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An mRNA Cap Binding Protein, ABH1, Modulates Early Abscisic Acid Signal Transduction in *Arabidopsis*

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Summary

The plant hormone abscisic acid (ABA) regulates important stress and developmental responses. We have isolated a recessive *ABA hypersensitive* mutant, *abh1*, that shows hormone specificity to ABA. *ABH1* encodes the *Arabidopsis* homolog of a nuclear mRNA cap binding protein and functions in a heterodimeric complex to bind the mRNA cap structure. DNA chip analyses show that only a few transcripts are downregulated in *abh1*, several of which are implicated in ABA signaling. Consistent with these results, *abh1* plants show ABA-hypersensitive stomatal closing and reduced wilting during drought. Interestingly, ABA-hypersensitive cytosolic calcium increases in *abh1* guard cells demonstrate amplification of early ABA signaling. Thus, ABH1 represents a modulator of ABA signaling proposed to function by transcript alteration of early ABA signaling elements.

Introduction

The phytohormone abscisic acid regulates many physiologically important stress and developmental responses throughout the life cycle of plants. In seeds, ABA is responsible for the acquisition of nutritive reserves, desiccation tolerance, maturation, and dormancy (Marcotte et al., 1992; Koornneef et al., 1998). During vegetative growth, ABA is a central signal that triggers plant responses to various adverse environmental conditions including drought, salt, and cold stresses (Marcotte et al., 1992; Koornneef et al., 1998; Leung and Giraudat, 1998).

During drought stress, a rapid response mediated by ABA is stomatal closure. Stomata on the leaf surface are formed by pairs of guard cells whose turgor regulates stomatal pore apertures. ABA is synthesized in response to drought and induces stomatal closure via efflux of K^+ and anions from guard cells and removal of organic osmolytes (MacRobbie, 1998; Schroeder et al., 2001). ABA triggers cytosolic calcium ($[Ca^{2+}]_{cyt}$) increases in guard cells which regulate ion channels that control ion efflux and stomatal closure (Schroeder and Hagiwara, 1989; McAinsh et al., 1990; MacRobbie, 1998). ABA-induced stomatal closure is vital for plants to limit transpirational water loss during periods of drought.

Guard cells provide a well-suited system to characterize whether and by which mechanisms genes affect early ABA signal transduction. Two protein phosphatase type 2C (PP2C) mutations (*abi1-1* and *abi2-1*; Leung and Giraudat, 1998) and a protein kinase mutant (*aapk*) that dominantly disrupt early events in ABA signaling have been characterized (Armstrong et al., 1995; Pei et al., 1997; Li et al., 2000). Recessive mutations that affect ABA responses at the transcriptional level in seed germination and development have been identified encoding three transcriptional regulators, *ABI3* (Giraudat et al., 1992), *ABI4* (Finkelstein et al., 1998), and *ABI5* (Finkelstein and Lynch, 2000). However, thus far only one recessive mutant gene, *era1*, has been isolated that affects ABA signal transduction upstream of transcription factors (Cutler et al., 1996; Pei et al., 1998). Loss of ERA1 function, which encodes a β subunit of a farnesyl transferase, enhances ABA signaling and also affects several other signaling pathways and developmental programs (Pei et al., 1998; Yalovsky et al., 2000; Ziegelhoffer et al., 2000; Bonetta et al., 2000).

In the present work, we describe and characterize a novel recessive ABA hypersensitive *Arabidopsis* mutant, named *abh1*, which shows ABA hypersensitivity in early ABA signaling. The reported findings indicate a novel functional link between mRNA processing and modulation of early ABA signal transduction.

Results

Isolation of *abh1* Mutant

The *abh1* mutant was isolated from 3,000 activation-tagged *Arabidopsis thaliana* lines (>30,000 seeds) based on ABA hypersensitive inhibition of seed germination at 0.3 μM ABA, a concentration that allowed germination of wild-type (WT) seeds (Figure 1). In the absence of exogenous ABA, *abh1* seeds showed WT germination rates after preexposure to 4°C for 4 days (Figure 1). Southern blot analysis revealed a single T-DNA insertion in *abh1* plants (data not shown). WT and *abh1* plants were crossed. F1 seeds germinated at the same rate as WT seeds on plates containing 0.3 μM ABA. F2 seeds from 6 different F1 plants showed a segregation of the *abh1* phenotype of 273 to 780 corresponding to a segregation of about 1 to 3 ($\chi^2 = 0.50$, $P > 0.47$). ABA hypersensitive seeds were transferred to soil and all showed resistance to the herbicide BASTA and were homozygous for the T-DNA ($n = 12$) as determined by Southern blot analyses (data not shown). BASTA-resistant seedlings which germinated on 0.3 μM ABA were heterozygous for the T-DNA insertion ($n = 10$). These data suggested that the *abh1* mutation is recessive and segregates as a single nuclear locus linked to the BASTA resistance marker, indicating that *abh1* is a loss-of-function mutation caused by T-DNA insertion. The ABA content of WT and *abh1* plants were similar ($n = 3$), suggesting that ABH1 affects ABA sensitivity rather than biosynthesis (e.g., 0.18 and 0.16 μg/g ABA in seeds, and 0.14 and 0.12 μg/g dry weight in vegetative tissues for WT and *abh1*, respectively).

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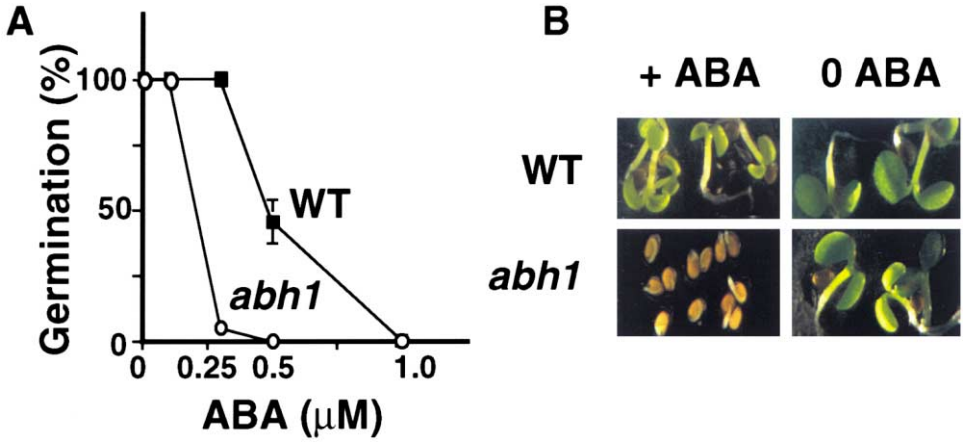

Figure 1. The *abh1* Mutant Shows ABA-Hypersensitive Inhibition of Seed Germination

(A and B) Comparison of germination rates of WT and *abh1* seeds after 5 days exposure to 0.3 μM ABA (+ ABA) or in the absence of ABA (0 ABA). Data represent the mean ± SD of 3 independent experiments (> 100 seeds were plated per line, per data point). Error bars are smaller than symbols when not visible.

