting that an inhibitor of ABA response, *ERA1*, encodes a subunit of farnesyl transferase. Although many of the specific targets of ERA1-dependent farnesylation are unknown, potential targets containing a C-terminal sequence conserved among yeast and animals have been identified by computerized screening (reviewed by Nambara and McCourt, 1999). These include cell cycle regulators, cell wall modifiers, ROPs, and transcription factors. Farnesylation of these proteins might affect their functions by regulating their membrane localization or potential for protein–protein interactions.

Although the studies described above focus on the growth-inhibiting effects of high ABA concentrations, the stunted growth of ABA-deficient plants even when well watered implies that the low endogenous ABA levels in unstressed plants actually promote growth. Recent studies in maize and tomato suggest that the stunted growth of ABA-deficient plants is caused by the overproduction of ethylene (Sharp et al., 2000; Spollen et al., 2000), which normally would be inhibited by ABA.

ABA SIGNALING IN VEGETATIVE STRESS RESPONSES

Genetic analyses of vegetative ABA responses have shown that the relative importance of the Arabidopsis ABA response loci changes after germination and commitment to seedling growth. The *ABI1*, *ABI2*, *ERA1*, *ABH1*, *HYL1*, *GCA1*, and *GCA2* loci affect many aspects of ABA-regulated vegetative growth, including cell elongation and/or stomatal regulation. Many additional Arabidopsis ABA response mutants with limited phenotypic effects have been identified based on alterations in ABA- or stress-regulated gene expression (Table 1) (reviewed by Finkelstein and Rock, 2001). In contrast, mutations in *ABI3*, *ABI4*, and *ABI5* have their greatest impact on gene expression during seed maturation, but all three genes are expressed to a limited degree in vegetative tissues (Finkelstein et al., 1998; Rohde et

al., 1999; Finkelstein and Lynch, 2000a) and appear to play a role in seedling salt or sugar response (Arenas-Huertero et al., 2000; Huijser et al., 2000; Laby et al., 2000; Quesada et al., 2000; Rook et al., 2001) (Figure 5). Both *abi4* and *abi5* also show some limited defects in ABA-regulated vegetative gene expression (Finkelstein and Lynch, 2000a; Söderman et al., 2000). In contrast to that of the other *ABI* genes, vegetative *ABI3* expression is localized to the meristem and appears to be involved in vegetative quiescence processes, plastid differentiation, and floral determination (Rohde et al., 1999, 2000a; Kurup et al., 2000).

A major role of ABA during vegetative growth is to optimize growth under adverse conditions by maintaining osmotic homeostasis. At the cellular level, ABA can promote the tolerance of some abiotic stresses, including cold, salinity, and drought. Although these stresses share the common element of inducing cellular dehydration, comparison of stress-induced gene expression in ABA biosynthesis and response mutants has demonstrated that there are both ABA-dependent and ABA-independent signaling pathways, with a complex array of interactions (reviewed by Rock, 2000; Shinozaki and Yamaguchi-Shinozaki, 2000; Xiong et al., 2002).

At the whole plant level, dehydrative stresses cause reduced stomatal aperture to minimize water loss via transpiration. In addition, mild water stress drives root growth but inhibits shoot growth, whereas severe stress inhibits both root and shoot growth. ABA is a major mediator of these stress responses, and its signaling mechanisms are best characterized with respect to its effects on stomata, where it both promotes stomatal closure and inhibits opening (reviewed by Schroeder et al., 2001). Although these effects lead to the same end result (i.e., closed stomata), opening and closing are not simple reversals of the same process, so the relevant signaling mechanisms differ. In addition to the electrophysiological changes leading to osmotic water loss, guard cells undergo a substantial change in volume accompanied by up to twofold changes in membrane surface area. These large volume and area changes involve vesicle secretion and endocytosis, possibly mediated by annexin-, syntaxin-, and Rho-like gene products (Kovacs et al., 1998; Leyman et al., 1999; Lemichez et al., 2001), and reorganization of the actin cytoskeleton (Eun and Lee, 1997). Like the electrophysiological changes, the ABA effects on actin reorganization are mediated by Ca^{2+} signaling and phosphorylation cascades, including *ABI1*-dependent signaling.

Although the best-characterized aspects of guard cell response are electrophysiological and ultrastructural changes, some of the ABA response loci have been shown to modify guard cell–specific gene expression. Of particular interest are the observations that *35S*::*ABI3* expression acts epistatically to *abi1-1* (Parcy and Giraudat, 1997) and that *abh1* mutants have altered guard cell expression of a small number of genes (Hugouvieux et al., 2001). Some of the regulatory targets of this transcription factor and CAP binding protein may play critical roles in stomatal function.

CONCLUSIONS AND PERSPECTIVES

The number of genes shown by genetic and/or molecular studies to regulate ABA responses has increased to nearly 50 in Arabidopsis alone. The list continues to expand, and the molecular and genetic studies are converging as the various ABA response loci are cloned, the putative interacting proteins are identified by two-hybrid screens, and the roles of suspected signaling intermediates are tested by manipulating their activities in transgenic plants. Studies of orthologs and functional tests in heterologous systems have shown that many of these genes have conserved functions. There also is substantial diversity in signaling within any given species, reflected in both redundant and independent ABA signaling mechanisms. As has been described for many other signaling systems, the ABA response depends on coordinated interactions between positive and negative regulators. Many interactions with pathways that mediate responses to other signals also have been described, but in many cases, it is unclear whether these interactions are direct or indirect.

Two of the best-characterized ABA responses are a developmental event and a response to environmental stress: regulation of seed maturation/germination inhibition and reduction of stomatal aperture, respectively. Although some loci appear to be critical regulators of both processes, most of the known embryonic regulators encode proteins that affect transcription, many of them acting combinatorially rather than in a regulatory hierarchy. In contrast, most of the identified genes that play major roles in vegetative growth encode proteins that affect processes such as protein phosphorylation or farnesylation, RNA processing, or phosphoinositide metabolism. These also may display combinatorial interactions among the many family members of Rops, kinases, phosphatases, and their potential activators and targets. However, events as disparate as stomatal regulation and ABA-regulated gene expression show many similarities in terms of the relevant secondary messengers and even a requirement for S-type anion channel activity.

Despite these major strides toward elucidating ABA signaling, we still have only a fragmented view of the relevant pathways. Some of the remaining specific questions include the identities of the receptors, the substrates of the various kinases and phosphatases, which loci interact directly or indirectly, and the identities of additional signaling elements linking the known elements into complete pathways or networks.

ACKNOWLEDGMENTS

We thank Jerome Giraudat and Francois Parcy for the generous gift of the *35S*::*ABI3* transgenic line, Donald McCarty and Ralph Quatrano for the VP1 and EMBP1 cDNAs, respectively, and Peter Quail for the Ubi-LUC construct. We also thank Terry Thomas for sharing unpublished data. Work in the laboratory of R.R.F. is supported by National

Science Foundation Grants IBN-9728297 and IBN-9982779. Work in the laboratory of C.D.R. is supported by Grants HKUST6134/99M and DAG00/01.SC10 from the Research Grants Council and Grant AoE/B-07/99 from the University Grants Council Area of Excellence in Plant and Fungal Biotechnology.

Received December 21, 2001; accepted March 4, 2002.

REFERENCES

Abe, H., Yamaguchi-Shinozaki, K., Urao, T., Iwasaki, T., Hosokawa, D., and Shinozaki, K. (1997). Role of Arabidopsis MYC and MYB homologs in drought- and abscisic acid–regulated gene expression. Plant Cell **9,** 1859–1868.

Ache, P., Becker, D., Ivashikina, N., Dietrich, P., Roelfsema, M.R.G., and Hedrich, R. (2000). GORK, a delayed outward rectifier expressed in guard cells of *Arabidopsis thaliana*, is a K^+-selective, K^+-sensing ion channel. FEBS Lett. **486,** 93–98.

Albinsky, D., Masson, J.E., Bogucki, A., Afsar, K., Vass, I., Nagy, F., and Paszkowski, J. (1999). Plant responses to genotoxic stress are linked to an ABA/salinity signaling pathway. Plant J. **17,** 73–82.

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

Allen, G.J., Chu, S.P., Schumacher, K., Shimazaki, C.T., Vafeados, D., Kemper, A., Hawke, S.D., Tallman, G., Tsien, R.Y., Harper, J.F., Chory, J., and Schroeder, J.I. (2000). Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in Arabidopsis *det3* mutant. Science **289,** 2338–2342.

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

Alonso, J., Hirayama, T., Roman, G., Nourizadeh, S., and Ecker, J. (1999). EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. Science **284,** 2148–2152.

