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The Arabidopsis Abscisic Acid Response Gene *ABI5* Encodes a Basic Leucine Zipper Transcription Factor

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The Arabidopsis abscisic acid (ABA)–insensitive *abi5* mutants have pleiotropic defects in ABA response, including decreased sensitivity to ABA inhibition of germination and altered expression of some ABA-regulated genes. We isolated the *ABI5* gene by using a positional cloning approach and found that it encodes a member of the basic leucine zipper transcription factor family. The previously characterized *abi5-1* allele encodes a protein that lacks the DNA binding and dimerization domains required for ABI5 function. Analyses of *ABI5* expression provide evidence for ABA regulation, cross-regulation by other *ABI* genes, and possibly autoregulation. Comparison of seed and ABA-inducible vegetative gene expression in wild-type and *abi5-1* plants indicates that *ABI5* regulates a subset of late embryogenesis–abundant genes during both developmental stages.

INTRODUCTION

Abscisic acid (ABA) regulates many agronomically important aspects of seed development, including synthesis of storage proteins and lipids (Finkelstein and Somerville, 1988; Rock and Quatrano, 1995) and acquisition of desiccation tolerance and dormancy (Black, 1983; Karssen et al., 1983; Koornneef et al., 1989). In addition, vegetative responses to ABA include induction of stomatal closure and tolerance of drought, salt, and cold stresses (reviewed in Leung and Giraudat, 1998). Molecular studies have identified many ABA-regulated genes and an array of corresponding transcriptional regulators (reviewed in Busk and Pages, 1998). Genetic studies, especially those with Arabidopsis plants, have identified a large number of loci involved in responses to ABA. Mutants with defects at these loci are being characterized physiologically, and as the affected genes are cloned, their products are being characterized biochemically.

To date, six genes required for wild-type ABA response have been reported cloned. These genes represent four classes of protein: two orthologous transcriptional regulators (VIVIPAROUS1 [VP1] of maize and ABA INSENSITIVE3 [ABI3] of Arabidopsis) (McCarty et al., 1991; Giraudat et al., 1992), two highly homologous members of the protein phosphatase 2C family (ABI1 and ABI2 of Arabidopsis) (Leung et al., 1994, 1997; Meyer et al., 1994), a member of the APETALA2 domain family (ABI4 of Arabidopsis) (Finkelstein et al., 1998), and a farnesyl transferase (ENHANCED RESPONSE TO ABA1 [ERA1] of Arabidopsis) (Cutler et al., 1996). Two additional genes demonstrated to interact with *ABI3* in regulating seed maturation, *FUSCA3* (*FUS3*) and *LEAFY COTYLEDON1* (*LEC1*), have been found to encode presumed transcription factors (Lotan et al., 1998; Luerssen et al., 1998). To fully describe the molecular events during ABA signaling, we need to identify the biochemical functions of many more of the genes that are required for ABA response.

The Arabidopsis *abi5* mutants, like many of the other ABA-insensitive mutants, were selected on the basis of ABA-resistant germination (Finkelstein, 1994). Initial physiologic and genetic analyses suggested that *ABI5* represented a new element of a signal transduction pathway involving two other ABA response loci: *ABI3* and *ABI4*. Mutations affecting all three loci resulted in defects in seed ABA sensitivity and seed-specific gene expression but did not alter vegetative growth. In addition, in digenic mutant analyses, the *abi3-1*, *abi4-1*, and *abi5-1* mutations all greatly enhanced the ABA resistance of *abi1* mutants with respect to ABA inhibition of seed germination (Finkelstein and Somerville, 1990; Finkelstein, 1994). In contrast, the *abi3-1 abi5-1* digenic mutant was only slightly more resistant to ABA than were its monogenic parents. Recently, we have found that *ABI5* function is essential for the ABA hypersensitivity conferred by ectopically expressed *ABI3* (R.R. Finkelstein, unpublished observations).

To address the molecular relationship between *ABI5* and other components of the ABA signal transduction pathways, we used a positional cloning approach to identify the *ABI5*

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gene. The predicted gene product showed structural similarities to the basic leucine zipper (bZIP) class of transcriptional regulators. Expression analyses showed that, like *ABI4*, *ABI5* is expressed in vegetative as well as seed tissues, albeit at much lower levels, and is required for some ABA-regulated gene expression in vegetative tissue. In addition, *ABI5* expression appears to be regulated by ABA, by most of the other known *ABI* genes, and possibly by itself.

RESULTS

Fine Mapping *ABI5*

Our initial mapping of *ABI5* localized it to the lower arm of chromosome 2, near the phenotypic marker *pyrimidine-requiring* (*py*) (Finkelstein, 1994). To generate fine-mapping populations with closely linked recombinations, we outcrossed *abi5-1* and *abi5-3* (in the Wassilewskija and Columbia [Col] backgrounds, respectively) to lines carrying the *erecta* (*er*) and *py* mutations (in the Landsberg *erecta* [L*er*] background) and screened for recombinants with these phenotypically scored markers and the molecular marker nga168. Recombinant families were subsequently scored at a series of molecular markers to identify the region of chromosome 2 that was most tightly linked to *ABI5*. This enabled us to fine map *ABI5* to a region of ~150 kb contained within two bacterial artificial chromosomes (BACs), TAMU 19H20 and TAMU 25C22 (Figure 1). The BAC fingerprint database (http://genome.wustl.edu/gsc/arab/arabidopsis.html) revealed a match with two of the BACs being sequenced, IGF 2H17 and IGF 1011, and the sequence data were made publicly available at the Institute for Genomic Research website (http://www.tigr.org/tdb/at/atgenome/atgenome.html). Likely genes were predicted by using GenScan analysis (Burge and Karlin, 1997) via the Massachusetts Institute of Technology server (http://CCR-081.mit.edu/GENSCAN.html). Predicted amino acid sequences were then used for BLAST (Altschul et al., 1997) searches of all nonredundant protein sequences to identify possible functions of the predicted genes.

Of the >20 predicted genes within the region between the two closest recombinations, only one appeared to be a strong candidate: a member of the bZIP transcription factor family, now designated gene F2H17.12 (GenBank accession number AC006921.5, PID g4510349). Consequently, we focused our attention on this gene. RNA gel blot analysis showed that transcript levels for this gene were severely decreased in the *abi5-1* and *abi5-2* mutants (Figure 2).

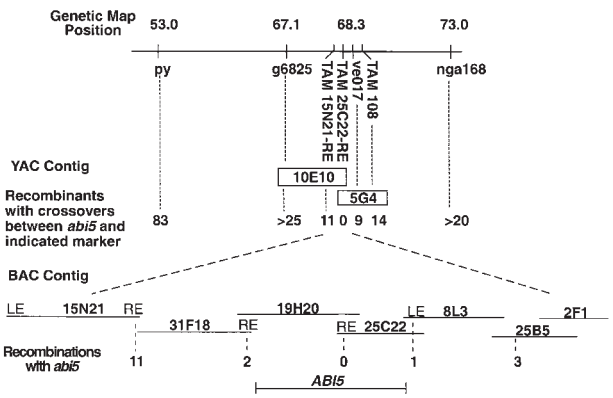

Figure 1. Fine Mapping of *ABI5* on Chromosome 2.