ABH1 Gene Isolation and Complementation

A 278 bp genomic fragment adjacent to the right border of the T-DNA insertion was isolated from *abh1* plants using plasmid rescue. Primers were generated to amplify genomic DNA flanking the rescued sequence and to amplify a corresponding cDNA, named *ABH1* (accession number AF272891). A sequence from the *Arabidopsis* genome project (Lin et al., 1999) (accession number AC007063) was later deposited that showed identity to the *ABH1* sequence. The *ABH1* gene is located on chromosome II and consists of 18 exons (Figure 2A). Based on Southern blot (data not shown) and database search analyses, *ABH1* is a single gene in the *Arabidopsis* genome. The T-DNA in *ABH1* is inserted at the end of the 8th intron (Figure 2A). Northern blot analyses showed that *ABH1* transcript was absent in *abh1* but present in WT leaves (Figure 2B).

We transformed *abh1* plants with the *ABH1* gene under the control of its own promoter (*abh1*:*ABH1)* and with the *ABH1* cDNA under the control of the CaMV 35S promoter (*abh1*:35SABH1). Seeds from 3 independent homozygous *abh1*:*ABH1* lines showed WT germination rates in the presence of 0.3 μM ABA, showing *abh1* complementation (Figures 2C and 2D). Seeds from *abh1*:*35SABH1* lines also germinated at 0.3 μM ABA (Figures 2C and 2D), but with a delay of 3 days, suggesting that ectopic *ABH1* expression affects the ABA response.

ABH1 Encodes a Functional mRNA Cap Binding Protein

ABH1 encodes a protein of 848 amino acids with significant similarity to a specific class of eukaryotic nuclear RNA cap binding proteins named CBP80, thus far not described in plants. ABH1 shares 33.8% and 45% similarity with the yeast (P34160) and human (NP_002477) CBP80, respectively (data not shown). In humans and yeast, CBP80 is a subunit of a heterodimeric nuclear cap *b*inding *c*omplex (CBC), together with CBP20 (Lewis and Izaurralde, 1997). The yeast and human dimeric nuclear CBCs play important roles in mRNA processing (Izaurralde et al., 1994, 1995; Lewis et al., 1996; Flaherty et al., 1997), and in HeLa cells, CBC has been suggested to function in nerve growth factor and stress-activated signal transduction pathways (Wilson et al., 1999). An *Arabidopsis* CBP20 homolog (*AtCBP20*) was identified on chromosome V (AAD29697). AtCBP20 exhibits 57.8% and 72.5% similarity with the yeast (AAF21454) and human (CAA58962) CBP20, respectively (data not shown).

Yeast two-hybrid experiments were pursued to test whether ABH1 and AtCBP20 can physically interact. Figure 3A shows that the WT YRG2 yeast strain was able to grow on plates without histidine only when expressing both ABH1 and AtCBP20. These data indicate that ABH1 can interact with AtCBP20 and ABH1 may be a subunit of an *Arabidopsis* CBC.

Nuclear CBCs bind to the monomethylated cap (m^7GpppN) structure of RNA transcribed by RNA polymerase II (Izaurralde et al., 1994; Kataoka et al., 1995; Lewis et al., 1996). To examine whether ABH1 and AtCBP20 function as a cap binding complex, we carried out biochemical assays by testing cap binding activity from the YRG2 yeast strain expressing ABH1 and AtCBP20, or each subunit alone. Whole-cell extracts from YRG2 yeast cells expressing both ABH1 and AtCBP20 subunits showed mRNA cap binding activity in gel mobility shift assays (Figure 3B, lanes 2 and 6). This cap binding activity was not detectable in control YRG2 WT yeast strain extracts (Figure 3B, lane 1) or when only one of the two CBC subunits was expressed alone (Figure 3B, lanes 3 [ABH1] and 4 [AtCBP20]), showing that the detected cap binding activity requires the presence of both ABH1 and AtCBP20. Moreover, the cap binding activity was abolished when m^7GpppN cap structure was added as a competitor (Figure 3B, lane 5), but not when an ApppN cap analog was added (Figure 3B, lane 6). No binding activity was observed when an A-primed RNA was used as RNA probe (Figure 3B, lane 7). These results strongly suggest that ABH1 functions as a subunit of an *Arabidopsis* CBC.

Analysis of Plant Hormone Responses in *abh1*

We investigated effects of *abh1* on responses to other known plant hormones: auxin (NAA), cytokinin (BA),