Anderson, B., Ward, J., and Schroeder, J. (1994). Evidence for an extracellular reception site for abscisic acid in *Commelina* guard cells. Plant Physiol. **104,** 1177–1183.

Anderson, J., Sorrells, M., and Tanksley, S. (1993). RFLP analysis of genomics regions associated with resistance to pre-harvest sprouting in wheat. Crop Sci. **33,** 453–459.

Arabidopsis Genome Initiative. (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature **408,** 796–815.

Arenas-Huertero, F., Arroyo, A., Zhou, L., Sheen, J., and Léon, P. (2000). Analysis of Arabidopsis glucose insensitive mutants, *gin5* and *gin6*, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugar. Genes Dev. **14,** 2085–2096.

Arroyo Becerra, A., Arenas, F., Jimenez, A., Cantero, A., and Léon-Mejia, P. (2001). The participation of ABA in the glucose-mediated regulation in Arabidopsis. Presented at the Twelfth International Conference on Arabidopsis Research (Madison, WI).

Assmann, S.M. (2002). Heterotrimeric and unconventional GTP binding proteins in plant cell signaling. Plant Cell **14** (suppl.), S355–S373.

Assmann, S.M., and Shimazaki, K.-i. (1999). The multisensory guard cell: Stomatal responses to blue light and abscisic acid. Plant Physiol. **119,** 809–815.

Astle, M., and Rubery, P. (1983). Carriers for abscisic acid and indole-3 acetic acid in primary roots: Their regional localization and thermodynamic driving forces. Planta **157,** 53–63.

Bailey, P.C., McKibbin, R.S., Lenton, J.R., Holdsworth, M.J., Flintham, J.E., and Gale, M.D. (1999). Genetic map locations for orthologous Vp1 genes in wheat and rice. Theor. Appl. Genet. **98,** 281–284.

Banno, H., and Chua, N.-H. (2000). Characterization of the Arabidopsis formin-like protein AFH1 and its interacting protein. Plant Cell Physiol. **41,** 617–626.

Barry, G.F. (2001). The use of the Monsanto draft rice genome sequence in research. Plant Physiol. **125,** 1164–1165.

Beaudoin, N., Serizet, C., Gosti, F., and Giraudat, J. (2000). Interactions between abscisic acid and ethylene signaling cascades. Plant Cell **12,** 1103–1115.

Berger, S., Bell, E., and Mullet, J. (1996). Two methyl jasmonate-insensitive mutants show altered expression of AtVSP in response to methyl jasmonate and wounding. Plant Physiol. **111,** 525–531.

Bhalerao, R.P., Salchert, K., Bakó, L., Ökrész, L., Szabados, L., Muranaka, T., Machida, Y., Schell, J., and Koncz, C. (1999). Regulatory interaction of PRL1 WD protein with Arabidopsis SNF1-like protein kinases. Proc. Natl. Acad. Sci. USA **96,** 5322–5327.

Bianco-Colomas, J., Barthe, P., Orlandini, M., and Le Page-Degivry, M.T. (1991). Carrier-mediated uptake of abscisic acid by suspension cultured *Amaranthus tricolour* cells. Plant Physiol. **95,** 990–996.

Bies, N., Aspart, L., Carles, C., Gallois, P., and Delseny, M. (1998). Accumulation and degradation of Em proteins in *Arabidopsis thaliana*: Evidence for post-transcriptional controls. J. Exp. Bot. **49,** 1925–1933.

Bies-Etheve, N., da Silva Conceicao, A., Giraudat, J., Koornneef, M., Leon-Kloosterziel, K., Valon, C., and Delseny, M. (1999). Importance of the Arabidopsis ABI3 protein for Em and 2S albumin gene regulation. Plant Mol. Biol. **40,** 1045–1054.

Blatt, M.R. (2000). Ca^{2+} signalling and control of guard-cell volume in stomatal movements. Curr. Opin. Plant Biol. **3,** 196–204.

Bobb, A.J., Eiben, H.G., and Bustos, M.M. (1995). PvAlf, an embryo-specific acidic transcriptional activator enhances gene expression from phaseolin and phytohemagglutinin promoters. Plant J. **8,** 331–343.

Burner, H., Benz, R., Gimmler, H., Hartung, W., and Stillwell, W. (1993). Abscisic acid-lipid interactions: A phospholipid monolayer study. Biochim. Biophys. Acta **1150,** 165–172.

Burnett, E.C., Desikan, R., Moser, R.C., and Neill, S.J. (2000). ABA activation of an MBP kinase in *Pisum sativum* epidermal peels correlates with stomatal responses to ABA. J. Exp. Bot. **51,** 197–205.

Busk, P.K., and Pages, M. (1998). Regulation of abscisic acid-induced transcription. Plant Mol. Biol. **37,** 425–435.

Carlberg, C. (1999). Lipid soluble vitamins in gene regulation. Biofactors **10,** 91–97.

Carrari, F., Perez-Flores, L., Lijavetzky, D., Enciso, S., Sanchez, R., Benech-Arnold, R., and Iusem, N. (2001). Cloning and expression of a sorghum gene with homology to maize vp1: Its potential involvement in pre-harvest sprouting resistance. Plant Mol. Biol. **45,** 631–640.

Carson, C., Hattori, T., Rosenkrans, L., Vasil, V., Vasil, I.K., Peterson, P.A., and McCarty, D.R. (1997). The quiescent/colorless alleles of viviparous1 show that the conserved B3 domain of VP1 is not essential for ABA-regulated gene expression in the seed. Plant J. **12,** 1231–1240.

Chandler, J.W., and Bartels, D. (1997). Structure and function of the vp1 gene homologue from the resurrection plant *Craterostigma plantagineum* Hochst. Mol. Gen. Genet. **256,** 539–546.

Chern, M.-S., Bobb, A.J., and Bustos, M.M. (1996). The Regulator Of Mat2 (ROM2) protein binds to early maturation promoters and represses PvALF-activated transcription. Plant Cell **8,** 305–321.

Choi, H., Hong, J., Ha, J., Kang, J., and Kim, S. (2000). ABFs, a family of ABA-responsive element binding factors. J. Biol. Chem. **275,** 1723–1730.

Coruzzi, G.M., and Zhou, L. (2001). Carbon and nitrogen sensing and signaling in plants: Emerging 'matrix effects.' Curr. Opin. Plant Biol. **4,** 247–253.

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

Debeaujon, I., and Koornneef, M. (2000). Gibberellin requirement for *Arabidopsis* seed germination is determined both by testa characteristics and embryonic abscisic acid. Plant Physiol. **122,** 415–424.

Debeaujon, I., Leon-Kloosterziel, K.M., and Koornneef, M. (2000). Influence of the testa on seed dormancy, germination, and longevity in Arabidopsis. Plant Physiol. **122,** 403–413.

Delseny, M., Bies-Etheve, N., Carles, C., Hull, G., Vicient, C., Raynal, M., Grellet, F., and Aspart, L. (2001). Late Embryogenesis Abundant (LEA) protein regulation during Arabidopsis seed maturation. J. Plant Physiol. **158,** 419–427.

Desikan, R., Hagenbeek, D., Neill, S.J., and Rock, C.D. (1999). Flow cytometry and surface plasmon resonance analyses demonstrate that the monoclonal antibody JIM19 interacts with a rice cell surface component involved in abscisic acid signalling in protoplasts. FEBS Lett. **456,** 257–262.

Eastmond, P.J., Germain, V., Lange, P.R., Bryce, J.H., Smith, S.M., and Graham, I.A. (2000). Postgerminative growth and lipid catabolism in oilseeds lacking the glyoxylate cycle. Proc. Natl. Acad. Sci. USA **97,** 5669–5674.

Ephritikhine, G., Fellner, M., Vannini, C., Lapous, D., and Barbier-Brygoo, H. (1999). The *sax1* dwarf mutant of *Arabidopsis thaliana* shows altered sensitivity of growth responses to abscisic acid, auxin, gibberellins and ethylene and is partially rescued by exogenous brassinosteroid. Plant J. **18,** 303–314.

Eun, S.-O., and Lee, Y. (1997). Actin filaments of guard cells are reorganized in response to light and abscisic acid. Plant Physiol. **115,** 1491–1498.

Estévez, J.M., Cantero, A., Reindl, A., Reichler, S., and León, P. (2001). 1-Deoxy-D-xylulose-5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. J. Biol. Chem. **276,** 22901–22909.

Ezcurra, I., Wycliffe, P., Nehlin, L., Ellerstrom, M., and Rask, L. (2000). Transactivation of the *Brassica napus* napin promoter by ABI3 requires interaction of the conserved B2 and B3 domains of ABI3 with different *cis*-elements: B2 mediates activation through an ABRE, whereas B3 interacts with an RY/G-box. Plant J. **24,** 57–66.