We screened five distinct mapping populations and isolated 106 recombinants across a 20-centimorgan interval surrounding *abi5*. Mapping results with a series of molecular markers across the interval are summarized schematically, indicating that *ABI5* was in a region of ~150 kb. The yeast artificial chromosome (YAC) clones indicated are from the CIC library; BAC clones are from the TAMU library. Markers shown to have >*n* recombinants either were not polymorphic or were scored in all mapping populations. Dashed lines denote the positions of the molecular markers used for mapping. LE, left end of BAC; RE, right end of BAC.

Identification of *ABI5*

After determining that the bZIP family member described above was underexpressed in some of the *abi5* mutants, we subcloned a 6-kb HindIII fragment encompassing only the bZIP gene and found that this fragment was sufficient to complement the mutation (Table 1). To determine the nature of the lesions in the mutants, we sequenced the mutant alleles. We found a single base pair change in the coding sequence of *abi5-1:* a G-to-T substitution at nucleotide 34,017 of IGF 2H17 (Figure 3A). This substitution resulted in an early translation termination such that the mutant protein lacked 81 C-terminal amino acids, including the conserved basic and leucine zipper domains required for DNA binding and dimerization.

In addition to the base change within the coding sequence, *abi5-1* has a small duplication that can be detected by DNA gel blot analysis. The duplicated region is contained within a 1-kb BglII-PstI fragment comprising 272 bp at the 5′ end of the coding sequence and an additional 758 bp extending into the promoter region (data not shown); the precise endpoints of the duplication are not known. However, the *abi5-1* genomic sequence is identical to that of its progenitor line for at least 1184 bp 5′ to the initiating codon, extending into a region beyond the BglII site delimiting the duplicated region. This indicates that the duplication is not immediately adjacent to the *ABI5* locus.

The *abi5-1* and *abi5-2* mutants have identical genomic rearrangements, indicating that they are probably siblings that were redistributed into independent T-DNA pools. Another allele, *abi5-3*, has a small rearrangement adjacent to the 5′ splice site of the final exon, extending from nucleotides 33,454 to 33,439 of IGF 2H17 (Figure 3A). Although the

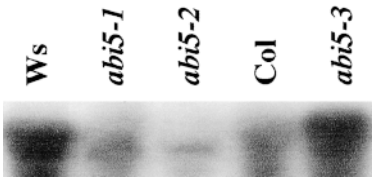

Figure 2. *ABI5* Transcript Levels in Wild-Type and *abi5* Siliques.

Each lane contains 15 μg of total RNA isolated from pooled stages of developing siliques. The hybridization probe spanned 826 bp of the *ABI5* coding region, starting at codon 63 and ending before the basic domain. Col, wild-type Columbia; Ws, wild-type Wassilewskija.

abi5-3 mutation results in a failure to splice the final intron, it does not reduce the transcript accumulation (Figure 2). The combination of identified sequence mutations and functional evidence from complementation indicates that this gene is indeed *ABI5*.

ABI5 Shows Homology to bZIP Domain Proteins

The annotated database submission and the sequence of several independent cDNA clones obtained by the 3′ rapid amplification of cDNA ends technique (Frohman, 1995) indicate that the *ABI5* gene is composed of four exons that encode a 442–amino acid protein (Figure 3). The first intron separates the regions that encode the basic and leucine zipper portions of the bZIP domain; the leucine zipper is assembled from sequences spread over the next two exons (Figure 3B). The 3′ ends of the cDNA clones are heterogeneous, indicating that any of several possible polyadenylation signals can be used.

Comparison of the predicted ABI5 amino acid sequence with those of other gene products in databases showed that ABI5 shares greatest sequence similarities with the bZIP class of proteins. The protein most similar to ABI5 (62% of predicted amino acids similar or identical) was a member of the Dc3–promoter binding factor family (DPBF-1) from sunflower embryos (Kim et al., 1997). The predicted amino acid sequence of TRAB1 (for transcription factor responsible for ABA regulation), a rice protein that interacts with VP1 (Hobo et al., 1999), was 55% similar to that of ABI5. Somewhat weaker similarity of predicted amino acid sequences was observed in comparisons with other members of the DPBF family (53 to 55% similar) (Kim and Thomas, 1998), with another rice seed transcription factor (OSE2; 44% similar) (GenBank accession number U25283), with an Arabidopsis G-box binding factor thought to participate in light-regulated transcription (GBF4; 46% similar) (Menkens and Cashmore, 1994), and with two predicted bZIP factors (50 to 59% similar) whose genes were discovered during sequencing of the Arabidopsis genome.

Whereas overall amino acid similarity with the closest homolog, DPBF-1, was only 62%, the highly conserved bZIP regions of ABI5 and DPBF-1 are 96% similar (Figure 4). This domain is thought to be involved in DNA binding and potential dimerization of bZIP transcription factors (reviewed in Hurst, 1995). Additional conserved domains are present at both 5′ and 3′ of the bZIP domain, and three of six predicted serine/threonine phosphorylation sites (Woodget et al., 1986; Pinna, 1990) are located in these conserved regions. Therefore, we hypothesize that the ABI5 protein is also a transcription factor and may be the Arabidopsis ortholog of DPBF-1. Consistent with this hypothesis, *ABI5* is identical to *AtDPBF-1*, a clone that was isolated on the basis of hybridization to *DPBF-1* (T. Thomas, personal communication). An *ABI5* cDNA has not appeared in the Arabidopsis expressed sequence tag collection, but that is consistent with its presence as a low-abundance transcript.

To determine whether *ABI5* is likely to have more closely related family members than those present in the sequence databases, we performed reduced stringency DNA gel blot hybridizations of genomic DNA. The hybridization probe was a region of the coding sequence that excluded the sequence encoding the highly conserved portion of the bZIP domain. This probe hybridized very weakly, regardless of stringency, to only four to seven fragments other than the one containing *ABI5*, indicating that few Arabidopsis genes are homologous to *ABI5* beyond the region encoding the bZIP domain (data not shown). Both of the two predicted Arabidopsis genes identified by BLAST searches (GenBank accession numbers AC004261 and AL031032) contain four regions encoding conserved domains of 20 to 30 amino acids each (data not shown), which probably correspond to some of the weakly hybridizing fragments. When the Arabidopsis genome sequence is complete, it will be possible to definitively identify the closest homolog by searching the database. Whether any of the more weakly homologous

Table 1. Complementation of *abi5-1* Mutation by Transgenes

Line	Intact Genes Transferred	Kanamycin Resistance (%)[a]	Germination on 3 μM ABA (%)[a]
Ws	NA[b]	NA	0
abi5-1	NA	NA	90
bZIP-1a	*ABI5*	100	8
bZIP-1b	*ABI5*	88	0
bZIP-7a	*ABI5*	91	18

[a] Seeds from three independent primary transformants, which were isolated from two distinct seed lots, were plated on media supplemented with either 50 μg/mL kanamycin or 3 μM ABA. ABA-resistant germination was scored after 4 days; kanamycin resistance was scored after 10 days. The high percentages of kanamycin resistance are consistant with the presence of two independent transgene insertions that segregated in the T_2 generation.