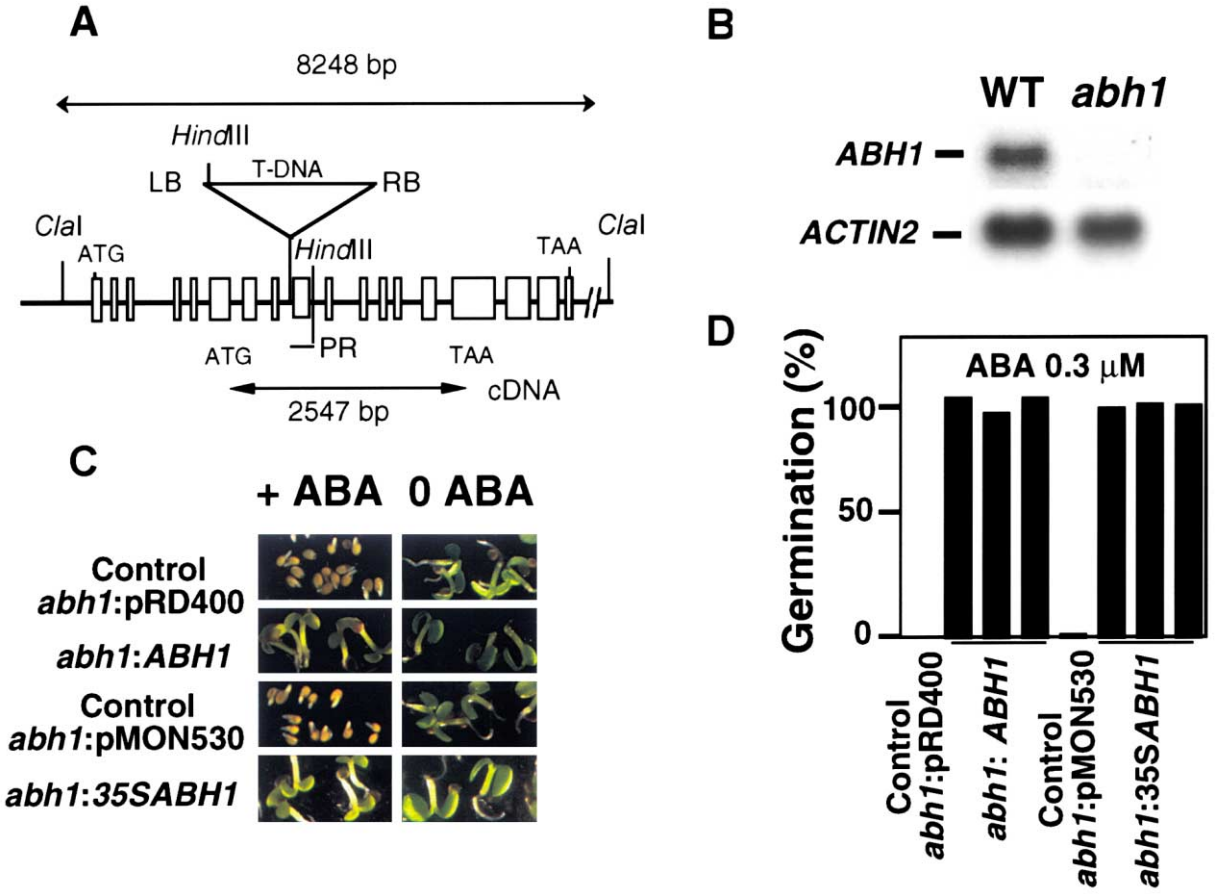

Figure 2. *ABH1* Gene Isolation and Complementation of *abh1*

(A) Map of *ABH1* gene (AC007063) and corresponding cDNA (AF272891). Hind III sites used during plasmid rescue (PR) are shown. Exons are represented by boxes.

(B) Northern blot analysis of WT and *abh1* leaf poly(A$^+$) mRNA (2 μg) probed with radiolabeled *ABH1* cDNA. Densitometry analysis (NIH Image Program) of the *ABH1* hybridization signal showed a reduction of at least 290 times in *abh1*. *ACTIN2* probe was used as a loading control.

(C and D) *ABH1* gene and cDNA complement the *abh1* hypersensitivity to ABA. Seeds (n > 100 per experiment) of 3 independent *abh1* transgenic lines expressing the *ABH1* gene (*abh1:ABH1*) or cDNA (*abh1*:35S*ABH1*) germinated in the presence of 0.3 μM of ABA, whereas vector (pRD400 and pMON530) transformed *abh1* lines did not. Germination was scored after 5 days (*abh1:ABH1*) and 8 days (*abh1:35SABH1*).

methyl jasmonate (MeJ), brassinosteroid (BR), ethylene (using the precursor 1-aminocyclopropane-1-carboxylic acid [ACC]), and gibberellic acid (GA) (using GA and the GA biosynthesis inhibitor tetcyclacis [TET]). For positive controls, known hormone signaling mutants that show cross-talk among hormones were analyzed in parallel

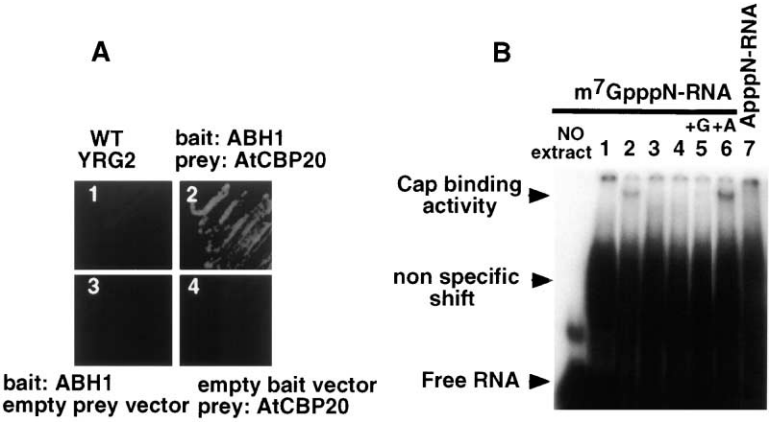

Figure 3. ABH1 Together with AtCBP20 Shows mRNA Cap Binding Activity

(A) Yeast two-hybrid experiment shows ABH1 and AtCBP20 interaction. Protein interactions were analyzed using a growth assay without histidine in the YRG2 background strain. Yeast expressing ABH1 as bait and an unknown prey showed no growth on histidine (not shown).

(B) Whole cell extracts from yeast WT strain (YRG2, lane 1) or yeast expressing both ABH1 and AtCBP20 (lanes 2 and 5–7), ABH1 alone (lane 3), or AtCBP20 alone (lane 4) were used for gel mobility shift assays as described (Kataoka et al., 1995) with either m^{7}GpppN (lanes 1–6)- or ApppN (lane 7)-primed radiolabeled RNA. Competitor (200 μM) m7GpppN (+G, lane 5) or ApppG (+A, lane 6) cap analogs were added. Experiments showed the same results in 4 replicates for all lanes.

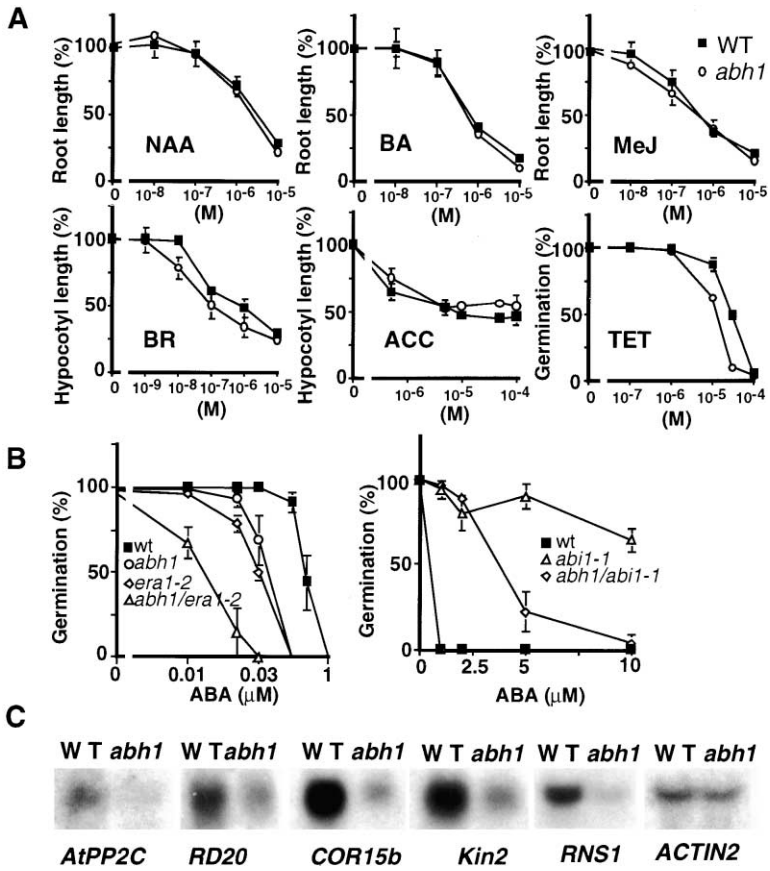

Figure 4. Hormone Responses of *abh1* Mutant and Epistasis Analyses

(A) Effects of known plant hormones on *abh1* (open circles) and WT (filled squares). The hormones analyzed were auxin (NAA), cytokinin (BA), methyl jasmonate (MeJ), epibrassinosteroid (BR), ethylene (using precursor ACC), and gibberellic acid (GA) (using the GA biosynthesis inhibitor tetcyclacis, TET). Inhibition of root and hypocotyl growth is expressed relative to the mean growth without hormones.