Fan, L., Zheng, S., and Wang, X. (1997). Antisense suppression of phospholipase Dα retards abscisic acid- and ethylene-promoted senescence of postharvest Arabidopsis leaves. Plant Cell **9,** 2183–2196.

Fan, L., Zheng, S., Cui, D., and Wang, X. (1999). Subcellular distribution and tissue expression of phospholipase Dα, Dβ, and Dγ in *Arabidopsis*. Plant Physiol. **119,** 1371–1378.

Fath, A., Bethke, P., Lonsdale, J., Meza-Romero, R., and Jones, R.L. (2000). Programmed cell death in cereal aleurone. Plant Mol. Biol. **44,** 255–266.

Finkelstein, R., and Gibson, S.I. (2002). ABA and sugar interactions regulating development: "cross-talk" or "voices in a crowd"? Curr. Opin. Plant Biol. **5,** 26–32.

Finkelstein, R., and Lynch, T. (1997). *Abi8*: A near-lethal ABA response mutant. Paper presented at the 8th International Conference on Arabidopsis Research; June 25–29, 1997; Madison, WI.

Finkelstein, R., and Lynch, T. (2000a). The Arabidopsis abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. Plant Cell **12,** 599–609.

Finkelstein, R., and Lynch, T. (2000b). Abscisic acid inhibition of radicle emergence but not seedling growth is suppressed by sugars. Plant Physiol. **122,** 1179–1186.

Finkelstein, R., and Rock, C. (2002). Abscisic acid biosynthesis and signaling. In The Arabidopsis Book, C.R. Somerville and E.M. Meyerowitz, eds (Rockville, MD: American Society of Plant Biologists, doi/10.1199/tab.0058, http.www.aspb.org/publications/arabidopsis, in press).

Finkelstein, R., and Somerville, C. (1990). Three classes of abscisic acid (ABA)-insensitive mutations of *Arabidopsis* define genes that control overlapping subsets of ABA responses. Plant Physiol. **94,** 1172–1179.

Finkelstein, R., Tenbarge, K., Shumway, J., and Crouch, M. (1985). Role of abscisic acid in maturation of rapeseed embryos. Plant Physiol. **78,** 630–636.

Finkelstein, R.R. (1994). Mutations at two new *Arabidopsis* ABA response loci are similar to the *abi3* mutations. Plant J. **5,** 765–771.

Finkelstein, R.R., Wang, M.L., Lynch, T.J., Rao, S., and Goodman, H.M. (1998). The Arabidopsis abscisic acid response locus *ABI4* encodes an APETALA2 domain protein. Plant Cell **10,** 1043–1054.

Foley, M.E. (2001). Seed dormancy: An update on terminology, physiological genetics, and quantitative trait loci regulating germinability. Weed Sci. **49,** 305–317.

Foster, R., and Chua, N.-H. (1999). An Arabidopsis mutant with deregulated ABA gene expression: Implications for negative regulator function. Plant J. **17,** 363–372.

Furini, A., Koncz, C., Salamini, F., and Bartels, D. (1997). High level transcription of a member of a repeated gene family confers dehydration tolerance to callus tissue of *Craterostigma plantagineum*. EMBO J. **16,** 3599–3608.

Gampala, S., Hagenbeek, D., and Rock, C. (2001). Functional interactions of lanthanum and phospholipase D with the abscisic acid signaling effectors VP1 and ABI1-1 in rice protoplasts. J. Biol. Chem. **276,** 9855–9860.

Gampala, S.S.L., Finkelstein, R.R., Sun, S.M., and Rock, C.D. (2002). ABI5 interacts with abscisic acid signaling effectors in rice protoplasts. J. Biol. Chem. **277,** 1689–1694.

Garciarrubio, A., Legaria, J.P., and Covarrubias, A.A. (1997). Abscisic acid inhibits germination of mature Arabidopsis seeds by limiting the availability of energy and nutrients. Planta **203,** 182–187.

Gazzarrini, S., and McCourt, P. (2001). Genetic interactions between ABA, ethylene and sugar signaling pathways. Curr. Opin. Plant Biol. **4,** 387–391.

Ghassemian, M., Nambara, E., Cutler, S., Kawaide, H., Kamiya, Y., and McCourt, P. (2000). Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. Plant Cell **12,** 1117–1126.

Ghelis, T., Dellis, O., Jeannette, E., Bardat, F., Cornel, D., Miginiac, E., Rona, J.-P., and Sotta, B. (2000a). Abscisic acid specific expression of RAB18 involves activation of anion channels in *Arabidopsis thaliana* suspension cells. FEBS Lett. **474,** 43–47.

Ghelis, T., Dellis, O., Jeannette, E., Bardat, F., Miginiac, E., and Sotta, B. (2000b). Abscisic acid plasmalemma perception triggers a calcium influx essential for RAB18 gene expression in *Arabidopsis thaliana* suspension cells. FEBS Lett. **483,** 67–70.

Gibson, S.I. (2000). Plant sugar-response pathways: Part of a complex regulatory web. Plant Physiol. **124,** 1532–1539.

Gibson, S.I., Laby, R.J., and Kim, D. (2001). The *sugar-insensitive1* (*sis1*) mutant of *Arabidopsis* is allelic to *ctr1*. Biochem. Biophys. Res. Commun. **280,** 196–203.

Gilroy, S., and Jones, R. (1992). Gibberellic acid and abscisic acid coordinately regulate cytoplasmic calcium and secretory activity in barley aleurone protoplasts. Proc. Natl. Acad. Sci. USA **89,** 3591–3595.

Gilroy, S., and Jones, R. (1994). Perception of gibberellin and abscisic acid at the external face of the plasma membrane of barley (*Hordeum vulgare* L.) aleurone protoplasts. Plant Physiol. **104,** 1185–1192.

Gilroy, S., Read, N.D., and Trewavas, A.J. (1990). Elevation of cytoplasmic calcium by caged calcium or caged inositol trisphosphate initiates stomatal closure. Nature **343,** 769–771.

Giraudat, J., Hauge, B., Valon, C., Smalle, J., Parcy, F., and Goodman, H. (1992). Isolation of the *Arabidopsis ABI3* gene by positional cloning. Plant Cell **4,** 1251–1261.

Gómez-Cadenas, A., Verhey, S.D., Holappa, L.D., Shen, Q., Ho, T.-H.D., and Walker-Simmons, M.K. (1999). An abscisic acid-induced protein kinase, PKABA1, mediates abscisic acid-suppressed gene expression in barley aleurone layers. Proc. Natl. Acad. Sci. USA **96,** 1767–1772.

Gómez-Cadenas, A., Zentella, R., Walker-Simmons, M.K., and Ho, T.-H.D. (2001). Gibberellin/abscisic acid antagonism in barley aleurone cells: Site of action of the protein kinase PKABA1 in relation to gibberellin signaling molecules. Plant Cell **13,** 667–679.

Gosti, F., Beaudoin, N., Serizet, C., Webb, A., Vartanian, N., and Giraudat, J. (1999). ABI1 protein phosphatase 2C is a negative regulator of abscisic acid signaling. Plant Cell **11,** 1897–1909.

Grabov, A., and Blatt, M.R. (1998a). Membrane voltage initiates Ca^{2+} waves and potentiates Ca^{2+} increases with abscisic acid in stomatal guard cells. Proc. Natl. Acad. Sci. USA **95,** 4778–4783.

Grabov, A., and Blatt, M.R. (1998b). Co-ordination of signalling elements in guard cell ion channel control. J. Exp. Bot. **49,** 351–360.

Grabov, A., Leung, J., Giraudat, J., and Blatt, M.R. (1997). Alteration of anion channel kinetics in wild-type and *abi1-1* transgenic *Nicotiana benthamiana* guard cells by abscisic acid. Plant J. **12,** 203–213.

Grappin, P., Bouinot, D., Sotta, B., Miginiac, E., and Jullien, M. (2000). Control of seed dormancy in *Nicotiana plumbaginifolia*: Post-imbibition abscisic acid synthesis imposes dormancy maintenance. Planta **210,** 279–285.

Gu, S., Thompson, D., Srikumari, C., Lorenz, B., Finckh, U., Nicoletti, A., Murthy, K., Rathmann, M., Kumaramanickavel, G., Denton, M., and Gal, A. (1997). Mutations in RPE65 cause autosomal recessive childhood-onset severe retinal dystrophy. Nat. Genet. **17,** 194–197.

Gubler, F., Kalla, R., Roberts, J.K., and Jacobsen, J.V. (1995). Gibberellin-regulated expression of a myb gene in barley aleurone cells: Evidence of myb transactivation of a high-pI α-amylase gene promoter. Plant Cell **7,** 1879–1891.

