

Lesions in the mRNA cap-binding gene *ABA HYPERSENSITIVE 1* suppress *FRIGIDA*-mediated delayed flowering in *Arabidopsis*

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Summary

Recessive mutations that suppress the late-flowering phenotype conferred by *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) and which also result in serrated leaf morphology were identified in T-DNA and fast-neutron mutant populations. Molecular analysis showed that the mutations are caused by lesions in the gene encoding the large subunit of the nuclear mRNA cap-binding protein, *ABH1* (*ABA hypersensitive1*). The suppression of late flowering is caused by the inability of *FRI* to increase *FLC* mRNA levels in the *abh1* mutant background. The serrated leaf morphology of *abh1* is similar to the *serrate* (*se*) mutant and, like *abh1*, *se* is also a suppressor of *FRI*-mediated late flowering although it is a weaker suppressor than *abh1*. Unlike *se*, in *abh1* the rate of leaf production and the number of juvenile leaves are not altered. The *abh1* lesion affects several developmental processes, perhaps because the processing of certain mRNAs in these pathways is more sensitive to loss of cap-binding activity than the majority of cellular mRNAs.

Keywords: flowering, leaf development, abscisic acid, winter annual, *FRIGIDA*, *FLC*.

Introduction

In many plant species, flowering is promoted by the sensing of environmental cues that accompany seasonal changes, such as changes in day-length or temperature. Within a given species, there can be variation in the response to environmental cues. In *Arabidopsis thaliana*, this variation has been well characterized. In rapid-flowering (summer-annual) accessions of *Arabidopsis* the major environmental cue for flowering is day-length; in these accessions the promotion of flowering occurs through a photoperiod pathway that perceives long days as inductive (Koornneef *et al.*, 1998a,b; Mouradov *et al.*, 2002; Simpson and Dean, 2002). At a molecular level, this pathway operates through a coincidence mechanism in which the presence of *CONSTANS* during the light period leads to the activation of genes involved in the initiation of flowering (Suarez-Lopez *et al.*, 2001; Valverde *et al.*, 2004; Yanovsky and Kay, 2002).

There are also winter-annual accessions of *Arabidopsis*. In these accessions flowering is promoted by prolonged exposure to cold temperature (vernalization) in addition to exposure to long days (for review see Michaels and

Amasino, 2000; Simpson and Dean, 2002). Winter annuals typically begin growing in the fall and, after exposure to the vernalizing temperatures of winter, flower early in the spring. A requirement for vernalization in winter annuals ensures that flowering does not occur prematurely.

In winter-annual accessions of *Arabidopsis*, the block to flowering in plants that have not been vernalized is largely due to the action of two genes, *FLOWERING LOCUS C* (*FLC*) and *FRIGIDA* (*FRI*) (Clarke and Dean, 1994; Koornneef *et al.*, 1994; Lee *et al.*, 1994). *FLC* encodes a MADS domain-containing transcription factor which is a repressor of flowering, and *FRI* acts to increase *FLC* expression to levels that inhibit flowering (Johanson *et al.*, 2000; Michaels and Amasino, 1999; Sheldon *et al.*, 1999). Vernalization results in the repression of *FLC* expression, and this *FLC* repression is mitotically stable in the absence of cold. Rapid-flowering accessions of *Arabidopsis* appear to have arisen from winter annuals due to allelic variation at *FRI* and/or *FLC*. Specifically, many summer-annual types flower rapidly without vernalization because they lack an active allele of *FRI* and

thus have low levels of *FLC* expression (Johanson *et al.*, 2000; Michaels and Amasino, 2001) or have an allele of *FLC* that is not upregulated by *FRI* (Michaels *et al.*, 2003). Three genes have been identified, *VERNALIZATION 1* and 2 (*VRN1* and *VRN2*) and *VERNALIZATION INSENSITIVE 3* (*VIN3*), that are required to maintain the vernalization-mediated repression of *FLC*. *VRN1* encodes a B3 domain DNA-binding protein (Levy *et al.*, 2002) and *VRN2* encodes a protein related to polycomb-group proteins (Gendall *et al.*, 2001). *VIN3* encodes a PHD domain-containing protein that is involved in the initiation of vernalization-mediated modifications to *FLC* chromatin (Sung and Amasino, 2004).