(B) *abh1/era1-2* and *abh1/abi1-1* double-mutant analyses. Seeds were stratified for 8 days (left) and 4 days (right) (WT: ler and col-0). Dose-response curves in (A) and (B) show the mean ± SD of 3 replicates.

(C) Northern blot analysis performed on 20 μg total RNA extracted from *abh1* and WT showing the down-regulation of specific genes in *abh1* in accordance with gene chip data described in Table 1. Gene specific probes were amplified by PCR. *ACTIN2* probe was used as a loading control.

including *axr1-3* (auxin insensitive; Lincoln et al., 1990), *ein2-1* (ethylene insensitive; Alonso et al., 1999), *gai-1* (GA insensitive; Koornneef et al., 1985), *era1-2* (ABA hypersensitive; Cutler et al., 1996), and *jar1-1* (JA insensitive; Staswick et al., 1992) (data not shown). Inhibition of root growth in the presence of NAA ($P > 0.5$ at 10^{-6} M), BA ($P > 0.2$ at 10^{-6} M), and MeJ ($P > 0.7$ at 10^{-6} M) were similar in WT and *abh1* (Figure 4A). The inhibition of hypocotyl growth in the dark, in the presence of BR, showed a possible slight increased sensitivity in *abh1* (Figure 4A). However, the half-maximal inhibition of hypocotyl growth was statistically similar in WT and *abh1* ($P > 0.2$; 10^{-7} M). The hypocotyl triple response to ACC (represented as hypocotyl growth inhibition [Guzman and Ecker, 1990]) ($P > 0.5$ at 5 μM) was similar in WT and *abh1* (Figure 4A).

GA is antagonistic to ABA and promotes seed germination and therefore, mutants in either pathway can affect the sensitivity to both hormones (Nambara et al., 1992; Koornneef and Karssen, 1994; Bethke and Jones, 1998; Koornneef et al., 1998; Steber et al., 1998). Consistent with these findings, *abh1* showed a slight GA insensitivity in hypocotyl elongation (data not shown). GA biosynthetic inhibitors such as paclobutrazol and TET prevent germination (Karssen et al., 1989). Seed germination assays in the presence of TET showed a slight increase in the TET sensitivity of *abh1* ($P < 0.02$ at 30 μM) (Figure 4A), which correlates with the observed increased resistance to GA biosynthetic inhibitors in ABA-deficient and ABA-insensitive mutants (Koornneef et al., 1998). Consistent with the lack of dramatic pleiotropic hormone sensitivities, the *abh1* mutant showed only slightly slowed growth and moderately serrated leaves (presented later) that were restored to WT phenotypes in complemented lines (data not shown). No other visible pleiotropic whole-plant phenotypes were observed. Together, these data show that *abh1* enhances ABA sensitivity, shows a slight cross-talk with GA signaling, and shows no significant effects in responses to other known hormones.

Epistasis Analyses

Homozygous double mutants were generated between *abh1* and *era1-2* and *abi1-1* plants. ABA-induced inhibition of seed germination was enhanced in the *abh1/era1-2* double mutant compared to the response in homozygous *abh1* or *era1-2* alone (Figure 4B). The *abh1/abi1-1* double mutant showed reduced ABA insensitivity compared to the response in *abi1-1* alone (Figure 4B). These data suggest that ABH1 does not target the same protein(s) as the ERA1 farnesyl transferase in the ABA signaling pathway and showed that the dominant ABA insensitivity of *abi1-1* is partially suppressed by *abh1*.

DNA Chip and Northern Analyses of *abh1*

In yeast, *GCR3* (*CBP80* homolog) is a nonessential gene that is only necessary for the accumulation of certain transcripts and the processing of some pre-mRNAs (Uemura and Jigami, 1992; Fortes et al., 1999). Because no strong pleiotropic phenotypes were found for *abh1*

Table 1. Genes Showing Reduced mRNA Levels in *abh1* Identified by DNA Gene Chip Profiling

Accession number	Gene name	Product	Fold wild type/*abh1**^
D38109	*AtPP2C*	Phosphatase type 2C	3.4*^
AAD23000	*COR15b*	Cold regulated protein	3.9*^
X55053	*KIN2/COR6*	Cold regulated protein	3*^
U05206	*RNS1*	Ribonuclease	7.4*^^
AF033206	*AtPMEpcrB*	Putative pectin methyl esterase	3
AF144391		Thioredoxine like 5	3.2
CAB41717	*PEARLI1 like*	PEARLI 1-like protein	7.7
CAA17130		Putative protein	5.5
AAB80656	*RD20*	Putative Ca^{2+} binding EF-hand protein	4.2*^
L36246	*PSAA1*	Anoxia induced protein	4.1^^
AJ249794	*LOX3*	Lipoxygenase	3.9
AAC95214		Putative proline rich protein	3.9
L41244	*THI2.1*	Thionine	3.3
AAB71443		Unknown	4.9^^
AAD34693		Similar to cytochrome P450 (*Sinapis alba*)	4
AAD31061		P450 cytochrome monoxygenase	3.5
AB017977	*APS2*	Unknown	7.9
AAC002628		Similar to MADS Box transcription factor	3.1

* Confirmed by Northern blot (see Figure 4C).
^ Published inducible by ABA.
^^ Inducible by ABA (V.H., J.I.S., data not shown).

plants (Figures 4A and 5C, presented later), we pursued DNA chip experiments to compare genomic-scale mRNA levels between WT and *abh1* and to determine which genes show the most reduced transcript levels in *abh1*. Among over 8000 genes represented on the chips, only 0.2% ($n = 18$) genes showed a significantly lower level of transcript accumulation in *abh1* with an expression ratio at least 3-fold lower in *abh1* versus WT (Table 1). Furthermore, only 13 genes showed a significantly higher expression level in *abh1* versus WT with an expression ratio ≥ 3 (data not shown). Among the 18 genes showing the most reduced transcript levels in *abh1*, 7 genes are induced by ABA in the WT (Table 1) (Gilmour et al., 1992; Wilhelm and Thomashow, 1993; Rodriguez et al., 1998; Foster and Chua, 1999). The down-regulation in *abh1* of the most highly expressed genes in the WT (*AtPP2C*, *RD20*, *KIN2*, *COR15b*, and *RSN1)* was confirmed by Northern blot analyses (Figure 4C). The functions of *KIN2* and *COR15b* remain unknown. Other genes down-regulated in *abh1* include the Ca^{2+} binding protein RD20 (Takahashi et al., 2000), the stress related *RSN1* gene (MacIntosh et al., 2001), and several genes implicated in oxidative responses (Table 1), which correlate to recent studies linking oxidative responses and ABA signaling (Bueno et al., 1998; Pei et al., 2000; Guan et al., 2000; Vranova et al., 2000). Interestingly, the *PP2C* encoding gene, *AtPP2C*, has been characterized as a negative regulator of ABA signaling based on overexpression analyses (Sheen, 1998). Gene chip analyses show a specificity of the *abh1* mutation in modulating transcript levels of a limited number of genes.