[b] NA, not applicable.

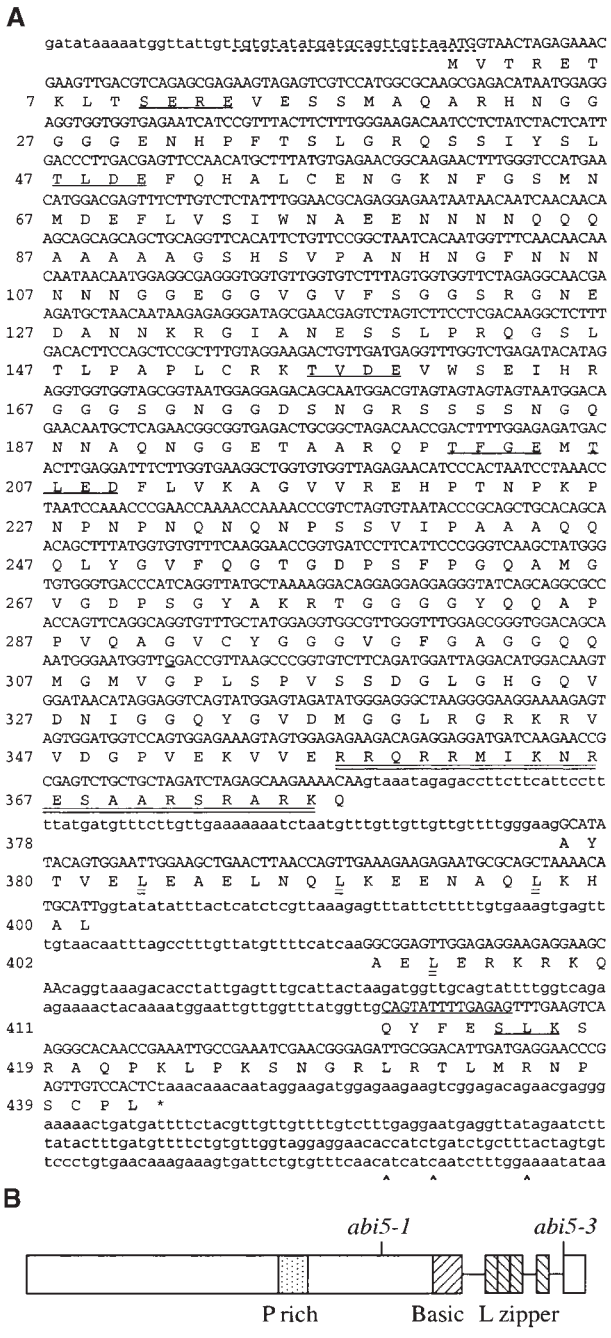

Figure 3. Sequence and Domain Structure of the *ABI5* Gene.

(A) The sequence displayed corresponds to the reverse complement of nucleotides 33,132 to 34,991 of BAC F2H17 (GenBank accession number AC006921.5). The coding sequence is shown in capital letters, with the predicted amino acid sequence below. Introns, identified by sequencing cDNAs, and untranslated regions are in lowercase letters. The basic domain and leucine repeats of the bZIP domain are double underlined, possible kinase recognition sites are single underlined, the position of the nested primer used for rapid amplification of cDNA ends is indicated by dashes, and the locations of poly(A)$^+$ tracts in three independent cDNAs are indicated by carets. Nucleotides altered in the mutants are underlined. The asterisk denotes the position of the stop codon.

(B) Domain structure of the ABI5 protein, including the bZIP (Basic L zipper) domain, a proline-rich (P rich) region, and the positions of the defects in two mutant alleles.

family members participate in the same regulatory processes as *ABI5* is not known. However, the *DPBF* subfamily of sunflower is also rather divergent outside the conserved basic domain, yet all members of this subfamily were identified by using a one-hybrid activation screen, indicating that they bind to the same promoter fragment and may be functionally redundant.

ABI5 Expression

As described earlier, our previous genetic and physiological studies suggested that *ABI5* expression was likely to be most abundant in seeds of wild-type plants and possibly regulated by ABA, *ABI3*, or *ABI4*. To test whether accumulation of *ABI5* transcripts fit these predictions, we compared *ABI5* transcript levels in developing siliques and dry seeds of a variety of genotypes and in vegetative tissues of wild-type plants (Figure 5). Although the hybridization probe included some of the conserved regions of the gene, the stringency used for RNA gel blot analyses produced gene-specific hybridization during DNA gel blot analyses. We found that the *ABI5* transcript is much more abundant in developing siliques than in vegetative tissue, with the greatest amounts being observed in desiccating and dry seeds (Figure 5A). A comparison of *ABI5* transcript levels in dry seeds showed that accumulation is diminished, to various extents, in ABA-deficient (*aba1-1*) seeds and all of the *abi* mutant seeds tested (Figure 5B). Consistent with this is the finding that ectopic expression of *ABI3* confers ABA-inducible vegetative expression of *ABI5* to levels even higher than those found in wild-type siliques (Figure 5C). These results indicate cross-regulation of *ABI5* expression by other known ABA response loci.

ABI5-Regulated Gene Expression

Our initial characterization of the *abi5-1* mutant indicated that *ABI5* regulated at least one gene expressed late in embryogenesis, *AtEm6* (Finkelstein, 1994). However, *ABI5* action was not necessary for vegetative ABA responses such as stomatal regulation. Having found that *ABI5* encoded a member of the bZIP family of transcriptional regulators and was expressed in both vegetative tissue and seeds, we compared gene expression in wild-type and *abi5-1* mutants at three ages: late embryogenesis, dry seed, and 13-day-old