In addition to the *VRN* genes, another group of genes that act to repress *FLC* expression are the autonomous-pathway genes *LUMINIDEPENDENS* (*LD*), *FLOWERING LOCUS D* (*FLD*), *FPA*, *FVE*, *FY*, and *FCA* (Koornneef *et al.*, 1998a,b; Michaels and Amasino, 1999; Sheldon *et al.*, 1999). However the autonomous-pathway genes are not likely to be involved in the vernalization response because autonomous-pathway mutants, which are delayed in flowering, are fully vernalization responsive (Michaels and Amasino, 2000). Autonomous-pathway mutations cause rapid-flowering types of *Arabidopsis* to behave like winter annuals because, although *FRI* activity is absent, the loss of any autonomous-pathway gene permits *FLC* to be expressed to levels that strongly inhibit flowering (Michaels and Amasino, 1999; Sheldon *et al.*, 1999).

The first positive regulator of *FLC*, *FRI*, was identified in studies of natural variation in flowering time as discussed above. Recently four additional positive regulators of *FLC* have been identified; mutations in these genes attenuate *FLC* expression. *FRIGIDA-LIKE 1* (*FRL1*) is a gene related to *FRI* that is required for *FRI* to upregulate *FLC* (Michaels *et al.*, 2004). *PHOTOPERIOD INDEPENDENT 1* encodes an ATP-dependent, chromatin-remodeling protein of the SWI2/SNF2 family (Noh and Amasino, 2003). *VERNALIZATION-INDEPENDENT 4* (*VIP4*) encodes a hydrophilic protein with repeated Trp-Asp (WD) motifs which is related to proteins in yeast and animals (Zhang and van Nocker, 2002; Zhang *et al.*, 2003). Mutations in *EARLY IN SHORT DAYS4* (*ESD4*), a gene that encodes a small protease of the SUMO class, cause a modest decrease in *FLC* levels (Murtas *et al.*, 2003; Reeves *et al.*, 2002).

Additional positive regulators of *FLC* are likely to exist and thus we initiated a screen for such regulators. In this paper, we report the characterization of several mutations in *ABSCISIC ACID HYPERSENSITIVE 1* (*ABH1*) that suppress the high levels of *FLC* expression typically found in an *FRI*-containing, winter-annual genetic background. Thus an *abh1* lesion converts a winter-annual type of *Arabidopsis* into one with rapid-flowering behavior. *ABH1* encodes the large subunit of the mRNA cap-binding complex. The loss of *ABH1* causes additional phenotypes such as serrated leaves and ABA hypersensitivity as previously reported

(Hugouvieux *et al.*, 2001, 2002) and, in non-inductive photoperiods, an increase in the number of cauline leaves (i.e. bracts) on the elongating stem after the initiation of bolting.

Results

A lesion in ABH1 suppresses the late-flowering phenotype conferred by FRI and FLC

The Columbia (Col) accession of *Arabidopsis thaliana* is rapid flowering because it lacks a functional allele of *FRI*. Introgression of an active *FRI* allele from the San-Feliu (Sf-2) accession into Col (to create the Col *FRI*-Sf2 line as described in Lee *et al.*, 1994) increases expression of *FLC* and thus converts Col into a winter annual that is severely delayed in flowering in the absence of vernalization. (This Col *FRI*-Sf2 line will be referred to here as Col *FRI*.) In a screen of both fast-neutron and T-DNA-mutagenized lines of Col *FRI*, five mutants that were early-flowering without vernalization, and which exhibited the additional phenotype of serrated leaves, were identified (Figure 1a,b and Table 1). Thus, these mutations cause a conversion of the winter-annual phenotype of Col *FRI* into that of a rapid-flowering