ABA Hypersensitive Stomatal Closure in *abh1*

WT transgenic seedlings expressing the GUS reporter gene under the control of the *ABH1* promoter showed that the *ABH1* promoter was active in guard cells (Figure 5A) and other vegetative tissues (V.H., unpublished data). Northern blot analysis on total RNA extracted from guard cell-enriched leaf epidermal strips further confirmed the expression of *ABH1* in guard cells (Figure 5B).

Experiments were pursued to determine whether *abh1* affected whole plant phenotypes linked to stomatal movements. When plants were subjected to water stress, ABA content increased to similar levels in WT and *abh1* in two independent experiments (1.33 and 1.26 μg/g of dry weight [experiment 1] and 1.05 and 1.26 μg/g [experiment 2] in WT and *abh1*, respectively). After 3 weeks without watering, *abh1* rosette and cauline leaves remained green and turgid whereas WT leaves showed wilting (Figure 5C; 3 independent experiments, $n \geq 20$ *abh1* and WT plants per experiment). After 10 days of drought, *abh1* plants already showed enhanced stomatal closing compared to control watered *abh1* plants ($P < 0.01$); whereas WT plants did not ($P > 0.5$) (10 days drought, stomatal apertures: 1.14 ± 0.04 μm in *abh1*, $n = 60$ stomata; 1.41 ± 0.07 μm in WT, $n = 60$ stomata; watered controls: 1.25 ± 0.08 μm in *abh1*, $n = 60$ stomata; 1.42 ± 0.05 μm in WT, $n = 60$ stomata). These data suggest that drought-induced stomatal closing contributes to reduced desiccation and wilting of *abh1* leaves.

Guard cells provide a well-suited system to determine whether, how, and when a mutation modulates early ABA signal transduction. Stomata were opened by exposing plants for 12 hr to high humidity (95%). Under these conditions, stomatal apertures were similar in WT and *abh1* (WT 2.03 ± 0.19 μm (SEM), $n = 3$ experiments, 180 stomata; *abh1*: 1.92 ± 0.21 μm (SEM), $n = 3$ experiments, 180 stomata; $P > 0.38$). In the presence of 0.25 μM ABA, *abh1* stomatal apertures started to decrease and at 0.5 μM of ABA, stomatal closure was significantly enhanced compared to WT ($P < 0.001$) (Figure 5D). These data show ABA hypersensitivity of *abh1* in guard cells. When plants were not exposed to high humidity, *abh1* plants showed smaller apertures than WT, indicating responses to endogeneous ABA (unpublished data). The ABA hypersensitivity of *abh1* guard cells recorded at 0.5 μM ABA was complemented by the *ABH1* gene

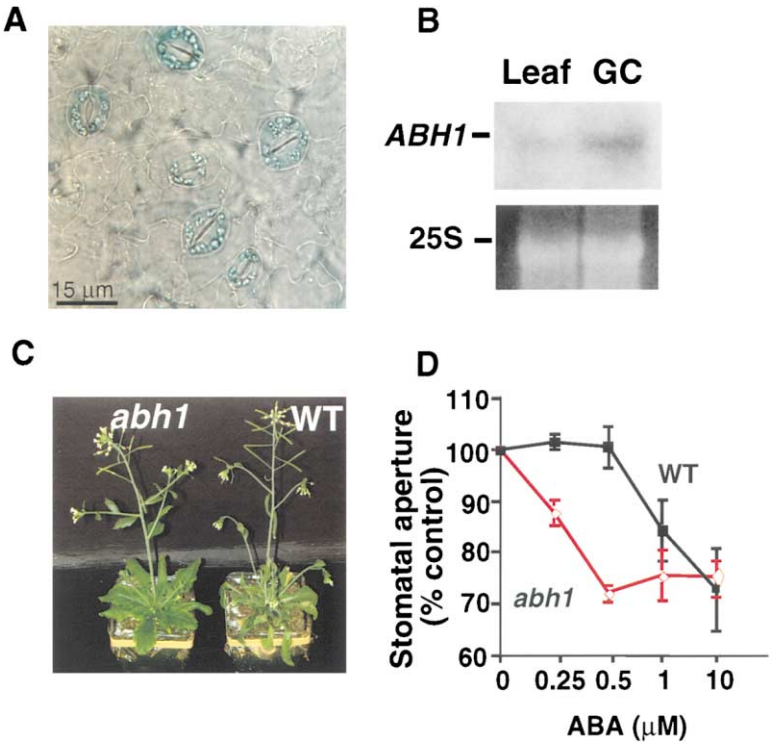

Figure 5. ABH1 Modulates Guard Cell ABA Signaling

(A) GUS activity in guard cells of WT plants expressing the GUS reporter gene under the control of the *ABH1* promoter.

(B) *ABH1* transcript detected by Northern blot analysis on 10 μg guard cell-enriched total RNA (GC). Ten μg of total RNA from whole leaves was run in parallel as control (Leaf).

(C) Reduced wilting during drought stress in *abh1* plants. Plants are shown after 3 weeks without watering. WT and *abh1* plants were grown under normal watering conditions in a growth chamber for two weeks. Plants selected at an identical developmental stage and size were subjected to drought stress by completely terminating irrigation and minimizing soil evaporation by covering pots with Saran Wrap.

(D) Stomatal closing is ABA hypersensitive in *abh1*. Data represent the mean ± SD of n = 3 independent experiments, 60 stomata per data point.

which restored the WT response (n = 60 stomata, 2 complemented lines, $P > 0.32$, data not shown).

ABA Hypersensitive Cytosolic Calcium Elevations in *abh1* Guard Cells

ABA-induced $[Ca^{2+}]_{cyt}$ elevations are presently among the earliest measurable ABA signaling events (McAinsh et al., 1990). Therefore, we directly investigated whether *abh1* modulates ABA-induced $[Ca^{2+}]_{cyt}$ elevations in time-resolved $[Ca^{2+}]_{cyt}$ imaging experiments (Allen et al., 1999b). We first investigated ABA-induced $[Ca^{2+}]_{cyt}$ elevations in guard cells at 0.5 μM, a hormone concentration that enhanced stomatal closing in *abh1* (Figure 5D). In WT, only 7% of the cells (n = 4 of 57) showed 3 to 4 $[Ca^{2+}]_{cyt}$ increases in response to 0.5 μM ABA during the 45 min of recording (Figure 6A bottom trace, 6C). In contrast, 30% of *abh1* cells (n = 19 of 64) responded by three or more repetitive $[Ca^{2+}]_{cyt}$ increases (Figure 6B bottom trace, 6C) with 13% (8 cells) showing between 5 and 8 transients. Furthermore in WT, 56% (n = 32 of 57) of guard cells showed no $[Ca^{2+}]_{cyt}$ increases in response to 0.5 μM ABA (Figure 6A top trace, 6C) versus only 36% in *abh1* (n = 23) (Figure 6B top trace, 6C). Note that even at saturating ABA concentrations a background rate of about 25% to 30% of guard cells showed no measurable ABA-induced $[Ca^{2+}]_{cyt}$ elevations (Figure 6C; Allan et al., 1994; Allen et al., 1999b). Statistical analyses demonstrated that the number of cells that showed $[Ca^{2+}]_{cyt}$ increases in response to 0.5 μM ABA was significantly increased in *abh1* versus WT guard cells ($\chi^2 = 4.96$, $P < 0.03$). Furthemore, the average ABA-induced peak $[Ca^{2+}]_{cyt}$ changes (*abh1*: 280 ± 22 nM; WT: 170 ± 25 nM; $P < 0.01$; Figure 6D) and the number of ABA-induced $[Ca^{2+}]_{cyt}$ transients ($P < 0.001$; Figure 6E) were significantly increased in *abh1* versus WT guard cells at 0.5 μM ABA.