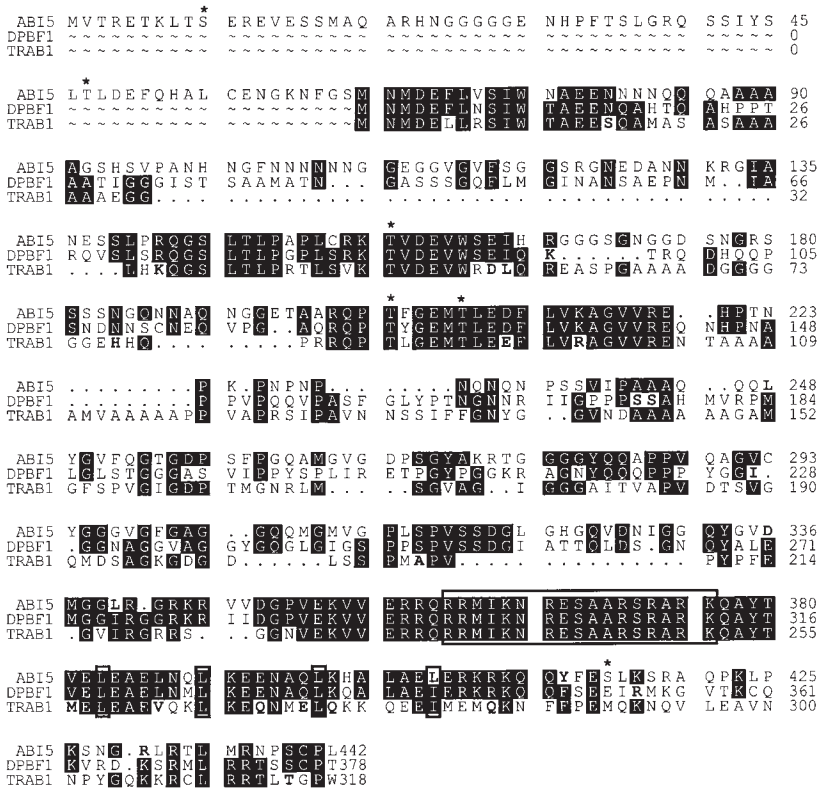

Figure 4. Comparison of ABI5 and Its Two Closest Homologs.

Alignment of the predicted amino acid sequences for ABI5 and its two closest homologs currently in the database, DPBF-1 and TRAB1. Alignment was done by the Pileup and PrettyBox programs. Identical residues are indicated by black boxes, and conservative substitutions are indicated in boldface. Potential phosphorylation sites are marked by asterisks; the canonical basic domain and the leucine repeats are outlined by boxes. Dashes denote positions of amino acids absent from the N termini of DPBF-1 and TRAB1.

plants (Figure 6). Of seven *LATE EMBRYOGENESIS ABUNDANT* (*LEA*) genes assayed, three were underexpressed in *abi5-1* plants (*AtEm1*, *AtEm6,* and the *LeaD34* homolog), three showed little or no change (the *RAB18*, *vicilin*, and *oleosin2* homologs), and one had substantially increased expression (M17) (Figure 6A). Of the two ABA-inducible genes assayed in young plants, only *AtEm1* appeared to depend on *ABI5* function for wild-type induction (Figure 6B). These results indicate that *ABI5* is important for regulation of some but not all *LEA* genes and that it may act as a positive or negative regulator, depending on the target gene. In addition, the low amount of vegetative *AB15* expression appears to be physiologically relevant because ABA induction of vegetative *AtEm1* expression is decreased in the *abi5-1* mutant.

DISCUSSION

Regulation of seed development and ABA signaling has been analyzed by using biochemical and genetic approaches. Many transcription factors correlated with seed-specific and ABA-responsive gene expression have been identified biochemically (reviewed in Busk and Pages, 1998). The strategies used have included identification of products of stress-induced transcripts and identification of factors that bind to *cis*-elements required for seed-specific or ABA-inducible gene expression. However, no available genetic evidence indicates whether most of these are required for the correlated response or any other ABA-regulated processes. In fact, although several of the bZIP family factors show similar DNA binding specificities in vitro, they are probably involved in transducing different signals. For example, ABA-responsive elements and sequences required for light regulation both contain an ACGT core, also known as the G-box. Consequently, at least one factor (GBF3) initially identified in a biochemical screen for regulators of light-induced expression was subsequently implicated in ABA-regulated expression (Lu et al., 1996).

Genetic studies have identified signaling elements required for seed maturation, ABA responses, or both. Several of these have now been shown to encode probable transcription

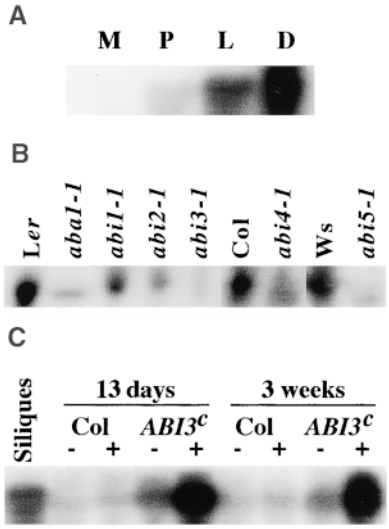

Figure 5. Expression of *ABI5*.

(A) Developmental time course of embryonic *ABI5* expression. RNA was isolated from maturation phase (M), postabscission (P), late embryogenesis (L), or dry seeds (D) of wild-type L*er* plants. Each lane contains 15 μg of total RNA.

(B) Comparison of *ABI5* expression in seeds of ABA-deficient (*aba*) or ABA-insensitive (*abi*) mutants and their respective wild-type backgrounds. RNA was isolated from dry seeds of the indicated genotypes. Each lane contains 5 μg of total RNA. Ws, Wassilewskija.

(C) *ABI5* expression in siliques compared with *ABI5* expression in untransformed and *EN35S*::*ABI3* (*ABI3*^{c}, for constitutive *ABI3*) vegetative tissue. Results with both control and ABA-treated tissue are shown. Each lane contains 15 μg of total RNA. RNA was isolated from pooled stages of developing siliques of wild-type Wassilewskija plants and from 13-day-old or 3-week-old plants. The plants used to compare Col and *ABI3*^{c} were incubated with (+) or without (−) 50 μM ABA for 48 hr before harvest. Comparable expression was observed in wild-type plants of the Col and C24 ecotypes (data not shown).

factors. These transcription factors include three members of the B3 domain family (*Vp1*, *ABI3*, and *FUS3*) (McCarty et al., 1991; Giraudat et al., 1992; Luerssen et al., 1998), a homolog of the CAAT-box binding factors (*LEAFY COTYLEDON1*) (Lotan et al., 1998), and an APETALA2 domain family member (*ABI4*) (Finkelstein et al., 1998). In this study, we report the positional cloning of the *ABI5* gene, which encodes a member of the bZIP domain transcription factor family.

A comparison with other members of the bZIP family shows four large blocks and several small blocks of homology between ABI5 and its closest homologs, DPBF-1 and TRAB1 (Figure 4). However, ABI5 differs from these proteins by the presence of a 64–amino acid N-terminal peptide that shows some homology to another predicted Arabidopsis bZIP factor (GenBank accession number AL031032). It is not clear whether this peptide is truly missing from DPBF-1 and TRAB1 or whether the available predicted amino acid sequences are based on less than full-length cDNA clones. In addition to the bZIP domain, the conserved regions include a proline-rich (33% of residues 221 to 241) domain that could function in transcriptional activation and three possible casein kinase II phosphorylation sites (Pinna, 1990) that could modulate ABI5 activity by changes in phosphorylation status. Several casein kinases have been identified from plants, and a variety of plant bZIP proteins have been shown to be either activated (e.g., GBF1) or inactivated (e.g., Opaque2) by phosphorylation of specific residues (reviewed in Schwechheimer et al., 1998). The conservation of the serine or threonine residues (or both) at the putative casein kinase II target sites suggests that these residues may be functionally relevant.