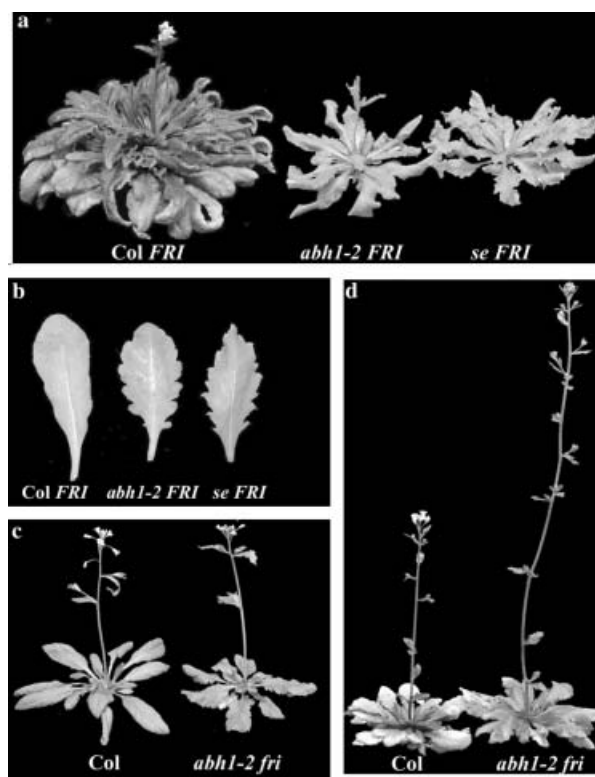


Figure 1. Phenotype of *abh1-2* and *se*. (a) Early flowering phenotypes of *abh1-2* and *se* in Col *FRI*. (b) Leaf morphology of *abh1-2* and *se* in Col *FRI* (fourth leaf shown at full expansion). (c) Col and *abh1-2 fri* grown in long days. (d) Col and *abh1-2 fri* grown in short days.

Table 1 Leaf number (rosette and cauline leaves formed by the primary meristem) of long-day and short-day grown plants. Each value is the mean \pm 1 SD. A minimum of 10 plants from each genotype was analyzed

Genotype ^a	Long days		Short days	
	Rosette	Cauline	Rosette	Cauline
<i>ABH1 FRI</i> (Col <i>FRI</i>)	71.3 \pm 1.5	8.6 \pm 0.6	>100	ND
<i>abh1-2 FRI</i>	18.0 \pm 1.4	7.6 \pm 0.6	50.5 \pm 4.4	19.0 \pm 1.1
<i>abh1-3 FRI</i>	17.3 \pm 1.2	7.0 \pm 1.8	52.8 \pm 2.6	21.6 \pm 2.7
<i>abh1-4 FRI</i>	17.3 \pm 1.6	7.0 \pm 1.0	44.8 \pm 6.1	21.5 \pm 1.8
<i>abh1-5 FRI</i>	14.0 \pm 0.9	3.5 \pm 1.4	53.0 \pm 4.2	23.7 \pm 2.1
<i>abh1-6 FRI</i>	18.5 \pm 0.5	7.8 \pm 0.8	53.0 \pm 3.4	24.5 \pm 0.6
<i>ABH1 fri</i> (Col)	14.6 \pm 1.0	3.8 \pm 1.0	54.2 \pm 3.8	9.2 \pm 0.4
<i>abh1-2 fri</i>	12.3 \pm 0.8	2.7 \pm 0.8	32.2 \pm 2.6	15.6 \pm 1.6
<i>se ABH1 FRI</i>	27.8 \pm 2.5	15 \pm 1.3	50.7 \pm 3.0	20.3 \pm 4.0

ND, not determined.

^aAll genotypes are in the Col background.

line. Complementation tests (including the F2 generation) revealed that all five mutants were allelic (data not shown) and, as discussed below, the mutants were designated *abh1-2*, *-3*, *-4*, *-5*, and *-6* (Table 1).

A region of DNA flanking the T-DNA insertion of one allele (*abh1-5*) revealed that the T-DNA was inserted into a gene encoding the large subunit of the mRNA cap-binding protein complex (At2g13540). This gene is present in a single copy in the *Arabidopsis* genome, and has been previously named *ABH1* because the *abh1-1* mutant is abscisic acid (ABA) hypersensitive (Hugouvieux *et al.*, 2001, 2002). *abh1-2*, *-5* and *-6* were identified in T-DNA mutant populations. *abh1-2*, which was the allele used for most of the studies described below, did not contain a T-DNA insertion but rather had a G–A transition which destroys a splicing site for the first intron (Figure 2). *abh1-5* has a T-DNA insertion in the middle of exon 8 and *abh1-6* has a 14 base pair duplication in the last exon, which causes a C-terminal frameshift (Figure 2). *abh1-3* and *-4* were identified in fast neutron-mutagenized populations and these alleles were not further characterized.

Effect of the *abh1* lesion on rosette and cauline leaf number

In long days (LD; 16 h light/8 h dark) the *abh1* mutants in Col *FRI* were much earlier flowering than the parental Col *FRI* line (Table 1). The mutants initiated the elongation of an inflorescence stem (bolting) with only a slightly higher rosette leaf number compared with the rapid-flowering Col accession.

However, the elongated inflorescence stem (bolt) contained many additional nodes at which cauline leaves were formed [cauline leaves can be considered as bracts subtending coflorescence meristems (Ratcliffe *et al.*, 1998)]. The number of cauline leaves formed by the mutants in the Col *FRI* background in LD was more similar to the Col *FRI* parent than to Col (Table 1). To determine whether the increase in cauline leaf number was due to an interaction of *FRI* and *abh1*, *abh1-2* was introduced into Col, which naturally lacks an active *FRI* allele (Johanson *et al.*, 2000). In the Col background, the *abh1* lesion did not increase cauline leaf number; rather the *abh1-2* mutant (*abh1-2 fri*) flowered with slightly fewer rosette and cauline leaves compared with Col (Figure 1c and Table 1).