At 5 μM ABA, a concentration that induces similar stomatal closure in both *abh1* and WT, the number of guard cells that responded to ABA was increased to 74% in WT (n = 14 out of 19; Figure 6C), and 70% in *abh1* (n = 14 out of 20) (Figure 6C). Statistical analyses showed that the number of guard cells that responded to 5 μM ABA was similar in WT and *abh1* ($\chi^2 = 0.069$, $P > 0.7$; Figure 6C). Furthemore, the average peak calcium increase ($P > 0.2$) and the number of $[Ca^{2+}]_{cyt}$ transients per cell were similar in WT and *abh1* at 5 μM ABA ($P > 0.5$, Figure 6E). $[Ca^{2+}]_{cyt}$ imaging analyses (Figure 6) and stomatal aperture measurements in response to ABA (Figure 5D) demonstrate that the *abh1* mutation enhances early signaling transduction upstream of ABA-induced $[Ca^{2+}]_{cyt}$ elevations.

Discussion

ABH1 is a novel type of modulator of ABA signaling in *Arabidopsis*. ABH1 modulates the ABA sensitivities of seed germination, of ABA-induced stomatal closing, of ABA-induced guard cell $[Ca^{2+}]_{cyt}$ elevations as well as whole plant transpirational water loss during drought. *ABH1* and *AtCBP20* gene homologs have not been previously described in plants and, as shown in gel mobility shift assays, ABH1 and AtCBP20 can function as subunits of an *Arabidopsis* CBC. Using DNA chip analysis, the *ABH1* mutation was shown to reduce the steady-state level of a limited number of mRNAs including ABA signaling-related genes, notably a reported negative regulator of ABA signaling and other candidates. Calcium imaging data demonstrate that ABH1 modulates

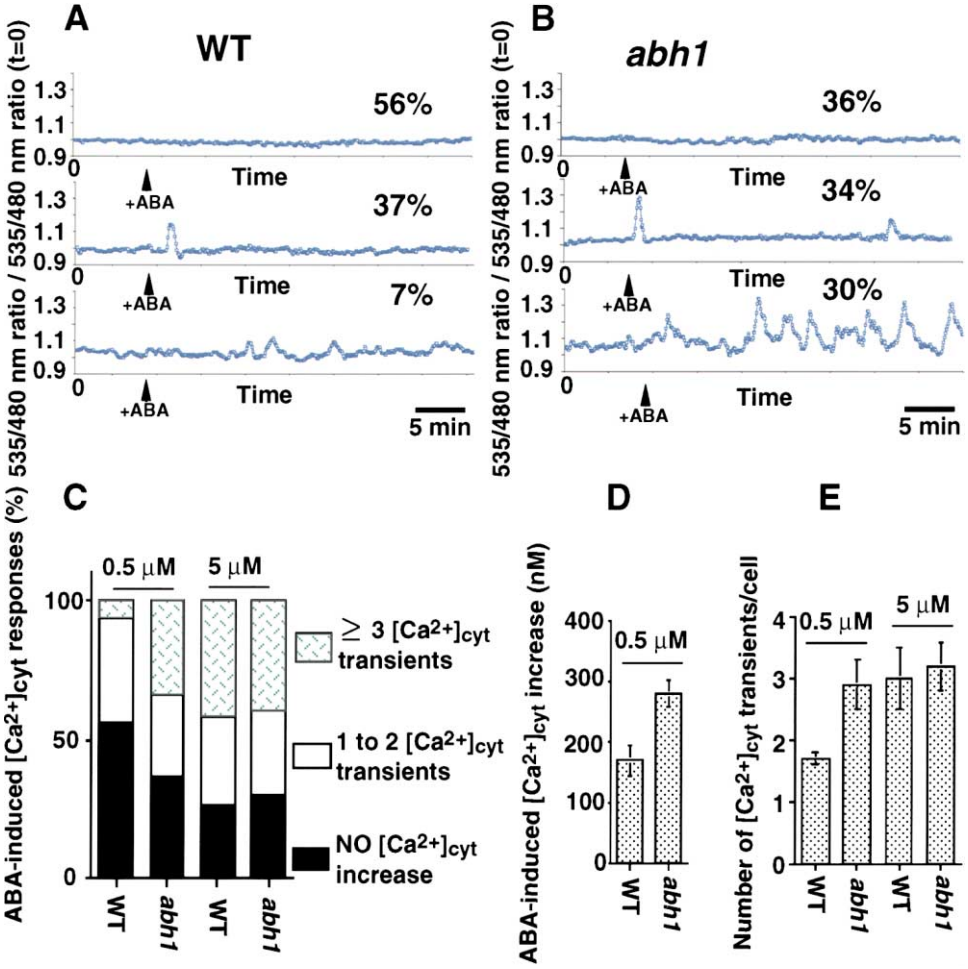

Figure 6. Enhancement of ABA-Induced $[Ca^{2+}]_{cyt}$ Elevations in *abh1* Guard Cells

(A) Fluorescence emission ratio (535/480 nm, normalized) of WT guard cells illustrating ABA-induced $[Ca^{2+}]_{cyt}$ elevation responsiveness at 0.5 μM ABA (n = 57 cells) (see text for details).

(B) Fluorescence emission ratio (535/480 nm, normalized) of *abh1* guard cells illustrating ABA-induced $[Ca^{2+}]_{cyt}$ elevation responsiveness at 0.5 μM ABA (n = 64 cells) (see text for details).

(C) Stack column representation of ABA-induced $[Ca^{2+}]_{cyt}$ increases in guard cells at 0.5 and 5 μM ABA.

(D) Average peak $[Ca^{2+}]_{cyt}$ increase in WT and *abh1* guard cells in response to 0.5 μM ABA.

(E) Average number of $[Ca^{2+}]_{cyt}$ transients in WT and *abh1* guard cells in response to 0.5 and 5 μM ABA. Cells showing no ABA-induced $[Ca^{2+}]_{cyt}$ transients were not included in analysis. Data represent the mean ± SEM.

early ABA signal transduction events. We have shown the *abh1* mutant to be a plant mutant that enhances stimulus-induced $[Ca^{2+}]_{cyt}$ elevations. These results show that ABH1 represents a novel mechanism for modulation of early ABA signal transduction.