The leucine zipper domain of bZIP proteins is involved in dimerization, which precedes DNA binding and therefore affects the relative affinities for possible binding sites. The potential interactions of each bZIP protein are determined by the charge distribution of the ZIP region as well as the iden-

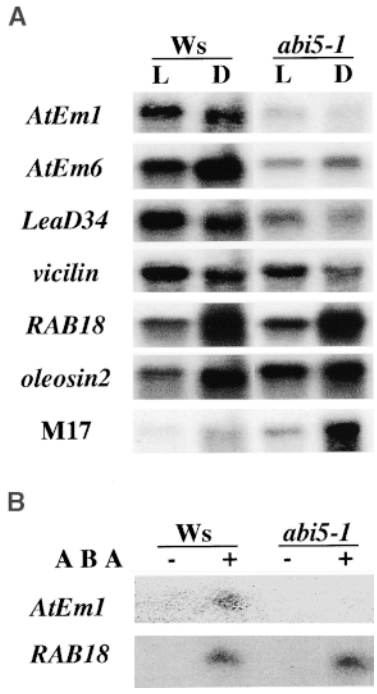

Figure 6. *ABI5*-Regulated Gene Expression.

RNA was hybridized sequentially with probes corresponding to genes of the identity or homology indicated at left.

(A) Comparison of *LEA* gene expression in late embryogenesis (L; 17 to 21 DPA) and mature dry seeds (D) of wild-type (Wassilewskija [Ws]) and *abi5-1* mutant plants.

(B) Comparison of ABA-inducible *LEA* gene expression in 13-day-old wild-type (Ws) and *abi5-1* plants incubated with (+) or without (−) 50 μM ABA for 48 hr before harvest.

tities of the available partners in any given tissue (Hurst, 1995). The residues present at the positions involved in stabilizing the ABI5 leucine zipper by interhelical salt bridges have a heterogeneous charge distribution, compatible with either homodimer or heterodimer formation. Consistent with function as a homodimer, the highly homologous DPBF-1 was identified by interaction with a target promoter fragment in a one-hybrid screen, where it presumably bound as a homodimer (Kim et al., 1997). In addition, DPBF-1 was shown to form heterodimers with DPBF-2. Identification of the *ABI5* and *AtDPBF* gene products, in combination with the availability of the *abi5-1* mutant, should allow us to determine whether ABI5 is truly the ortholog of DPBF-1 and whether the orthologs of the other DPBFs are functionally redundant in vivo, as implied by the similarity of their binding specificity.

RNA gel blot analyses (Figure 5) showed that *ABI5* expression is strongest during the later stages of embryogenesis. This differs from *ABI3*, which is expressed at a relatively constant level throughout embryo development (Parcy et al., 1994) and at low levels in some vegetative tissues (Rhode et al., 1999). Low-level *ABI5* expression was also observed in vegetative tissue. Thus, although the *abi5* mutations were initially characterized as having seed-specific effects (Finkelstein, 1994), *ABI5* expression is not seed specific. Consistent with this result, we have found that *ABI5* function is required for full induction of some LEA genes expressed at low levels in ABA-treated vegetative tissue. In addition, the ABA hypersensitivity conferred by ectopic *ABI3* expression in vegetative tissues appears to be partially mediated by hyperinduction of *ABI5* expression (Figure 5C; R.R. Finkelstein, unpublished observations). Comparison of *ABI5* transcript amounts in various mutant backgrounds also supports the hypothesis that *ABI5* expression is regulated by ABA and by most, if not all, of the known *ABI* genes. Products of three of these genes, *ABI3*, *ABI4,* and *ABI5* itself, are presumed to be transcription factors (Giraudat et al., 1992; Finkelstein et al., 1998) and could regulate *ABI5* expression directly. The other two genes, *ABI1* and *ABI2*, encode members of the serine/threonine protein phosphatase 2C family (Leung et al., 1994, 1997) and could regulate *ABI5* expression by altering phosphorylation and, concomitantly, activity of these or other transcription factors. Although specific substrates for ABI1 and ABI2 have not been identified, the cloning of genes demonstrated to interact genetically with *ABI1* and *ABI2* should allow us to test for such interactions.

Analysis of the *abi5-1* allele indicated that it encodes a truncated product lacking the bZIP domain required for dimerization and DNA binding. This suggests that the *abi5-1* gene product is probably inactive unless it can interact with other classes of transcriptional regulators through any of the domains remaining in the truncated protein. However, probably very little ABI5-1 protein is present because the sibling alleles *abi5-1* and *abi5-2* have low *abi5* transcript levels. This could reflect autoregulation, as has been documented for numerous other transcription factors (e.g., DEFICIENS; Schwarz-Sommer et al., 1992), or the mRNA could be destabilized as a result of poor translation (reviewed in Abler and Green, 1996). The *abi5-3* allele has a small rearrangement at the 5′ splice site of the fourth exon. Sequence analysis of cDNA from the *abi5-3* mutant shows that it fails to splice the third intron and thereby replaces most of the amino acids encoded by the last exon with the intron-encoded product. Although this splice defect disrupts the coding sequence, it would be unlikely to eliminate function, which is consistent with the weak phenotype of this mutant. Consequently, the strong expression of the *abi5-3* allele does not rule out the possibility of autoregulation.

Unlike the severe alleles of *ABI3* (e.g., *abi3-4),* which fail to complete seed maturation and consequently produce green, desiccation-intolerant seeds that are very insensitive to ABA (Ooms et al., 1993), the *abi5-1* mutant has a relatively weak phenotype (Finkelstein, 1994). *abi5-1* mutant seeds are desiccation tolerant and weakly dormant, but they are only slightly resistant to ABA inhibition of germination, and their accumulation of embryonic or ABA-inducible transcripts is altered for only a subset of the transcripts. Although it is not clear why *ABI5* acts as a positive regulator of some genes but negatively regulates other coordinately expressed genes, there is ample precedent for specific bZIP factors to act as both positive and negative regulators (reviewed in Hurst, 1995). Further analysis of the promoter structures of various *ABI5*-regulated genes and identification of other transcriptional regulators controlling their expression could clarify the mechanism of this differential regulation.

The fact that the *abi5-1* mutant has a weak phenotype, even though the *abi5-1* allele is grossly underexpressed and encodes a transcription factor lacking its presumed DNA binding and dimerization domains, suggests that loss of *ABI5* function may be masked by the presence of proteins having at least partially redundant activity. There are a large number of candidates for the source of this redundancy. Many bZIP factors have been implicated in ABA-regulated seed gene expression, including EmBP-1, DPBF factors, and TRAB1 (Guiltinan et al., 1990; Kim and Thomas, 1998; Hobo et al., 1999). Other bZIP factors such as GBF3 have been implicated in ABA-regulated but not seed-specific gene expression (Lu et al., 1996). Conversely, the bZIP factor Opaque2 is required for endosperm-specific but not ABA-regulated zein expression (Müller et al., 1997); presumably, comparable factors are required for seed-specific but hormone-independent gene expression in Arabidopsis. In addition, transcription factors belonging to other protein families (e.g., ABI3, ABI4, and FUS3) have been shown to regulate many of the same genes as ABI5 (Baeumlein et al., 1994; Keith et al., 1994; Parcy et al., 1994, 1997; R.R. Finkelstein, unpublished observations). VP1 has been shown to interact with EmBP-1, TRAB1, and various other transcription factors (Hattori et al., 1992; Hill et al., 1996; Hobo et al., 1999), indicating that this coordinate regulation may involve direct interactions among disparate classes of transcription factors, as has been described for a variety of yeast and mammalian

transcriptional regulators (reviewed in Wolberger, 1998). The modular nature of promoters (e.g., Shen et al., 1996) also provides opportunities for different factors to bind adjacent sites independently, according to their recognition specificity. Altogether, gene expression in seeds appears to depend on the combinatorial action of a large number of transcription factors. This high degree of genetic redundancy could permit subtle variations in gene expression during seed set that might be critical for plants growing outside of the controlled environment of the laboratory.