Guiltinan, M., Marcotte, W.R., Jr., and Quatrano, R. (1990). A plant leucine zipper protein that recognizes an abscisic acid response element. Science **250,** 267–271.

Hagenbeek, D., Quatrano, R., and Rock, C. (2000). Trivalent ions activate abscisic acid-inducible promoters through an *ABI1*-dependent pathway in rice protoplasts. Plant Physiol. **123,** 1553–1560.

Hamilton, D.W.A., Hills, A., Kohler, B., and Blatt, M.R. (2000). Ca^{2+} channels at the plasma membrane of stomatal guard cells are activated by hyperpolarization and abscisic acid. Proc. Natl. Acad. Sci. USA **97,** 4967–4972.

Hattori, T., Terada, T., and Hamasuna, S.T. (1994). Sequence and functional analyses of the rice gene homologous to the maize Vp1. Plant Mol. Biol. **24,** 805–810.

Hedrich, R., Moran, O., Conti, F., Busch, H., Becker, D., Gambale, F., Dreyer, I., Kuch, A., Neuwinger, K., and Palme, K. (1995). Inward rectifier potassium channels in plants differ from their animal counterparts in response to voltage and channel modulators. Eur. Biophys. J. **24,** 107–115.

Heimovaara-Dijkstra, S., Mundy, J., and Wang, M. (1995). The effect of intracellular pH on the regulation of the Rab 16A and the α-amylase 1/6-4 promoter by abscisic acid and gibberellin. Plant Mol. Biol. **27,** 815–820.

Hemerly, A.S., Ferreira, P., De Almeida Engler, J., Van Montagu, M., Engler, G., and Inzé, D. (1993). Cdc2a expression in Arabidopsis is linked with competence for cell division. Plant Cell **5,** 1711–1723.

Hill, A., Nantel, A., Rock, C., and Quatrano, R. (1996). A conserved domain of the Viviparous-1 gene product enhances the DNA binding activity of the bZIP protein EmBP-1 and other transcription factors. J. Biol. Chem. **271,** 3366–3374.

Himmelbach, A., Iten, M., and Grill, E. (1998). Signalling of abscisic acid to regulate plant growth. Philos. Trans. R. Soc. Lond. B Biol. Sci. **353,** 1439–1444.

Hirayama, T., Ohto, C., Mizoguchi, T., and Shinozaki, K. (1995). A

gene encoding a phosphatidylinositol-specific phospholipase C is induced by dehydration and salt stress in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **92,** 3903–3907.

Hobo, T., Kowyama, Y., and Hattori, T. (1999). A bZIP factor, TRAB1, interacts with VP1 and mediates abscisic acid-induced transcription. Proc. Natl. Acad. Sci. USA **96,** 15348–15353.

Hocking, T., Clapham, J., and Cattell, K.J. (1978). Abscisic acid binding to subcellular fractions from leaves of *Vicia faba*. Planta **138,** 303–304.

Hoecker, U., Vasil, I.K., and McCarty, D.R. (1995). Integrated control of seed maturation and germination programs by activator and repressor functions of Viviparous-1 of maize. Genes Dev. **9,** 2459–2469.

Hoecker, U., Vasil, I.K., and McCarty, D.R. (1999). Signaling from the embryo conditions Vp1-mediated repression of α-amylase genes in the aleurone of developing maize seeds. Plant J. **19,** 371–377.

Holdsworth, M., Kurup, S., and McKibbin, R. (1999). Molecular and genetic mechanisms regulating the transition from embryo development to germination. Trends Plant Sci. **4,** 275–280.

Holdsworth, M., Lenton, J., Flintham, J., Gale, M., Kurup, S., McKibbin, R., Bailey, P., Larner, V., and Russell, L. (2001). Genetic control mechanisms regulating the initiation of germination. J. Plant Physiol. **158,** 439–445.

Hong, S.W., Jon, J.H., Kwak, J.M., and Nam, H.G. (1997). Identification of a receptor-like protein kinase gene rapidly induced by abscisic acid, dehydration, high salt, and cold treatments in *Arabidopsis thaliana*. Plant Physiol. **113,** 1203–1212.

Hornberg, C., and Weiler, E. (1984). High-affinity binding sites for abscisic acid on plasmalemma of *Vicia faba* guard cells. Nature **310,** 321–324.

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

Huijser, C., Kortstee, A., Pego, J., Weisbeek, P., Wisman, E., and Smeekens, S. (2000). The Arabidopsis *SUCROSE UNCOUPLED-6* gene is identical to *ABSCISIC ACID INSENSITIVE-4*: Involvement of abscisic acid in sugar responses. Plant J. **23,** 577–585.

Hwang, I., and Goodman, H.M. (1995). An *Arabidopsis thaliana* root-specific kinase homolog is induced by dehydration, ABA, and NaCl. Plant J. **8,** 37–43.

Ingram, J., and Bartels, D. (1996). The molecular basis of dehydration tolerance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47,** 377–403.

Ishitani, M., Xiong, L., Stevenson, B., and Zhu, J.-K. (1997). Genetic analysis of osmotic and cold stress signal transduction in Arabidopsis: Interactions and convergence of abscisic acid–dependent and abscisic acid–independent pathways. Plant Cell **9,** 1935–1949.

Izawa, T., Foster, R., and Chua, N.-H. (1993). Plant bZIP protein DNA binding specificity. J. Mol. Biol. **230,** 1131–1144.

Jacob, T., Ritchie, S., Assmann, S., and Gilroy, S. (1999). Abscisic acid signal transduction in guard cells is mediated by phospholipase D activity. Proc. Natl. Acad. Sci. USA **96,** 12192–12197.

Jacobsen, J.V. (1995). Gibberellin action in germinated cereal grains. In Plant Hormones: Physiology, Biochemistry and Molecular Biology, P.J. Davies, ed (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 246–271.

Jeannette, E., Rona, J.-P., Bardat, F., Cornel, D., Sotta, B., and Miginiac, E. (1999). Induction of RAB18 gene expression and activation of K^+ outward rectifying channels depend on extracellular perception of ABA in *Arabidopsis thaliana* suspension cells. Plant J. **18,** 13–22.

Jones, H., Kurup, S., Peters, N., and Holdsworth, M. (2000). Identification and analysis of proteins that interact with the *Avena fatua* homologue of the maize transcription factor VIVIPAROUS 1. Plant J. **21,** 133–142.

Jones, H.D., Peters, N.C.B., and Holdsworth, M.J. (1997). Genotype and environment interact to control dormancy and differential expression of the *VIVIPAROUS 1* homologue in embryos of *Avena fatua*. Plant J. **12,** 911–920.

Karssen, C., Brinkhorst-van der Swan, D., Breekland, A., and Koornneef, M. (1983). Induction of dormancy during seed development by endogenous abscisic acid: Studies of abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. Planta **157,** 158–165.

Katagiri, T., Takahashi, S., and Shinozaki, K. (2001). Involvement of a novel *Arabidopsis* phospholipase D, AtPLDδ, in dehydration-inducible accumulation of phosphatidic acid in stress signalling. Plant J. **26,** 595–605.

Keith, K., Kraml, M., Dengler, N.G., and McCourt, P. (1994). *FUSCA3*: A heterochronic mutation affecting late embryo development in Arabidopsis. Plant Cell **6,** 589–600.

Kieber, J.J., Rothenberg, M., Roman, G., Feldmann, K.A., and Ecker, J.R. (1993). *CTR1*, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the Raf family of protein kinases. Cell **72,** 427–441.

Kim, B.T., Min, Y.K., Asami, T., Park, N.K., Kwon, O.Y., Cho, K.Y., and Yoshida, S. (1999). 2-Fluoroabscisic acid analogs: Their synthesis and biological activities. J. Agric. Food Chem. **47,** 313–317.

Kim, S.Y., and Thomas, T.L. (1998). A family of novel basic leucine zipper proteins binds to seed-specification elements in the carrot *Dc3* gene promoter. J. Plant Physiol. **152,** 607–613.

Kim, S.Y., Chung, H.-J., and Thomas, T.L. (1997). Isolation of a novel class of bZIP transcription factors that interact with ABA-responsive and embryo-specification elements in the Dc3 promoter using a modified yeast one-hybrid system. Plant J. **11,** 1237–1251.

Koornneef, M., Jorna, M.L., Brinkhorst-van der Swan, D.L.C., and Karssen, C.M. (1982). The isolation of abscisic acid (ABA)-deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of *Arabidopsis thaliana* (L.) Heynh. Theor. Appl. Genet. **61,** 385–393.

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

Koornneef, M., Hanhart, C., Hilhorst, H., and Karssen, C. (1989). In vivo inhibition of seed development and reserve protein accumulation in recombinants of abscisic acid biosynthesis and responsiveness mutants in *Arabidopsis thaliana*. Plant Physiol. **90,** 463–469.