When the *abh1* mutants in Col *FRI* were grown in non-inductive short days (SD; 8 h light/16 h dark), conditions under which the parental Col *FRI* line flowers after the primary meristem has formed greater than 100 leaves, the mutants flowered with a primary rosette leaf number similar to Col (Table 1). However, as was the case in LD, the number of cauline leaves formed by the mutants in SD was much greater than the number formed by Col (Table 1). Thus in both LD and SD the *abh1* lesion suppresses most of the effect of *FRI* on rosette leaf number, but does not suppress the effect of *FRI* on the number of cauline leaves.

In SD in the Col background (i.e. lacking *FRI* activity), *abh1* formed fewer rosette leaves but had more cauline leaves than Col (Table 1). Thus in the Col background in SD, but not in LD, *abh1* increases the number of cauline leaves formed independent of *FRI*, and *abh1* affects rosette and cauline leaf numbers in opposite ways. This increase in cauline leaf number results in an inflorescence in which the flowers open later after bolting in *abh1* than in Col wild type (Figure 1d). The prolonged cauline leaf-forming phase of *abh1* inflorescence development under SD, but not LD, in the Col background is similar to that previously reported for *serrate* (*se*), another mutant with serrated leaves (Clarke *et al.*, 1999). *SE* encodes a zinc-finger protein that may be involved in chromatin modification (Prigge and Wagner, 2001).

Serrate can partially suppress the delayed flowering caused by *FRI*

The similarity of the phenotypes of *abh1* and *se* mutants led us to determine whether the *se* mutation could suppress the

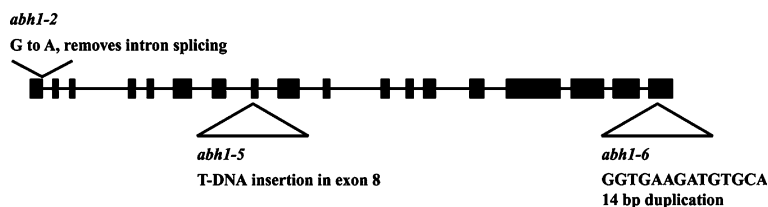


Figure 2. *ABH1* gene structure and position of the *abh1* mutations.

late-flowering of Col *FRI*. Accordingly, the *se* lesion was introduced into Col *FRI*. In LD, the rosette leaf number of Col *FRI* is reduced by the presence of *se*, but this suppression of