METHODS

Plant Material

The abscisic acid (ABA)–insensitive *abi5-1* and *abi5-2* mutants were isolated from T-DNA insertion lines of the *Arabidopsis thaliana* ecotype Wassilewskija, as described previously (Finkelstein, 1994); the mutations did not cosegregate with the T-DNA insertions. The *abi5-3* mutant was isolated from a mutagenized population derived from fast-neutron–irradiated seeds of the *A. thaliana* ecotype Columbia (Col; obtained from Lehle Seeds, Round Rock, TX). The *EN35S*::*ABI3* transgenic line (isolate C7A19), which contains an ABI3 cDNA controlled by a double-enhanced cauliflower mosaic virus 35S promoter, was constructed as described by Parcy et al. (1994). Marker lines used for mapping were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus).

For germination assays scoring ABA sensitivity, 20 to 100 seeds per treatment were surface sterilized in 5% hypochlorite and 0.02% Triton X-100. Seeds were then rinsed three or four times with sterile water before plating on minimal medium (Haughn and Somerville, 1986) containing 0.7% agar and ABA (mixed isomers; Sigma) at 3 or 5 μM in 15 × 100-mm Petri dishes. For scoring kanamycin resistance, the medium included 0.5 × Murashige and Skoog salts (Murashige and Skoog, 1962), 1% sucrose, 0.05% Mes, and 50 μg/mL kanamycin. The dishes were incubated for 1 to 3 days at 4°C to break any residual dormancy, and then they were transferred to 22°C in continuous light (50 to 70 μE m^{-2} sec^{-1}).

For DNA isolation, plants were grown in pots of soil (a 1:1:1 mix of vermiculite, perlite, and peat moss) supplemented with nutrient salts at 22°C in continuous light or in 16-hr light/8-hr dark cycles; shoots and rosette leaves were harvested when the shoots began bolting. For RNA isolation from siliques or 3-week-old plants, plants were grown as described for DNA isolation. Siliques were harvested as a pooled mixture of developmental stages spanning the full period of embryogeny (i.e., all stages from flower buds to dry seeds) or as subpools corresponding to four developmental stages: maturation (8 to 11 days postanthesis [DPA]), postabscission (12 to 16 DPA), late embryogenesis (17 to 21 DPA), and dry seed (>21 DPA). Three-week-old plants were sprayed to runoff with 0.05% Triton X-100 supplemented with 0 or 50 μM ABA and then incubated another 2 days before harvest. Seedlings were grown aseptically on Murashige and Skoog medium (Murashige and Skoog, 1962) containing 1% sucrose and 0.55% agar for 11 days at 22°C in continuous light (50 to 70 μE m^{-2} sec^{-1}); then they were transferred to fresh Murashige and Skoog medium containing 1% sucrose, 0.7% agar, and 0 or 50 μM ABA for an additional 2 days before harvest. All tissues harvested for nucleic acid extraction were weighed, frozen in liquid nitrogen, and stored frozen at –70°C until extracted.

Isolation of Recombinant Plants

The *abi5* mutants were outcrossed to a Landsberg *erecta* (L*er*) marker line carrying the *er* and *py* mutations. Mapping populations were produced by selecting ABA-insensitive F_2 progeny and then screening the resulting F_3 families for recombinations with the markers *er* and *py*, which could be scored phenotypically. In addition, F_2 progeny of the *abi5-3* outcross were screened for recombination with nga168, a simple sequence length polymorphism that could be scored using polymerase chain reaction (PCR) (Bell and Ecker, 1994). To allow direct selection of recombinants between the *ABI5* and *PY* loci, we backcrossed an *abi5-1 py* recombinant to wild-type L*er*; ABA-insensitive F_2 progeny that were viable without a thiamine supplement were *abi5 PY* recombinants. Those with recombinations tightly linked to *ABI5* were identified by scoring their genotypes with the cleaved amplified polymorphic sequence (CAPS) marker m323a.

Restriction Fragment Length Polymorphism Analysis

Restriction fragment length polymorphism mapping was conducted with F_3 and F_4 recombinant families. Plant DNA was extracted (Jhingan, 1992), and ~2 μg was digested with an appropriate enzyme to distinguish between the parental DNAs. The digested DNA was fractionated according to size on an 0.8% agarose gel, denatured, and transferred to Zeta Probe (Bio-Rad) membranes as described previously (Finkelstein, 1993). Mapping probes included cosmid clones obtained from the Arabidopsis Biological Resource Center and bacterial artificial chromosome (BAC) ends derived from plasmid rescue or inverse PCR for right and left ends, respectively (Woo et al., 1994). Cosmid and plasmid DNA was isolated as described by Sambrook et al. (1989). DNA templates were labeled by random priming (Hodgson and Fisk, 1987). Filters were hybridized in 5 × SSPE (1 × SSPE is 0.15 M NaCl, 10 mM sodium phosphate, 1 mM EDTA, pH 7.4), 5 × Denhardt's solution (1 × Denhardt's solution is 0.02% each of Ficoll, PVP, and BSA), 0.5% SDS, and 200 μg/mL herring testes DNA (Sambrook et al., 1989) with a probe added at 10^6 to 4 × 10^6 cpm/mL.

CAPS Analysis

Two CAPS markers were used to fine map *ABI5*: m323a and veo17a. Map positions of these markers, as well as the primers and enzymes used to score them, were as described by E. Drenkard and F. Ausubel (http://www.arabidopsis.org/aboutcaps.html). Reaction and cycling conditions were as described by Konieczny and Ausubel (1993).

Simple Sequence Length Polymorphism Analysis

The microsatellite sequence nga168 (Bell and Ecker, 1994) is polymorphic between the Col and L*er* ecotypes, which are the genetic backgrounds for the *abi5-3* mutant and the marker line, respectively. DNA from F_2 individuals (10 to 50 mg of leaf tissue per plant) and F_3 or F_4 families was used as a template in PCR amplification of the satellite sequences. PCR mixtures contained ~10 ng of DNA, 2.5 pmol of each primer (MapPairs; Research Genetics, Huntsville, AL), 10 mM

Tris, pH 9.0, 50 mM KCl, 2 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, 200 μM deoxynucleotide triphosphates, and 0.25 units of Taq polymerase in a 10-μL reaction volume. Reaction products were size-fractionated by electrophoresis through a 6% polyacrylamide-Tris-borate-EDTA gel (Sambrook et al., 1989).