Kovacs, I., Ayaydin, F., Oberschall, A., Ipacs, I., Bottka, S., Pongor, S., Dudits, D., and Toth, E.C. (1998). Immunolocalization of a

novel annexin-like protein encoded by a stress and abscisic acid responsive gene in alfalfa. Plant J. **15,** 185–197.

Kovtun, Y., Chiu, W.-L., Tena, G., and Sheen, J. (2000). Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc. Natl. Acad. Sci. USA **97,** 2940–2945.

Kudla, J., Xu, Q., Harter, K., Gruissem, W., and Luan, S. (1999). Genes for calcineurin B-like proteins in *Arabidopsis* are differentially regulated by stress signals. Proc. Natl. Acad. Sci. USA **96,** 4718–4723.

Kuo, A., Cappelluti, S., Cervantes-Cervantes, M., Rodriguez, M., and Bush, D.S. (1996). Okadaic acid, a protein phosphatase inhibitor, blocks calcium changes, gene expression, and cell death induced by gibberellin in wheat aleurone cells. Plant Cell **8,** 259–269.

Kurup, S., Jones, H., and Holdsworth, M. (2000). Interactions of the developmental regulator ABI3 with proteins identified from developing Arabidopsis seeds. Plant J. **21,** 143–155.

Kutschera, U., and Schopfer, P. (1986). Effect of auxin and abscisic acid on cell wall extensibility in maize coleoptiles. Planta **167,** 527–535.

Laby, R., Kincaid, M., Kim, D., and Gibson, S. (2000). The Arabidopsis sugar-insensitive mutants *sis4* and *sis5* are defective in abscisic acid synthesis and response. Plant J. **23,** 587–596.

Leckie, C.P., McAinsh, M.R., Allen, G.J., Sanders, D., and Hetherington, A.M. (1998). Abscisic acid-induced stomatal closure mediated by cyclic ADP-ribose. Proc. Natl. Acad. Sci. USA **95,** 15837–15842.

Lee, H., Xiong, L., Gong, Z., Ishitani, M., Stevenson, B., and Zhu, J.-K. (2001). The *Arabidopsis HOS1* gene negatively regulates cold signal transduction and encodes a ring finger protein that displays cold-regulated nucleo-cytoplasmic partitioning. Genes Dev. **15,** 912–924.

Lee, S., Choi, H., Suh, S., Doo, I.-S., Oh, K.-Y., Choi, E.J., Schroeder Taylor, A.T., Low, P.S., and Lee, Y. (1999). Oligogalacturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from guard cells of tomato and *Commelina communis*. Plant Physiol. **121,** 147–152.

Lee, S.H., Lee, M.H., Chung, W.I., and Liu, J.R. (1998). *WAPK*, a Ser/Thr protein kinase gene of *Nicotiana tabacum*, is uniquely regulated by wounding, abscisic acid and methyl jasmonate. Mol. Gen. Genet. **259,** 516–522.

Lee, Y.-H., and Chun, J.-Y. (1998). A new homeodomain-leucine zipper gene from *Arabidopsis thaliana* induced by water stress and abscisic acid treatment. Plant Mol. Biol. **37,** 377–384.

Lekstrom-Himes, J., and Xanthopoulos, K. (1998). Biological role of the CCAAT/enhancer-binding protein family of transcription factors. J. Biol. Chem. **273,** 28545–28548.

Lemichez, E., Wu, Y., Sanchez, J.-P., Mettouchi, A., Mathur, J., and Chua, N.-H. (2001). Inactivation of AtRAC1 by abscisic acid is essential for stomatal closure. Genes Dev. **15,** 1808–1816.

Lemtiri-Chlieh, F., MacRobbie, E.A.C., and Brearley, C.A. (2000). Inositol hexakisphosphate is a physiological signal regulating the K^+-inward rectifying conductance in guard cells. Proc. Natl. Acad. Sci. USA **97,** 8687–8692.

Leon-Kloosterziel, K., Van De Bunt, G., Zeevaart, J., and Koornneef, M. (1996). Arabidopsis mutants with a reduced seed dormancy. Plant Physiol. **110,** 233–240.

Le Page-Degivry, M.T., and Garello, G. (1992). In situ abscisic acid synthesis: A requirement for induction of embryo dormancy in *Helianthus annuus*. Plant Physiol. **98,** 1386–1390.

Leube, M.P., Grill, E., and Amrhein, N. (1998). ABI1 of *Arabidopsis* is a protein serine/threonine phosphatase highly regulated by the proton and magnesium ion concentration. FEBS Lett. **424,** 100–104.

Leung, J., and Giraudat, J. (1998). Abscisic acid signal transduction. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49,** 199–222.

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

Leung, J., Merlot, S., and Giraudat, J. (1997). The Arabidopsis *ABSCISIC ACID-INSENSITIVE2* (*ABI2*) and *ABI1* genes encode homologous protein phosphatases 2C involved in abscisic acid signal transduction. Plant Cell **9,** 759–771.

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

Leyman, B., Geelen, D., and Blatt, M.R. (2000). Localization and control of expression of Nt-Syr1, a tobacco snare protein. Plant J. **24,** 369–381.

Li, G., Bishop, K., Chandrasekharan, M., and Hall, T. (1999). β-Phaseolin gene activation is a two-step process: PvALF-facilitated chromatin modification followed by abscisic acid-mediated gene activation. Proc. Natl. Acad. Sci. USA **96,** 7104–7109.

Li, H., Shen, J.J., Zheng, Z.L., Lin, Y.K., and Yang, Z.B. (2001). The Rop GTPase switch controls multiple developmental processes in Arabidopsis. Plant Physiol. **126,** 670–684.

Li, J., and Assmann, S.M. (1996). An abscisic acid-activated and calcium-independent protein kinase from guard cells of fava bean. Plant Cell **8,** 2359–2368.

Li, J., and Chory, J. (1997). A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell **90,** 929–938.

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

Lijavetzky, D., Martinez, M.C., Carrari, F., and Hopp, H.E. (2000). QTL analysis and mapping of pre-harvest sprouting resistance in sorghum. Euphytica **112,** 125–135.

Liu, J.-H., Luo, M., Cheng, K.-J., Mohapatra, S.S., and Hill, R.D. (1999). Identification and characterization of a novel barley gene that is ABA-inducible and expressed specifically in embryo and aleurone. J. Exp. Bot. **50,** 727–728.

Liu, Y., Bergervoet, J.H.W., Ric De Vos, C.H., Hilhorst, H.W.M., Kraak, H.L., Karssen, C.M., and Bino, R.J. (1994). Nuclear replication activities during imbibition of abscisic acid- and gibberellin-deficient tomato (*Lycopersicon esculentum* Mill.) seeds. Planta **194,** 368–373.

Llorente, F., Oliveros, J.C., Martinez-Zapater, J.M., and Salinas, J. (2000). A freezing-sensitive mutant of Arabidopsis, *frs1*, is a new *aba3* allele. Planta **211,** 648–655.

Lopez-Molina, L., and Chua, N.-H. (2000). A null mutation in a bZIP factor confers ABA-insensitivity in *Arabidopsis thaliana*. Plant Cell Physiol. **41,** 541–547.

Lopez-Molina, L., Mongrand, S., and Chua, N.-H. (2001). A post-germination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **98,** 4782–4787.

Lotan, T., Ohto, M.-A., Yee, K.M., West, M.A.L., Lo, R., Kwong, R.W., Yamagishi, K., Fischer, R.L., Goldberg, R.B., and Harada, J.J. (1998). Arabidopsis *LEAFY COTYLEDON1* is sufficient to induce embryo development in vegetative cells. Cell **93,** 1195–1205.

Lovegrove, A., and Hooley, R. (2000). Gibberellin and abscisic acid signalling in aleurone. Trends Plant Sci. **5,** 102–110.

Lu, C., and Fedoroff, N. (2000). A mutation in the Arabidopsis *HYL1* gene encoding a dsRNA binding protein affects responses to abscisic acid, auxin, and cytokinin. Plant Cell **12,** 2351–2365.

Lu, G., Paul, A.-L., McCarty, D.R., and Ferl, R.J. (1996). Transcription factor veracity: Is GBF3 responsible for ABA-regulated expression of *Arabidopsis Adh*? Plant Cell **8,** 847–857.

Luerssen, H., Kirik, V., Herrmann, P., and Misera, S. (1998). *FUSCA3* encodes a protein with a conserved VP1/ABI3-like B3 domain which is of functional importance for the regulation of seed maturation in *Arabidopsis thaliana*. Plant J. **15,** 755–764.