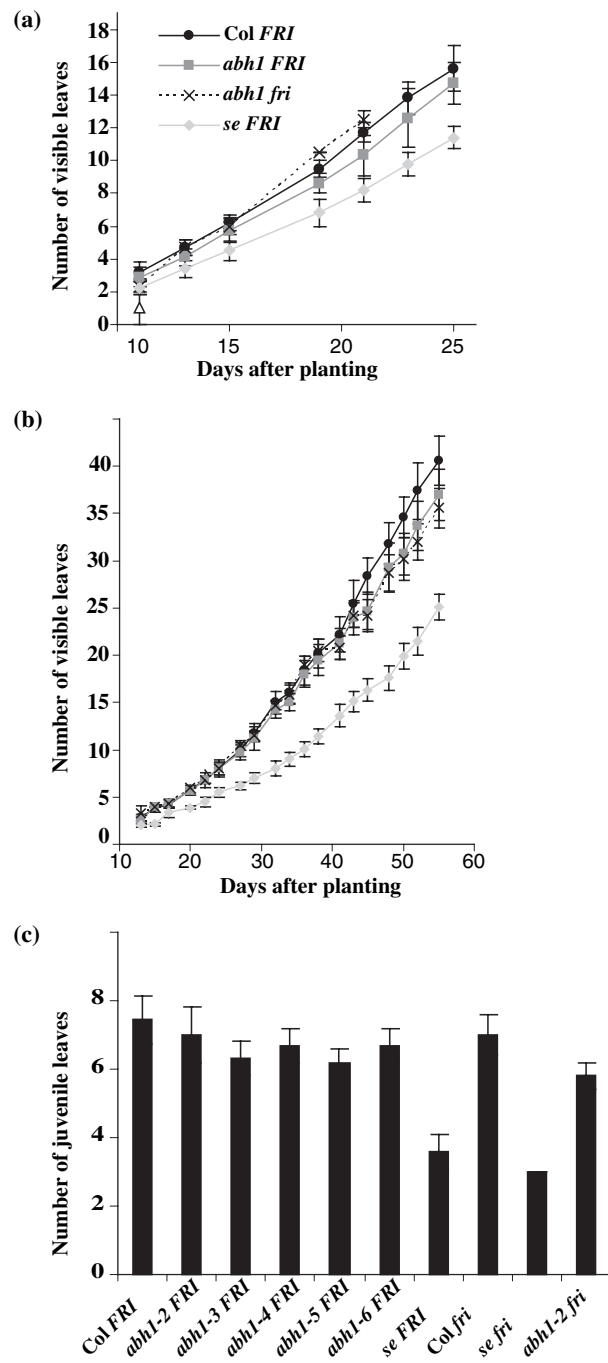


Figure 3. Rate of leaf emergence and juvenile phase length. (a) Comparison of leaf emergence rates *abh1-2* and *se* in Col *FRI* and *abh1-2* in Col in LD, and (b) in SD. Data points indicate the average number of visible leaves. (c) The number of juvenile leaves in *abh1* alleles and *se* in Col *FRI* and *abh1-2* and *se* in Col in LD. Data points depict the average number of juvenile leaves (defined as primary rosette leaves without abaxial trichomes). Error bars indicate the standard deviation ($n = 18-25$).

FRI-mediated late flowering conferred by *se* mutation is not as strong as the suppression caused by *abh1* (Table 1). In addition, the *se FRI* line produced even more cauline leaves than the Col *FRI* parent in LD which was not observed in the *abh1* alleles in an *FRI* background. In SD, the *se FRI* line behaved similar to the *abh1 FRI* lines with respect to leaf numbers at flowering (Table 1).

Effect of *abh1* on leaf emergence and phase change

The *se* mutant exhibits a slower rate of leaf production (Clarke *et al.*, 1999; Prigge and Wagner, 2001). Thus it was of interest to evaluate this parameter in *abh1*. In both LD and SD, there was little difference in the rate of leaf emergence in the parental Col *FRI* line compared with *abh1* with or without the presence of *FRI* (Figure 3a,b). In these conditions, *se* in Col *FRI* had a slower rate of leaf production similar to previous reports of the effect of *se* in Col (Clarke *et al.*, 1999; Prigge and Wagner, 2001).

SERRATE is also involved in phase change; in *se* mutants the number of juvenile leaves (i.e. leaves that lack trichomes on their abaxial surface) is reduced (Clarke *et al.*, 1999; Prigge and Wagner, 2001). Thus, whether phase change was altered by the *abh1* lesion was also examined. The *abh1* lesion does not affect the number of juvenile leaves formed (Figure 3c). Moreover, the presence of *FRI* did not affect the number of juvenile leaves formed in wild type, *abh1* or *se*. Thus although the presence of *FRI* causes a substantial delay in flowering in wild-type Col, *FRI* does not alter phase change. In addition, leaf phyllotaxy is altered in *se* (Clarke *et al.*, 1999; Prigge and Wagner, 2001), but it is normal in *abh1-2* in the presence or absence of *FRI* (data not shown).

FLC mRNA levels in an *FRI* background are reduced by *abh1*

The delayed flowering of the non-vernalized Col *FRI* line is due to the upregulation of *FLC* by *FRI* (Michaels and Amasino, 1999; Sheldon *et al.*, 1999). Thus it was of interest to determine whether the early-flowering phenotype of *abh1* in Col *FRI* was due to an effect on *FLC* mRNA levels. Indeed, *abh1-2* in Col *FRI* has reduced *FLC* mRNA levels when

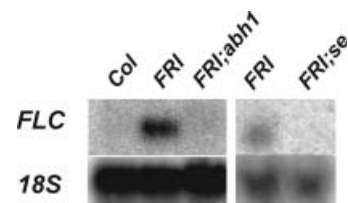


Figure 4. Expression of *FLC*. RNA blot analysis of Col, Col *FRI*, *abh1 FRI* and *se FRI*. Total RNA was isolated from seedlings and probed with *FLC* cDNA (not containing the MADS-box region). An *18S* probe was used as loading control.