Construction of Clones for Complementation Studies

The 6-kb HindIII fragment from a complete HindIII digest of TAMU 19H20 was isolated from a gel and then purified with a QIAQuick Gel extraction kit (Qiagen, Chatsworth, CA). The pBIN19 (Bevan, 1984) binary plasmid vector DNA (~2 μg) was digested with HindIII and dephosphorylated. The digested BAC DNA was ligated to the dephosphorylated vector DNA and transformed into *Escherichia coli* DH5α cells. Transformants were selected on kanamycin/X-gal/Luria-Bertani agar plates; white colonies were screened for the presence of appropriate inserts by restriction mapping of plasmid DNA.

Construction of Transgenic Plants

abi5-1 plants were grown at a density of five to 10 plants per 5-inch pot under photoperiods of 14 hr of light and 10 hr of darkness to produce large, leafy plants. Plants were vacuum-infiltrated with an *Agrobacterium tumefaciens* culture carrying an appropriate plasmid essentially as described by Bent et al. (1994). Seeds were harvested from individual pots and plated on selection medium (0.5 × Murashige and Skoog salts, 1% sucrose, and 50 μg/mL kanamycin) to identify transgenic progeny. ABA sensitivity and antibiotic resistance were scored in the next generation.

RNA Isolation and Gel Blot Analysis

RNA was isolated from dry seeds, 13-day-old plants, and 3-week-old plants by hot phenol extraction as described previously (Finkelstein et al., 1985). Silique RNA was isolated by grinding the siliques to a fine powder in liquid nitrogen, followed by incubation for 1 hr at 37°C in 3 to 5 mL of extraction buffer (0.2 M Tris, pH 9.0, 0.4 M NaCl, 25 mM EDTA, 1% SDS, 5 mg/mL polyvinylpolypyrrolidone, and 0.5 mg/mL proteinase K) per gram of tissue. Proteins and polysaccharides were precipitated by incubation on ice with 18.3 mg/mL $BaCl_2$ and 150 mM KCl. After the mixture was cleared by a 10-min centrifugation at 9000*g*, RNA was isolated from the supernatant by LiCl precipitation. The pellets were washed in 2 M LiCl and then resuspended and reprecipitated with ethanol and sodium acetate before a final resuspension in Tris-EDTA. The RNA concentration was estimated from the absorbance of the suspension at 260 and 280 nm.

Total RNA (5 to 15 μg per lane) was size-fractionated on 1% agarose Mops–formaldehyde gels (Sambrook et al., 1989) and then transferred to Nytran (Schleicher and Schuell) membranes with 20 × SSPE used as blotting buffer. RNA was bound to the filters by UV cross-linking (120 mJ cm^{-2} at 254 nm). Uniformity of loading and transfer was assayed qualitatively by methylene blue staining of the filters (Herrin and Schmidt, 1988). The *ABI5* mRNA was detected by hybridization with a PCR product corresponding to ~60% of the 5′ part of the coding sequence (nucleotides 33,878 to 34,703 of IGF 2H17; GenBank accession number AC006921.5) and labeled by random priming to a specific activity of 10^8 cpm/μg (Hodgson and Fisk, 1987). Hybridization conditions included incubation in 7% SDS, 0.5 M sodium phosphate, pH 7.2, 1 mM EDTA, and 1% BSA for 16 to 24 hr at 65°C (Church and Gilbert, 1984) in a rotisserie oven (Hyb-Aid, Teddington, UK), with probe being added at 2×10^6 to 4×10^6 cpm/mL. Washing was performed with 40 mM sodium phosphate, pH 7.2, 5% SDS, and 1 mM EDTA, and then with 40 mM sodium phosphate, pH 7.2, 1% SDS, and 1 mM EDTA, with a final wash in 0.2 × SSC (1 × SSC is 0.015 M NaCl and 0.015 M sodium citrate) and 0.1% SDS. Each wash step was performed for 10 to 60 min at 65°C. Exposure times were 2 to 8 days. The *LEA* gene transcripts were detected by hybridization with cDNA clones designated PAP085 (*vicilin* homolog), PAP140 (*LeaD34* homolog), PAP147 (*oleosin2* homolog), PAP023 (*RAB18* homolog), and *AtEm1* (generous gifts of M. Delseny), and a genomic clone comprising *AtEm6* (Finkelstein, 1993). Hybridization conditions for the *LEA* transcript analyses were either as given above or were 50% formamide, 5 × SSPE, 5 × Denhardt's solution, 0.1% SDS, and 200 μg/mL DNA at 43°C in a Hyb-Aid rotisserie oven. Filters were washed twice at 60°C in 2 × SSC and 0.1% SDS and once at 60°C in 0.2 × SSC and 0.1% SDS for 10 to 60 min each.

DNA Sequence Analysis

Plasmid DNA containing cDNA clones was isolated by using QIAprep Spin Miniprep kits (Qiagen) and then dissolved in double-distilled water to be used as DNA templates for sequencing. PCR primers were designed to amplify 5′, internal, and 3′ regions of the *ABI5* gene from genomic DNA of the mutant alleles and their progenitor lines. DNA was sequenced on a model 310 DNA sequencer (ABI Prism; ABI, Foster City, CA) using BigDye Terminator mix (ABI).

Comparison of Predicted Amino Acid Sequences for ABI5 and Closely Related Basic Leucine Zipper Proteins

Predicted amino acid sequences for the indicated proteins were compared using the Pileup and Gap programs of the Genetics Computer Group (Madison, WI) sequence analysis software package. Overall percentage similarity to *ABI5* was calculated using the Gap program. GenBank accession numbers for the genes compared are given in parentheses: *ABI5* (AC006921), *DPBF-1* (AF001453), *TRAB1* (AB023288), *DPBF-2* (AF001454), *DPBF-3* (AF061870), *OSE2* (U25283), and *GBF4* (P42777).

3′ Rapid Amplification of cDNA Ends

Total RNA (5 μg) was used as the template for reverse transcription with a kit for 3′ rapid amplification of cDNA ends (Gibco BRL). After first-strand synthesis and RNase H treatment, the cDNAs were amplified by using the universal adapter primer and a gene-specific primer annealing 51 nucleotides 5′ to the start of the *ABI5* open reading frame. Amplified products were size-fractionated on a 1% agarose gel, blotted, and hybridized with an internal fragment of the *ABI5* gene. A nested primer, annealing 23 nucleotides 5′ to the presumed initiating codon, was used to amplify cDNAs from a plug of sized DNA corresponding to the region of hybridization. The amplification reactions contained 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 200 nM each primer, 200 μM each deoxynucleotide triphosphate, and 0.05 units/mL Taq polymerase. The polymerase was added after a 3-min incubation at 94°C for "hot start" amplification. Cycling conditions for the first round of amplification were 30 cycles of 45 sec at 94°C, 1 min at 46°C, and 2 min at 72°C. Cycling

conditions for the nested amplifications used a 57°C annealing step but were otherwise identical to those in the first round.