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

MacRobbie, E.A.C. (2000). ABA activates multiple Ca^{2+} fluxes in stomatal guard cells, triggering vacuolar K^+ (Rb^+) release. Proc. Natl. Acad. Sci. USA **97,** 12361–12368.

McAinsh, M.R., Webb, A.A.R., Taylor, J.E., and Hetherington, A.M. (1995). Stimulus-induced oscillations in guard cell cytosolic free calcium. Plant Cell **7,** 1207–1219.

McCallum, C.M., Comai, L., Greene, E.A., and Henikoff, S. (2000). Targeting Induced Local Lesions IN Genomes (TILLING) for plant functional genomics. Plant Physiol. **123,** 439–442.

McCarty, D.R., Hattori, T., Carson, C.B., Vasil, V., Lazar, M., and Vasil, I. (1991). The *Viviparous-1* developmental gene of maize encodes a novel transcriptional activator. Cell **66,** 895–905.

McCourt, P. (1999). Genetic analysis of hormone signaling. Annu. Rev. Plant Physiol. Plant Mol. Biol. **50,** 219–243.

Meinke, D.W., Franzmann, L.H., Nickle, T.C., and Yeung, E.C. (1994). Leafy cotyledon mutants of Arabidopsis. Plant Cell **6,** 1049–1064.

Merlot, S., Gosti, F., Guerrier, D., Vavasseur, A., and Giraudat, J. (2001). The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. Plant J. **25,** 295–303.

Meurs, C., Basra, A., Karssen, C., and van Loon, L. (1992). Role of abscisic acid in the induction of desiccation tolerance in developing seeds of *Arabidopsis thaliana*. Plant Physiol. **98,** 1484–1493.

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

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

Mikami, K., Katagiri, T., Iuchi, S., Yamaguchi-Shinozaki, K., and Shinozaki, K. (1998). A gene encoding phosphatidylinositol-4-phosphate 5-kinase is induced by water stress and abscisic acid in *Arabidopsis thaliana*. Plant J. **15,** 563–568.

Mizoguchi, T., Irie, K., Hirayama, T., Hayashida, N., Yamaguchi-Shinozaki, K., Matsumoto, K., and Shinozaki, K. (1996). A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **93,** 765–769.

Nagpal, P., Walker, L., Young, J., Sonawala, A., Timpte, C., Estelle, M., and Reed, J. (2000). *Axr2* encodes a member of the AUX/IAA protein family. Plant Physiol. **123,** 563–573.

Nakamura, S., and Toyama, T. (2001). Isolation of a *VP1* homologue from wheat and analysis of its expression in embryos of dormant and non-dormant cultivars. J. Exp. Bot. **52,** 875–876.

Nakamura, S., Lynch, T., and Finkelstein, R. (2001). Physical interactions between ABA response loci of *Arabidopsis*. Plant J. **26,** 627–635.

Nambara, E., and McCourt, P. (1999). Protein farnesylation in plants: A greasy tale. Curr. Opin. Plant Biol. **2,** 388–392.

Nambara, E., Naito, S., and McCourt, P. (1992). A mutant of *Arabidopsis* which is defective in seed development and storage protein accumulation is a new *abi3* allele. Plant J. **2,** 435–441.

Nambara, E., Hayama, R., Tsuchiya, Y., Nishimura, M., Kawaide, H., Kamiya, Y., and Naito, S. (2000). The role of *ABI3* and *FUS3* loci in *Arabidopsis thaliana* on phase transition from late embryo development to germination. Dev. Biol. **220,** 412–423.

Nantel, A., and Quatrano, R. (1996). Characterization of three rice basic-leucine zipper factors, including two inhibitors of EmBP-1 DNA binding activity. J. Biol. Chem. **271,** 31296–31305.

Neill, S.J., Horgan, R., and Parry, A. (1986). The carotenoid and abscisic acid content of viviparous kernels and seedlings of *Zea mays* L. Planta **169,** 87–96.

Németh, K., et al. (1998). Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in *Arabidopsis*. Genes Dev. **12,** 3059–3073.

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

Niyogi, K.K., Grossman, A.R., and Bjorkman, O. (1998). Arabidopsis mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. Plant Cell **10,** 1121–1134.

Oeda, K., Salinas, J., and Chua, N. (1991). A tobacco bZIP transcription activator (TAF-1) binds to a G-box-like motif conserved in plant genes. EMBO J. **10,** 1793–1802.

Ogas, J., Cheng, J.-C., Sung, Z.R., and Somerville, C. (1997). Cellular differentiation regulated by gibberellin in the *Arabidopsis thaliana pickle* mutant. Science **277,** 91–94.

Ogas, J., Kaufmann, S., Henderson, J., and Somerville, C. (1999). PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **96,** 13839–13844.

Ooms, J.J.J., Leon-Kloosterziel, K.M., Bartels, D., Koornneef, M., and Karssen, C.M. (1993). Acquisition of desiccation tolerance and longevity in seeds of *Arabidopsis thaliana*. Plant Physiol. **102,** 1185–1191.

Papi, M., Sabatini, S., Bouchez, D., Camilleri, C., Costantino, P., and Vittorioso, P. (2000). Identification and disruption of an Arabidopsis zinc finger gene controlling seed germination. Genes Dev. **14,** 28–33.

Parasassi, T., Destasio, G., Miccheli, A., Bruno, F., Conti, F., and Gratton, E. (1990). Abscisic acid-induced microheterogeneity in phospholipid vesicles: A fluorescence study. Biophys. Chem. **35,** 65–73.

Parcy, F., and Giraudat, J. (1997). Interactions between the *ABI1* and the ectopically expressed *ABI3* genes in controlling abscisic acid responses in Arabidopsis vegetative tissues. Plant J. **11,** 693–702.

Parcy, F., Valon, C., Raynal, M., Gaubier-Comella, P., Delseny, M., and Giraudat, J. (1994). Regulation of gene expression programs during *Arabidopsis* seed development: Roles of the *ABI3* locus and of endogenous abscisic acid. Plant Cell **6,** 1567–1582.

Parcy, F., Valon, C., Kohara, A., Misera, S., and Giraudat, J. (1997). The *ABSCISIC ACID-INSENSITIVE3*, *FUSCA3*, and *LEAFY COTYLEDON1* loci act in concert to control multiple aspects of Arabidopsis seed development. Plant Cell **9,** 1265–1277.

Parinov, S., and Sundaresan, V. (2000). Functional genomics in Arabidopsis: Large-scale insertional mutagenesis complements the genome sequencing project. Curr. Opin. Biotechnol. **11,** 157–161.

Pedron, J., Brault, M., Nake, C., and Miginiac, E. (1998). Detection of abscisic-acid-binding proteins in the microsomal protein fraction of *Arabidopsis thaliana* with abscisic-acid-protein conjugates used as affinity probes. Eur. J. Biochem. **252,** 385–390.

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

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

Perras, M.R., Abrams, S.R., and Balsevich, J.J. (1994). Characterization of an abscisic acid carrier in suspension-cultured barley cells. J. Exp. Bot. **45,** 1565–1573.

Phillips, J., Artsaenko, O., Fiedler, U., Horstmann, C., Mock, H.-P., Muentz, K., and Conrad, U. (1997). Seed-specific immunomodulation of abscisic acid activity induces a developmental switch. EMBO J. **16,** 4489–4496.

Piao, H.L., Pih, K.T., Lim, J.H., Kang, S.G., Jin, J.B., Kim, S.H., and Hwang, I. (1999). An *Arabidopsis GSK3/shaggy*-like gene that complements yeast salt stress-sensitive mutants is induced by NaCl and abscisic acid. Plant Physiol. **119,** 1527–1534.

Pritchard, S., and Graham, I.A. (2001). The effect of phytohormones on storage reserve mobilization during germination in Arabidopsis. Presented at the Twelfth International Conference on Arabidopsis Research; June 23–27, 2001; Madison, WI.

Quesada, V., Ponce, M., and Micol, J. (2000). Genetic analysis of salt-tolerant mutants in *Arabidopsis thaliana*. Genetics **154,** 421–436.

Raskin, I., and Ladyman, J. (1988). Isolation and characterization of a barley mutant with abscisic-acid-insensitive stomata. Planta **173,** 73–78.

Raz, V., Bergervoet, J., and Koornneef, M. (2001). Sequential steps for developmental arrest in Arabidopsis seeds. Development **128,** 243–252.

Reidt, W., Wohlfarth, T., Ellerstroem, M., Czihal, A., Tewes, A., Ezcurra, I., Rask, L., and Baumlein, H. (2000). Gene regulation during late embryogenesis: The RY motif of maturation-specific gene promoters is a direct target of the FUS3 gene product. Plant J. **21,** 401–408.