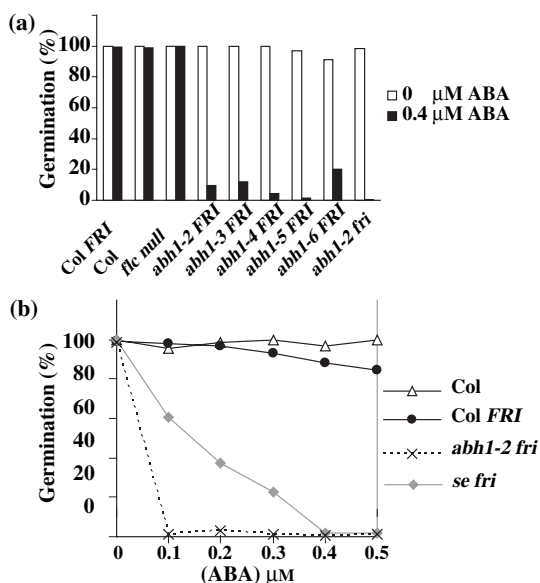


Figure 5. ABA hypersensitivity of *abh1* alleles.

(a) Percentage germination of *abh1* alleles and *se* in *Col FRI* and in *Col* after 4 days on medium containing 0.4 μM ABA. About 190–300 seeds were tested for each genotype. Similar results (not shown) were obtained with an independent batch of seed for each genotype.

(b) Percentage germination of *Col*, *Col FRI*, *abh1-2* and *se* after 4 days on medium containing either 0.1, 0.2, 0.3, 0.4, or 0.5 μM ABA. About 120–200 seeds were tested for each genotype. Similar results (not shown) were obtained with an independent batch of seed for each genotype.

compared with the parental *Col FRI* line, similar to that of *Col* (Figure 4); *se* is also a suppressor of the late-flowering phenotype, and the *se FRI* line also showed reduced levels of *FLC* (Figure 4). Thus, the suppression of the late-flowering effect of *FRI* by *abh1* and *se* appears to be due, at least in part, to the effect of these lesions on *FLC* expression.

ABA hypersensitivity is observed in all abh1 alleles and in se

Hugouvieux *et al.* (2001) reported that *abh1-1* exhibits ABA hypersensitivity. The additional *abh1* alleles presented in this study were also ABA hypersensitive with respect to the inhibition of germination (Figure 5a). Curiously, one allele, *abh1-6*, which has a C-terminal frameshift (Figure 2) was not as ABA hypersensitive as the other alleles in tests with two different lots of seeds, yet *abh1-6* was similar to other *abh1* alleles with respect to the suppression of *FRI*-mediated late flowering. The *se* mutant also exhibited some degree of ABA hypersensitivity although it was not as pronounced as that of *abh1*. (Figure 5b). The presence of *FRI* did not alter the ABA sensitivity of *abh1* alleles or *se* (Figure 5a).

Because *abh1* has reduced *FLC* levels, the ABA germination response of an *flc* null mutant was also examined (Figure 5a). The *flc* null behaved similar to wild type; thus

the effect of *abh1* on ABA sensitivity is independent of its effects on *FLC*.

Discussion

In a screen for mutants that cause a winter-annual type of *Arabidopsis* to exhibit a rapid flowering phenotype, we identified five alleles of *abscisic acid hypersensitive 1* (*abh1*). *ABH1* was first identified as a modulator of ABA sensitivity, and it encodes the large subunit of the eukaryotic nuclear mRNA cap-binding complex (Hugouvieux *et al.*, 2001). We find that loss of *ABH1* suppresses the late flowering associated with dominant alleles of *FRI*. Specifically, the *abh1* mutation causes a *FRI*-containing line (*Col FRI*) to initiate bolting after forming a number of rosette leaves comparable with a line that lacks *FRI* activity (*Col* wild type). *FRI* causes late flowering by increasing *FLC* expression (Michaels and Amasino, 1999, 2001; Sheldon *et al.*, 1999), and the *abh1* lesion suppresses the *FRI*-mediated increase in *FLC* mRNA levels. Preliminary results indicate that *abh1* does not suppress the late flowering of all autonomous-pathway mutants as effectively as it suppresses *FRI*-mediated late flowering.

The *abh1* suppression of the effects of *FRI* on rosette leaf number occurs in both inductive (LD) and non-inductive (SD) photoperiods. However in LD most of the *abh1* alleles in the *FRI* background produced almost as many cauline leaves as the *FRI*-containing parental line, whereas *abh1-2* in a *Col* background (i.e. lacking *FRI*) did not form an increased number of cauline leaves. Therefore, the *abh1* lesion *per se* does not cause an increased cauline leaf number in LD, but rather the lesion does not suppress the effect of *FRI* on cauline leaf number. *FRI*-containing lines produce high numbers of rosette and cauline leaves because *FRI* increases expression of the floral repressor *FLC* (Michaels and Amasino, 1999, 2001; Sheldon *et al.*, 1999). This differential effect of *abh1* on rosette and cauline leaf production might be due to differential effects on *FLC* expression at different stages of development and/or in different tissues. It is interesting to note that in SD the *abh1-2* mutation in a *Col* background actually forms more cauline leaves than *Col*. Thus in SD the loss of *ABH1* affects cauline leaf number independently of *FRI*.