The cDNA ends were blunted by fill-in reactions with the Klenow fragment of DNA polymerase I, treated with T4 polynucleotide kinase as described by Sambrook et al. (1989), and then gel-purified with QIAQuick Gel extraction kit (Qiagen) according to the manufacturer's instructions. The cDNAs were ligated into pBluescript KS+ (Stratagene, La Jolla, CA) after digestion with EcoRV and dephosphorylation and then transformed into *E. coli* DH5α. Transformants were selected on ampicillin/X-gal/Luria-Bertani agar plates; white colonies were screened for the presence of appropriate inserts by restriction mapping of plasmid DNA.

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REFERENCES

Abler, M.L, and Green, P.J. (1996). Control of mRNA stability in higher plants. Plant Mol. Biol. **32,** 63–78.

Altschul, S., Madden, T., Schäffer, A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. **25,** 3389–3402.

Baeumlein, H., Misera, S., Luerssen, H., Koelle, K., Horstmann, C., Wobus, U., and Mueller, A.J. (1994). The *FUS3* gene of *Arabidopsis thaliana* is a regulator of gene expression during late embryogenesis. Plant J. **6,** 379–387.

Bell, C.J., and Ecker, J.R. (1994). Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics **19,** 137–144.

Bent, A., Kunkel, B., Dahlbeck, D., Brown, K., Schmidt, R., Giraudat, J., Leung, J., and Staskawicz, B. (1994). RPS2 of *Arabidopsis thaliana*: A leucine-rich repeat class of plant disease resistance genes. Science **265,** 1856–1860.

Bevan, M. (1984). Binary *Agrobacterium* vectors for plant transformation. Nucleic Acids Res. **12,** 8711–8721.

Black, M. (1983). Abscisic acid in seed germination and dormancy. In Abscisic Acid, F. Addicott, ed (New York: Praeger Publishers), pp. 331–363.

Burge, C., and Karlin, S. (1997). Prediction of complete gene structures in human genomic DNA. J. Mol. Biol. **268,** 78–94.

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

Church, G., and Gilbert, W. (1984). Genomic sequencing. Proc. Natl. Acad. Sci. USA **81,** 1991–1995.

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.

Finkelstein, R. (1993). Abscisic acid–insensitive mutations provide evidence for stage-specific signal pathways regulating expression of an *Arabidopsis* late embryogenesis-abundant gene. Mol. Gen. Genet. **238,** 401–408.

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

Finkelstein, R., and Somerville, C. (1988). Abscisic acid and high osmoticum promote accumulation of long-chain fatty acids in developing embryos of *Brassica napus*. Plant Sci. **61,** 213–217.

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., 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.

Frohman, M.A. (1995). Rapid amplification of cDNA ends. In PCR Primer: A Laboratory Manual, C.W. Dieffenbach and G.S. Dveksler, eds (Plainview, NY: Cold Spring Harbor Laboratory Press), pp. 381–409.

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.

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

Hattori, T., Vasil, V., Rosenkrans, L., Hannah, L., McCarty, D., and Vasil, I. (1992). The *viviparous-1* gene and abscisic acid activate the *C1* regulatory gene for anthocyanin biosynthesis during seed maturation in maize. Genes Dev. **6,** 609–618.

Haughn, G., and Somerville, C. (1986). Sulfonylurea-resistant mutants of *Arabidopsis thaliana*. Mol. Gen. Genet. **204,** 430–434.

Herrin, D., and Schmidt, G. (1988). Rapid, reversible staining of Northern blots prior to hybridization. BioTechniques **6,** 196–200.

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.

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.

Hodgson, C., and Fisk, R. (1987). Hybridization probe size control: Optimized "oligolabelling." Nucleic Acids Res. **14,** 6295.

Hurst, H. (1995). bZIP proteins. Protein Profile **2,** 105–168.

Jhingan, A.K. (1992). A novel technology for DNA isolation. Methods Mol. Cell. Biol. **3,** 15–22.

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.

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.

Kim, S., and Thomas, T. (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.

Konieczny, A., and Ausubel, F.A. (1993). A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J. **4,** 403–410.

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.

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.

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.

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.

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.

Menkens, A.E., and Cashmore, A.R. (1994). Isolation and characterization of a fourth *Arabidopsis thaliana* G-box-binding factor, which has similarities to Fos oncoprotein. Proc. Natl. Acad. Sci. USA **91,** 2522–2526.

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

Müller, M., Dues, G., Balconi, C., Salamini, F., and Thompson, R.D. (1997). Nitrogen and hormonal responsiveness of the 22 kDa α-zein and b-32 genes in maize endosperm is displayed in the absence of the transcriptional regulator Opaque-2. Plant J. **12,** 281–291.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15,** 473–497.

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.

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., Miséra, 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.

Pinna, L. (1990). Casein kinase-2—An eminence-grise in cellular regulation. Biochim. Biophys. Acta **1054,** 267–284.

Rhode, A., Van Montagu, M., and Boerjan, W. (1999). The abscisic acid–insensitive gene *ABI3* is expressed during vegetative quiescence processes in *Arabidopsis*. Plant Cell Environ. **22,** 261–270.

Rock, C., and Quatrano, R. (1995). The role of hormones during seed development. In Plant Hormones: Physiology, Biochemistry and Molecular Biology, 2nd ed., P.J. Davies, ed (Norwell, MA: Kluwer Academic Publishers), pp. 671–697.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Schwarz-Sommer, Z., Hue, I., Huijser, P., Flor, P., Hansen, R., Tetens, F., Lonnig, W.-E., Saedler, H., and Sommer, H. (1992). Characterization of the *Antirrhinum* floral homeotic MADS-box gene *deficiens*: Evidence for DNA binding and autoregulation of its persistent expression throughout flower development. EMBO J. **11,** 251–263.

Schwechheimer, C., Zourelidou, M., and Bevan, M.W. (1998). Plant transcription factor studies. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49,** 127–150.

Shen, Q., Zhang, P., and Ho, T.-H.D. (1996). Modular nature of abscisic acid (ABA) response complexes: Composite promoter units that are necessary and sufficient for ABA induction of gene expression in barley. Plant Cell **8,** 1107–1119.

Wolberger, C. (1998). Combinatorial transcription factors. Curr. Opin. Genet. Dev. **8,** 552–559.

Woo, S.-S., Jiang, J., Gill, B., Paterson, A., and Wing, R. (1994). Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. Nucleic Acids Res. **22,** 4922–4931.

Woodget, J.R., Gould, K.L., and Hunter, T. (1986). Substrate specificity of protein kinase C: Use of synthetic peptides corresponding to physiological sites as probes for substrate recognition requirements. Eur. J. Biochem. **161,** 177–184.

The Arabidopsis Abscisic Acid Response Gene *ABI5* Encodes a Basic Leucine Zipper Transcription Factor

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