Riechmann, J., et al. (2000). Arabidopsis transcription factors: Genome-wide comparative analysis among eukaryotes. Science **290,** 2105–2110.

Ritchie, S., and Gilroy, S. (1998). Abscisic acid signal transduction in the barley aleurone is mediated by phospholipase D activity. Proc. Natl. Acad. Sci. USA **95,** 2697–2702.

Ritchie, S., and Gilroy, S. (2000). Abscisic acid stimulation of phospholipase D in the barley aleurone is G-protein-mediated and localized to the plasma membrane. Plant Physiol. **124,** 693–702.

Rivin, C., and Grudt, T. (1991). Abscisic acid and the developmental regulation of embryo storage proteins in maize. Plant Physiol. **95,** 358–365.

Robertson, D. (1955). The genetics of vivipary in maize. Genetics **40,** 745–760.

Robichaud, C., Wong, J., and Sussex, I. (1980). Control of in vitro growth of viviparous embryo mutants of maize by abscisic acid. Dev. Genet. **1,** 325–330.

Rock, C. (2000). Pathways to abscisic acid-regulated gene expression. New Phytol. **148,** 357–396.

Rock, C., and Quatrano, R. (1995). The role of hormones during seed development. In Plant Hormones: Physiology, Biochemistry and Molecular Biology, P.J. Davies, ed (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 671–697.

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

Rohde, A., Ardiles-Diaz, W., Van Montagu, M., and Boerjan, W. (1998). Isolation and expression analysis of an ABSCISIC ACID-INSENSITIVE 3 (ABI3) homologue from *Populus trichocarpa*. J. Exp. Bot. **49,** 1059–1060.

Rohde, A., Van Montagu, M., and Boerjan, W. (1999). The *ABSCISIC ACID-INSENSITIVE 3* (*ABI3*) gene is expressed during vegetative quiescence processes in Arabidopsis. Plant Cell Environ. **22,** 261–270.

Rohde, A., De Rycke, R., Beeckman, T., Engler, G., Van Montagu, M., and Boerjan, W. (2000a). *ABI3* affects plastid differentiation in dark-grown Arabidopsis seedlings. Plant Cell **12,** 35–52.

Rohde, A., Kurup, S., and Holdsworth, M. (2000b). *ABI3* emerges from the seed. Trends Plant Sci. **5,** 418–419.

Rojas, A., Almoguera, C., and Jordano, J. (1999). Transcriptional

activation of a heat shock gene promoter in sunflower embryos: Synergism between ABI3 and heat shock factors. Plant J. **20,** 601–610.

Rook, F., Corke, F., Card, R., Munz, G., Smith, C., and Bevan, M.W. (2001). Impaired sucrose-induction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signalling. Plant J. **26,** 421–433.

Russell, L., Larner, V., Kurup, S., Bougourd, S., and Holdsworth, M. (2000). The Arabidopsis *COMATOSE* locus regulates germination potential. Development **127,** 3759–3767.

Sanchez, J.-P., and Chua, N.-H. (2001). Arabidopsis PLC1 is required for secondary responses to abscisic acid signals. Plant Cell **13,** 1143–1154.

Sasaki, T., and Burr, B. (2000). International Rice Genome Sequencing Project: The effort to completely sequence the rice genome. Curr. Opin. Plant Biol. **3,** 138–141.

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

Schultz, T., Medina, J., Hill, A., and Quatrano, R. (1998). 14-3-3 proteins are part of an abscisic acid-VIVIPAROUS1 (VP1) response complex in the *Em* promoter and interact with VP1 and EmBP1. Plant Cell **10,** 837–847.

Schultz, T.F., and Quatrano, R.S. (1997). Evidence for surface perception of abscisic acid by rice suspension cells as assayed by Em gene expression. Plant Sci. **130,** 63–71.

Schwartz, A., Wu, W.-H., Tucker, E.B., and Assmann, S.M. (1994). Inhibition of inward K^+ channels and stomatal response by abscisic acids: An intracellular locus of phytohormone action. Proc. Natl. Acad. Sci. USA **91,** 4019–4023.

Schwartz, S.H., Tan, B.C., Gage, D.A., Zeevaart, J.A.D., and McCarty, D.R. (1997). Specific oxidative cleavage of carotenoids by VP14 of maize. Science **276,** 1872–1874.

Seo, M., Peeters, A., Koiwai, H., Oritani, T., Marion-Poll, A., Zeevaart, J., Koornneef, M., Kamiya, Y., and Koshiba, T. (2000). The *Arabidopsis* aldehyde oxidase 3 (*AAO3*) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. Proc. Natl. Acad. Sci. USA **97,** 12908–12913.

Sharp, R.E., LeNoble, M.E., Else, M.A., Thorne, E.T., and Gherardi, F. (2000). Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: Evidence for an interaction with ethylene. J. Exp. Bot. **51,** 1575–1584.

Shears, S.B. (1998). The versatility of inositol phosphates as cellular signals. Biochim. Biophys. Acta **1436,** 49–67.

Sheen, J. (1996). Ca^{2+}-dependent protein kinases and stress signal transduction in plants. Science **274,** 1900–1902.

Sheen, J. (1998). Mutational analysis of protein phosphatase 2C involved in abscisic acid signal transduction in higher plants. Proc. Natl. Acad. Sci. USA **95,** 975–980.

Sheen, J., Zhou, L., and Jang, J. (1999). Sugars as signaling molecules. Curr. Opin. Plant Biol. **2,** 410–418.

Shen, Q., Gómez-Cadenas, A., Zhang, P., Walker-Simmons, M., Sheen, J., and Ho, T. (2001). Dissection of abscisic acid signal transduction pathways in barley aleurone layers. Plant Mol. Biol. **47,** 437–448.

Shinozaki, K., and Yamaguchi-Shinozaki, K. (2000). Molecular responses to dehydration and low temperature: Differences and cross-talk between two stress signaling pathways. Curr. Opin. Plant Biol. **3,** 217–223.

Shiota, H., Satoh, R., Watabe, K.-I., Harada, H., and Kamada, H. (1998). *C-ABI3*, the carrot homologue of the Arabidopsis *ABI3* is expressed during both zygotic and somatic embryogenesis and functions in the regulation of embryogenesis-specific ABA-inducible genes. Plant Cell Physiol. **39,** 1184–1193.

Shripathi, V., Swamy, G.S., and Chandrasekhar, K.S. (1997). Microviscosity of cucumber (*Cucumis sativus* L.) fruit protoplast membranes is altered by triacontanol and abscisic acid. Biochim. Biophys. Acta **1323,** 263–271.

Skriver, K., Olsen, F.L., Rogers, J.C., and Mundy, J. (1991). *cis*-acting DNA elements responsive to gibberellin and its antagonist. Proc. Natl. Acad. Sci. USA **88,** 7266–7270.

Söderman, E., Mattsson, J., and Engstrom, P. (1996). The Arabidopsis homeobox gene *ATHB-7* is induced by water deficit and by abscisic acid. Plant J. **10,** 375–381.

Söderman, E., Hjellstrom, M., Fahleson, J., and Engstrom, P. (1999). The HD-Zip gene *ATHB6* in Arabidopsis is expressed in developing leaves, roots and carpels and up-regulated by water deficit conditions. Plant Mol. Biol. **40,** 1073–1083.

Söderman, E., Brocard, I., Lynch, T., and Finkelstein, R. (2000). Regulation and function of the Arabidopsis *ABA-insensitive4* (*ABI4*) gene in seed and ABA response signaling networks. Plant Physiol. **124,** 1752–1765.

Spollen, W.G., LeNoble, M.E., Samuels, T.D., Bernstein, N., and Sharp, R.E. (2000). Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. Plant Physiol. **122,** 967–976.

Staswick, P., Su, W., and Howell, S. (1992). Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. Proc. Natl. Acad. Sci. USA **89,** 6837–6840.

Steber, C., and McCourt, P. (2001). A role for brassinosteroids in germination in Arabidopsis. Plant Physiol. **125,** 763–769.

Steber, C.M., Cooney, S.E., and McCourt, P. (1998). Isolation of the GA-response mutant sly1 as a suppressor of ABI1-1 in *Arabidopsis thaliana*. Genetics **149,** 509–521.

Stone, S.L., Kwong, L.W., Yee, K.M., Pelletier, J., Lepiniec, L., Fischer, R.L., Goldberg, R.B., and Harada, J.J.A. (2001). *LEAFY COTYLEDON2* encodes a B3 domain transcription factor that induces embryo development. Proc. Natl. Acad. Sci. USA **98,** 11806–11811.

Sturaro, M., Vernieri, P., Castiglioni, P., Binelli, G., and Gavazzi, G. (1996). The *rea* (*red embryonic axis*) phenotype describes a new mutation affecting the response of maize embryos to abscisic acid and osmotic stress. J. Exp. Bot. **47,** 755–762.