The increase in cauline leaf number of the *abh1* mutant in SD, as well as the serrated leaf morphology first described by Hugouvieux *et al.* (2001), is reminiscent of the phenotype of the *serrate* (*se*) mutant (Clarke *et al.*, 1999). Thus, we evaluated whether *se* could act as a suppressor of *FRI*-mediated late flowering. Indeed, *se* partially suppressed the increase in rosette leaf number due to the presence of *FRI*, although the suppression was not as strong as that of *abh1*, and, like *abh1*, *se* did not reduce cauline leaf numbers in LD. The *se* mutant exhibits altered phyllotaxy, a slower rate of leaf emergence and a shortening of the juvenile phase (Clarke *et al.*, 1999; Prigge and Wagner, 2001). These

alterations are maintained when *se* is introduced into an *FRI*-containing line. However, *abh1* mutants do not share these characteristics of the *se* mutant; *abh1* mutants exhibit wild-type phyllotaxy, rate of leaf emergence and length of the juvenile phase.

A characteristic of the *abh1-1* allele is ABA hypersensitivity (Hugouvieux *et al.*, 2001). We find that the five additional *abh1* alleles described in this study are also ABA hypersensitive in a seed germination assay. This ABA hypersensitivity as well as the serrated leaf morphology is independent of the effect of the *abh1* lesion on *FLC* expression because loss of *FLC* does not affect either phenotype. Interestingly, the *se* mutant also exhibits ABA hypersensitivity, but the hypersensitivity of *se* to low levels of ABA (e.g. 0.1–0.2 μM ; Figure 5b) is not as strong as that of *abh1-2*.

Whether the common features of the *se* and *abh1* mutant phenotypes are due to a common molecular mechanism is not known. *SE* encodes a single C2H2-type zinc-finger protein that has been suggested to regulate in gene expression by modification of chromatin structure (Prigge and Wagner, 2001), whereas *ABH1* encodes the large subunit of the mRNA cap-binding complex. However, certain zinc-finger proteins function as RNA-binding proteins (Friesen and Darby, 1998; Iuchi, 2001) so it is possible that a common feature of RNA metabolism is affected in both mutants.

In eukaryotes, transcripts produced by RNA polymerase II possess a 7-methylguanosine cap structure attached by a 5'-5' phosphotriester linkage to the first encoded nucleotide of the transcript (Shatkin *et al.*, 1976). In the nucleus, this cap can be bound by cap-binding complex (CBC) which is a heterodimer consisting of a small subunit cap-binding protein (CBP20), and a large subunit, CBP80. The presence of CBC bound to the cap is thought to promote pre-mRNA splicing (Ohno *et al.*, 1987) and pre-mRNA 3' end formation by cleavage and polyadenylation (Flaherty *et al.*, 1997). Yeast mutants that lack the CBC do not have global defects in mRNA cleavage or polyadenylation (i.e. cbc mutants are not lethal), although such mutants are slower growing than wild type (Fortes *et al.*, 1999). In *Arabidopsis* *ABH1/CBP80* is a single-copy gene and thus our work and that of Hugouvieux *et al.* (2001) demonstrate that, similar to yeast, a loss of CBC is not lethal.

The distinct phenotypes of the *abh1* lesion in *Arabidopsis* may result from differential sensitivities of certain mRNAs to the lack of the CBC. Specifically certain mRNAs may not be properly processed in the absence of the CBC and these mRNAs might encode key regulators of certain pathways (such as the those for leaf development, ABA signal transduction, or flowering). Indeed, previous studies in yeast (Fortes *et al.*, 1999) and plants (Hugouvieux *et al.*, 2001) demonstrated that levels of certain mRNAs are more sensitive than others to loss of the CBC. A previous study of *abh1* (Hugouvieux *et al.*, 2001) did not reveal a change in *FLC* mRNA levels or a substantial effect on flowering time in

the *abh1/cpb80* mutant; that is because this study was performed in the rapid-flowering Col background which lacks *FRI* activity and thus has low levels of *FLC* expression. In the *FRI*-containing background, which has high levels of *FLC* expression, the effect of the *abh1/cpb80* lesion on flowering time and *FLC* expression becomes apparent.