ABH1 is a single gene in *Arabidopsis* and its mutation is not lethal and nonpleiotropic (Figure 5C). Similarly, loss of function in the only *CBP80* homolog, *GCR3* in *S. cerevisiae* is nonlethal (Uemura and Jigami, 1992). The whole plant *abh1* phenotype is not dramatically affected, showing a weak serrated leaf phenotype and a slightly slowed growth consistent with internal ABA hypersensitivity. Recently, several *Arabidopsis* mutants have illustrated that a mutation in one gene can affect responses to multiple hormones (Alonso et al., 1999; Ephritikhine et al., 1999; Beaudoin et al., 2000; Ghassemian et al., 2000; Lu and Fedoroff, 2000), suggesting cross-talk or feedback interactions of these loci with multiple signaling pathways. For example, the recently isolated *Arabidopsis* mutant *hyl1* shows a newly recognized cross-talk among ABA, cytokinin, and auxin signaling responses and shows several pleiotropic growth phenotypes (Lu and Fedoroff, 2000). Analyses of responses of *abh1* to other plant hormones showed that *abh1* responses were not distinguishable from WT responses (Figure 4A). These data are consistent with the whole plant phenotype of *abh1* (Figure 5C) and expression profiling data, suggesting that ABH1 has a nonpleiotropic function in mRNA processing linked to the plant stress hormone ABA.

Messenger RNAs transcribed with RNA polymerase II have a characteristic 5′ end consisting of an m^7GpppN-cap structure attached by a 5′-5′ phosphotriester linkage to the first nucleotide of the transcript that provides resistance to 5′-3′ exonucleases (Shatkin, 1976; Furuichi et al., 1977; Shimotohno et al., 1977; Gutierrez et al.,

1999). In yeast and HeLa cells, CBC can bind to the cap structure (Izaurralde et al., 1994; Kataoka et al., 1995; Lewis et al., 1996; Lewis and Izaurralde, 1997). The *S. cerevisiae* CBP80 homolog, GCR3, is required for expression of specific glycolytic genes (Uemura and Jigami, 1992) and a strain lacking CBC expression was found to be defective in the splicing of pre-mRNA that encodes specific ribosomal proteins (Fortes et al., 1999). However, the functions of the dimeric CBC in mRNA processing remain less well understood than other RNA processing mechanisms that control, for instance, mRNA stability (Gutierrez et al., 1999) and the initiation of translation by the cytosolic cap binding protein eIF4E (Shatkin, 1985; Sonenberg and Gingras, 1998). The CBP80/20 complex appears to have more than one function and has been implicated in several aspects of mRNA metabolism including pre-mRNA splicing in yeast and HeLa cells (Izaurralde et al., 1994; Lewis et al., 1996; Fortes et al., 1999), 3′ end formation (Flaherty et al., 1997), and RNA export in HeLa cells (Izaurralde et al., 1995). More recently, CBC has been suggested to function in growth factor and stress signaling in HeLa cells (Wilson et al., 1999) and to interact with the translation machinery in yeast (Fortes et al., 2000).

Genes showing reduced transcript levels in *abh1* represent putative targets for ABH1-dependent mRNA processing as shown in yeast (Fortes et al., 1999). It is possible that ABH1 modulates another protein(s) which in turn regulates the identified transcripts (Table 1). DNA chip experiments showed that only 18 genes had significant and 3-fold reduced transcript levels in the mutant, and 7 of these genes are ABA-regulated in the WT (Table 1). These data together with the relatively nonpleiotropic phenotype of *abh1*, suggest that further analysis of *abh1* down-regulated genes could lead to the identification of new negative regulators of early ABA signaling.

Interestingly, the PP2C, *AtPP2C*, was down-regulated in *abh1* (Table 1) and has been proposed to function as a negative regulator of ABA signaling based on overexpression studies (Sheen, 1998). The down-regulation of a negative regulator of ABA signaling could contribute to the ABA hypersensitivity of *abh1*. Dominant mutations in genes encoding the *ABI1* and *ABI2* PP2Cs were shown to disrupt ABA-induced calcium elevations (Allen et al., 1999a). We demonstrate that ABA induction of $[Ca^{2+}]_{cyt}$ increases is ABA hypersensitive in *abh1* (Figure 6). The down-regulation of *AtPP2C* in *abh1* may therefore contribute to the ABA hypersensitive induction of calcium elevations in guard cells.

Additional genes showing reduced transcript levels in *abh1* may function in early ABA signaling. For example, the *RD20* gene was recently shown to be induced by drought and ABA in *Arabidopsis*, and the recombinant RD20 protein is a Ca^{2+} binding protein (Takahashi et al., 2000; Seki et al., 2001). *RSN1* transcript, which is down-regulated 7.4-fold in *abh1* (Table 1) was recently shown to complement the yeast mutant *rny1* (MacIntosh et al., 2001). RSN1 was proposed to function in membrane permeability and stress responsiveness (MacIntosh et al., 2001). Several other genes with reduced transcript levels in *abh1* (Table 1: AF144391, L36246, AJ249794, AAD34693, and AAD31061) are predicted to function in regulating oxidative stress, which correlates to recent studies that show a role of reactive oxygen species in early ABA signaling (Guan et al., 2000; Pei et al., 2000).

ABA-induced repetitive $[Ca^{2+}]_{cyt}$ elevations in guard cells precede and mediate stomatal closing (Gilroy et al., 1990; McAinsh et al., 1990; Schroeder and Hagiwara, 1990; Grabov and Blatt, 1998; Allen et al., 1999a; Staxen et al., 1999). Repetitive $[Ca^{2+}]_{cyt}$ elevations have recently been shown to be necessary for mediating steady-state stomatal closure (Allen et al., 2000). We show that the *abh1* mutation enhances the probability of ABA-induced repetitive $[Ca^{2+}]_{cyt}$ elevations in guard cells at 0.5 μM ABA, a concentration that shows enhanced stomatal closure in *abh1*. Calcium imaging and stomatal movement data demonstrate that *abh1* amplifies early ABA signaling.

The isolation and characterization of *abh1* provides genetic evidence that an mRNA cap binding protein regulates the strength of ABA signaling in plants. ABH1 could provide a new control mechanism for manipulating the ABA responsiveness of crop plants during stress.

Experimental Procedures

Isolation of *abh1* Mutant and Generation of Double Mutants

Arabidopsis thaliana seeds (ecotype Columbia) transformed with pSK1015 T-DNA containing the BAR gene that confers resistance to the herbicide BASTA (Weigel et al., 2000) were plated on minimal medium (0.25× Murashige & Skoog medium [MS]) containing 0.3 μM ABA. After 4 days at 4°C, seeds were transferred to 28°C and continuous light. After 5 more days, nongerminated seeds were transferred to soil. Seeds used for comparative studies were from plants grown and harvested simultaneously. *abh1* was crossed with *era1-2* and *abi1-1*. Homozygous double mutants were isolated from F2 seeds based on PCR assays (Leung et al., 1997).