Subramanian, S., Rajagopal, B., and Rock, C.D. (2002). *Harlequin* (*hlq*) and *short blue root* (*sbr*), two Arabidopsis mutants that ectopically express an ABA- and auxin-inducible transgenic carrot promoter and have pleiotropic effects on morphogenesis. Plant Mol. Biol. **49,** 93–105.

Sutton, F., Paul, S.S., Wang, X.-Q., and Assmann, S.M. (2000).

Distinct abscisic acid signaling pathways for modulation of guard cell versus mesophyll cell potassium channels revealed by expression studies in *Xenopus laevis* oocytes. Plant Physiol. **124,** 223–230.

Suzuki, M., Kao, C., and McCarty, D. (1997). The conserved B3 domain of VIVIPAROUS1 has a cooperative DNA binding activity. Plant Cell **9,** 799–807.

Tahtiharju, S., and Palva, T. (2001). Antisense inhibition of protein phosphatase 2C accelerates cold acclimation in *Arabidopsis thaliana*. Plant J. **26,** 461–470.

Takahashi, S., Katagiri, T., Yamaguchi-Shinozaki, K., and Shinozaki, K. (2000). An Arabidopsis gene encoding a Ca^{2+} binding protein is induced by abscisic acid during dehydration. Plant Cell Physiol. **41,** 898–903.

Thomas, B.R., and Rodriguez, R.L. (1994). Metabolite signals regulate gene expression and source/sink relations in cereal seedlings. Plant Physiol. **106,** 1235–1239.

Thomas, T. (1993). Gene expression during plant embryogenesis and germination: An overview. Plant Cell **5,** 1401–1410.

Toyomasu, T., Yamane, H., Murofushi, N., and Inoue, Y. (1994). Effects of exogenously applied gibberellin and red light on the endogenous levels of abscisic acid in photoblastic lettuce seeds. Plant Cell Physiol. **35,** 127–129.

Toyomasu, T., Kawaide, H., Mitsuhashi, W., Inoue, Y., and Kamiya, Y. (1998). Phytochrome regulates gibberellin biosynthesis during germination of photoblastic lettuce seeds. Plant Physiol. **118,** 1517–1523.

Trotochaud, A.E., Hao, T., Wu, G., Yang, Z., and Clark, S.E. (1999). The CLAVATA1 receptor-like kinase requires CLAVATA3 for its assembly into a signaling complex that includes KAPP and a Rho-related protein. Plant Cell **11,** 393–406.

Ullah, H., Chen, J.-G., Young, J., Im, K.-H., Sussman, M., and Jones, A. (2001). Modulation of cell proliferation by heterotrimeric G protein in Arabidopsis. Science **292,** 2066–2069.

Ulmasov, T., Hagen, G., and Guilfoyle, T.J. (1999). Activation and repression of transcription by auxin-response factors. Proc. Natl. Acad. Sci. USA **96,** 5844–5849.

Uno, Y., Furihata, T., Abe, H., Yoshida, R., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2000). Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. Proc. Natl. Acad. Sci. USA **97,** 11632–11637.

Urao, T., Yakubov, B., Satoh, R., Yamaguchi-Shinozaki, K., Seki, M., Hirayama, T., and Shinozaki, K. (1999). A transmembrane hybrid-type histidine kinase in Arabidopsis functions as an osmosensor. Plant Cell **11,** 1743–1754.

Urao, T., Yamaguchi-Shinozaki, K., Urao, S., and Shinozaki, K. (1993). An Arabidopsis myb homolog is induced by dehydration stress and its gene product binds to the conserved MYB recognition sequence. Plant Cell **5,** 1529–1539.

Vasil, V., Marcotte, W.R.J., Rosenkrans, L., Cocciolone, S.M., Vasil, I.K., Quatrano, R.S., and McCarty, D.R. (1995). Overlap of viviparous 1 (VP1) and abscisic acid response elements in the Em promoter: G-box elements are sufficient but not necessary for VP1 transactivation. Plant Cell **7,** 1511–1518.

Walker-Simmons, M.K., Holappa, L.D., Abrams, G.D., and Abrams, S.R. (1997). ABA metabolites induce group 3 LEA mRNA and inhibit germination in wheat. Physiol. Plant. **100,** 474–480.

Wang, H., Qi, Q., Schorr, P., Cutler, A.J., Crosby, W., and Fowke, L.C. (1998). ICK1, a cyclin-dependent protein kinase inhibitor from *Arabidopsis thaliana* interacts with both Cdc2a and CycD3, and its expression is induced by abscisic acid. Plant J. **15,** 501–510.

Wang, M., Heimovaara-Dijkstra, S., Van Der Meulen, R.M., Knox, J.P., and Neill, S.J. (1995). The monoclonal antibody JIM19 modulates abscisic acid action in barley aleurone protoplasts. Planta **196,** 271–276.

Wang, X., Wang, C., Sang, Y., Zheng, L., and Qin, C. (2000). Determining functions of multiple phospholipase Ds in stress response of *Arabidopsis*. Biochem. Soc. Trans. **28,** 813–816.

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

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

West, M.A.L., Yee, K.M., Danao, J., Zimmerman, J.L., Fischer, R.L., Goldberg, R.B., and Harada, J.J. (1994). *LEAFY COTYLEDON1* is an essential regulator of late embryogenesis and cotyledon identity in Arabidopsis. Plant Cell **6,** 1731–1745.

White, C.N., Proebsting, W.M., Hedden, P., and Rivin, C.J. (2000). Gibberellins and seed development in maize. I. Evidence that gibberellin/abscisic acid balance governs germination versus maturation pathways. Plant Physiol. **122,** 1081–1088.

Wilson, A., Pickett, F., Turner, J., and Estelle, M. (1990). A dominant mutation in Arabidopsis confers resistance to auxin, ethylene and abscisic acid. Mol. Gen. Genet. **222,** 377–383.

Windsor, M.L., Milborrow, B.V., and Abrams, S.R. (1994). Stereochemical requirements of the saturable uptake carrier for abscisic acid in carrot suspension culture cells. J. Exp. Bot. **45,** 227–233.

Wolkers, W.F., Alberda, M., Koornneef, M., Leon-Kloosterziel, K.M., and Hoekstra, F.A. (1998). Properties of proteins and the glassy matrix in maturation-defective mutant seeds of *Arabidopsis thaliana*. Plant J. **16,** 133–143.

Wu, Y., Kuzma, J., Marechal, E., Graeff, R., Lee, H.C., Foster, R., and Chua, N.-H. (1997). Abscisic acid signaling through cyclic ADP-ribose in plants. Science **278,** 2126–2130.

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

Xiong, L., Ishitani, M., Lee, H., Zhang, C., and Zhu, J.-K. (2001b). *FIERY1* encoding an inositol polyphosphate 1-phosphatase is a negative regulator of abscisic acid and stress signaling in *Arabidopsis*. Genes Dev. **15,** 1971–1984.

Xiong, L., Ishitani, M., Lee, H., and Zhu, J.-K. (1999). *HOS5*: A negative regulator of osmotic stress-induced gene expression in *Arabidopsis thaliana*. Plant J. **19,** 569–578.

Xiong, L., Ishitani, M., Lee, H., and Zhu, J.-K. (2001c). The Arabidopsis *LOS5*/*ABA3* locus encodes a molybdenum cofactor sulfurase and modulates cold stress– and osmotic stress–responsive gene expression. Plant Cell **13,** 2063–2083.

Xiong, L., Schumaker, K., and Zhu, J.-K. (2002). Cell signaling during cold, drought and salt stress. Plant Cell **14** (suppl.), S165–S183.

Yang, Z. (2002). Small GTPases: Versatile signaling switches in plants. Plant Cell **14** (suppl.), S375–S388.

Young, T.E., and Gallie, D.R. (2000). Regulation of programmed cell death in maize endosperm by abscisic acid. Plant Mol. Biol. **42,** 397–414.

Zhang, D., Zhang, Z., Chen, J., and Jia, W. (1999). Specific abscisic acid-binding sites in mesocarp of grape berry: Properties and subcellular localization. J. Plant Physiol. **155,** 324–331.

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

Zheng, Z., Jin, Z., Xia, K., Zhou, X., and Ma, C. (1998). An ABA-binding protein with nucleic acid-binding properties. Sci. China Ser. C Life Sci. **41,** 209–216.

Ziegelhoffer, E.C., Medrano, L.J., and Meyerowitz, E.M. (2000). Cloning of the Arabidopsis *WIGGUM* gene identifies a role for farnesylation in meristem development. Proc. Natl. Acad. Sci. USA **97,** 7633–7638.