The mechanism by which loss of the CBC affects *FLC* mRNA levels is not known. It is possible that the effect is indirect and that the *abh1* lesion alters the expression of a key regulator of *FLC* such as *FRI*. Alternatively, CBC bound to the *FLC* mRNA cap may be required for high levels of *FLC* expression. In animals, the presence of CBC increases the rate of recognition and splicing of the cap-proximal intron (Lewis *et al.*, 1996). *FLC* contains a large first intron that may render the *FLC* pre-mRNA more sensitive to loss of CBC. Another possibility is that the specific regulation of *FLC* mRNA levels involves components that interact with a CBC-bound *FLC* mRNA or pre-mRNA. Among the regulators of *FLC* mRNA levels are three proteins with RNA-recognition motifs, FCA, FLK and FPA (Lim *et al.*, 2004; Macknight *et al.*, 1997; Schomburg *et al.*, 2001), and the presence of the CBC may modulate an interaction between the *FLC* mRNA and these regulators of *FLC*. Recent studies have also revealed the involvement of RNA-binding proteins in ABA signal transduction (Koiwa *et al.*, 2002; Lu and Fedoroff, 2000). Thus genes in pathways involving certain types of RNA-binding proteins may be most affected by loss of the CBC.

Experimental Procedures

Plant and growth conditions

The Col *FRI* line was previously described (Lee and Amasino, 1995); *se* seeds were obtained from ABRC (Ohio State University, CO, USA); *fca-9* seeds were kindly provided by Caroline Dean; *ld-1* seeds were previously described (Lee *et al.*, 1994); *fpa-7* was isolated in our laboratory from a T-DNA population. All genotypes used in this work are in the Columbia (Col) background. Plants were grown under cool-white fluorescent light (100 $\mu\text{mol m}^{-2} \text{sec}^{-1}$; bulbs from Sylvania, Danvers, MA, USA) at $22 \pm 1^\circ\text{C}$ in Fafard Germination Mix (Fafard Co., Agawan, MA, USA) and were fertilized with Dyna-Grow 7-9-5 fertilizer (Dyna-Grow Corp., San Pablo, CA, USA) 2 weeks after planting. Long-days (LDs) consisted of 16 h light and 8 h darkness, and short days (SDs) consisted of 8 h light followed by 16 h darkness.

Measurement of rate of leaf emergence and juvenile phase length

The rate of leaf emergence was evaluated by counting visible leaves at a series of time points throughout development as described by Telfer *et al.* (1997). Only leaves formed by the primary meristem were counted. Juvenile phase length was measured by counting the number of juvenile leaves which are defined as leaves that do not possess abaxial trichomes (Telfer *et al.*, 1997).

Fast-neutron and T-DNA mutagenesis

Fast-neutron mutagenized lines were obtained as previously described (Michaels and Amasino, 1999). For T-DNA mutagenesis, Col *FRI* plants were transformed using *Agrobacterium tumefaciens* containing the binary vector pSKI015 (Weigel *et al.*, 2000) and T₂ plants were screened for flowering time.

Cloning of ABH1 gene

The genomic DNA sequence flanking the T-DNA of *abh1-5* was obtained by the thermal asymmetrical interlaced (TAIL)-PCR method as previously described (Schomburg *et al.*, 2003). The location of the mutations on *abh1* alleles was determined by sequencing PCR products obtained by using *ABH1* (At2g13540)-specific gene primers.

RNA gel blot analysis

Total RNA was isolated from 15-day-old seedlings using TRIzol (Invitrogen, Carlsbad, CA, USA). Twenty micrograms of RNA was run in a 1.0% formaldehyde agarose gel (Sambrook *et al.*, 1989). RNA was transferred to a Hybond-N+ membrane according to the manufacturer's instructions. The membrane was probed with ³²P-dATP-labeled *FLC* cDNA fragment that did not contain the MADS-box domain (Michaels and Amasino, 2001). An 18S rRNA probe was used as RNA loading control.

ABA hypersensitivity assay

For seed germination assays on ABA plates, a minimal of two different seed lots were tested for each genotype and three replicates for each seed lot were performed. Approximately 100 seeds of each line were plated on minimal medium (0.25× Murashige and Skoog medium) containing 0.4 μM ABA. Plates were incubated for 4 days at 4°C and transferred to LDs. To avoid ABA breakdown, the light intensity was reduced by covering the plates with two layers of Kimwipes (Kimberly-Clark Co., Roswell, GA, USA). After 4 days, percentage germination was determined.

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