Growth Conditions

Plants were grown side-by-side in growth chambers: 40% humidity, 16 hr light/8hr dark cycle, temperature 23°C, and photon fluency rate of 80 μmol m^{-2} s^{-1}. To test the sensitivity of *abh1* seedlings to hormones other than ABA, seedlings were grown on vertical plates containing 1× MS medium, supplemented with 0.1% sucrose and the indicated hormones for 8 days. Sensitivity to NAA (α-naphthalene acetic acid, Sigma), BA (6-benzylamino purine, Sigma), or MeJ (methyl jasmonate, Aldrich) was determined by measuring root growth inhibition (Lincoln et al., 1990; Staswick et al., 1992; Cary et al., 1995). Sensitivity to brassinosteroid was studied by measuring the inhibition of hypocotyl growth with epibrassinolide (Sigma) of seedlings grown in the dark (Ephritikhine et al., 1999). Sensitivity to ethylene was studied using the precursor ACC (Sigma) by measuring the inhibition of hypocotyl elongation of seedlings grown in the dark (Guzman and Ecker, 1990). GA (Sigma) or the GA biosynthesis inhibitor (TET, BASF A.G.) was used to investigate the effect of GA on seed germination on 1× MS medium (Karssen et al., 1989).

Determination of ABA Content

ABA content was kindly determined by Dr. Jan Zeevaart (Michigan State University) using 1 g of seeds and two separate batches of watered or dehydrated plants as described (Schwartz et al., 1997).

ABH1 Cloning and Plant Transformation

A 8248 bp ClaI genomic fragment containing *ABH1* locus was cloned from BAC T10F2 (*Arabidopsis* Biological Research Center) into the plant expression vector pRD400. *ABH1* coding sequences were amplified from an *Arabidopsis* Columbia leaf cDNA library by rapid amplification of cDNA ends (Marathon cDNA Amplification Kit, Clontech) using the plasmid rescue sequence primer (5′ GAAGCTCAAC TCGTTGCTGGAAAG 3′) and its reverse. The full-length cDNA was then amplified using *Pfu* DNA polymerase (Stratagene), sequenced and cloned in pMON530. *ABH1* 5′ UTR (1250 bp) was amplified from genomic DNA by PCR using *Pfu* DNA polymerase and subcloned

in pCAMBIA1303 (GenBank AF23299) containing the GUS reporter gene. Sequences amplified by PCR were confirmed by sequencing (Retrogen, CA). *Agrobacterium tumefaciens* strain C58 was used to generate *Arabidopsis* transgenic plants. GUS activity was assayed on 10-day-old seedlings grown on MS plates, after vacuum infiltration, using 5-bromo-4-chloro-3-indolyl-D-glucuronide as substrate.

RNA Isolation and Blot Analyses

Total RNA was extracted from 4- to 5-week-old *Arabidopsis* leaves using Trizol reagent (Life Technologies) and poly(A^+) RNA was further purified using an mRNA purification kit (Poly(A^+) Quik, Stratagene). To extract total RNA from guard cell-enriched epidermal strips, leaves were blended in a Waring blender in cold water, ultrasonicated. Epidermal strips were collected after filtration. Epidermal strips with $\geq$ 95% guard cell purity were used to extract total RNA using Trizol. Total and poly(A^+) RNA were separated in a denaturing agarose gel and then transferred onto a Hybond-N^+ membrane (Amersham-Pharmacia) and hybridized with radiolabeled probe.

Yeast Two-Hybrid Constructs and Gel Mobility Shift Assays

ABH1 and *AtCBP20* full-length cDNAs were amplified from an *Arabidopsis* Columbia leaf cDNA library by PCR using *Pfu* DNA polymerase (Stratagene) and cloned in frame into pAS1 and pACT vectors, respectively (Matchmaker Gal4 Two-Hybrid system, Clontech). Yeast whole-cell extracts were obtained as described (Kataoka et al., 1995). A Poly(A^+) RNA of about 60 nt containing the mRNA cap structure (m^7GpppG) or an analog (ApppG) was synthetized from pSP64 Poly(A^+) vector (Promega) using SP6 RNA polymerase. Gel mobility shift assays were pursued as described (Kataoka et al., 1995).

DNA Chip Experiments

Ten μg of total RNA from 2 independently extracted samples (5 plants each) was mixed and used in DNA chip experiments using DNA chips (Affymetrix). cDNA synthesis, radiolabeling, and hybridization of the chips were carried out at the UCSD Gene Chip core. Data analyses were performed using Affymetrix and GeneSpring (Silicon Genetics) softwares.

Stomatal Movement Analyses

Stomata from 5- to 6-week-old plants were opened by exposing plants for 12 hr to light, 95% humidity, and incubation of the leaves for 1.5 hr in stomatal opening solution (Pei et al., 1997) containing 50 mM KCl, 10 μM $CaCl_2$, and 10 mM MES (pH 6.15) in the growth chamber. Stomatal apertures were measured 1.5 hr after ABA was added (Pei et al., 1997). Control experiments were performed in parallel with no ABA added. Standard errors (SEM), when given, were calculated relative to the square root of the number of epidermal peel experiments (Pei et al., 1997).

Calcium Imaging Analyses

Calcium imaging analyses were performed on 5- to 6-week-old plants as described (Allen et al., 1999b) on WT and *abh1* plants stably transformed with the cameleon construct p35SYC2.1. Stomatal pores were opened by keeping plants under light and 95% humidity for 12 hr. $[Ca^{2+}]_{cyt}$ transients were counted when an increase in the $[Ca^{2+}]_{cyt}$ ratio was 0.1 units above the baseline prior to normalization and distinguishable from the background. In both WT and *abh1*, a similar number of guard cells showed no stable resting $[Ca^{2+}]_{cyt}$ before ABA was added ($n = 18$ out 75 in WT and $n = 23$ out of 87 in *abh1*) as reported previously (Allen et al., 1999b). A slow baseline drift due to bleaching was subtracted. Cells that showed stable resting $[Ca^{2+}]_{cyt}$ during the first 8 min of recording (no ABA added) were tested for their response to ABA. Cells that did not respond to ABA were tested for their ability to respond to 10 mM external calcium at the end of the experiment. Cameleon ratios were normalized relative to the average baseline ratio prior to ABA application, which was statistically indistinguishable in *abh1* and WT ($P > 0.2$; baseline ratio 535/480 nm 0.83 $\pm$ 0.14 in WT and 0.79 $\pm$ 0.12 in *abh1*). Data represent the average of recordings performed on 3 independent cameleon-transformed WT lines and 2 independent *abh1* lines.

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References

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., Kuchitsu, K., Chu, S.P., Murata, Y., and Schroeder, J.I. (1999a). *Arabidopsis abi1–1* and *abi2–1* phosphatase mutations reduce abscisic acid-induced cytoplasmic calcium rises in guard cells. Plant Cell *11*, 1785–1798.

Allen, G.J., Kwak, J.M., Chu, S.P., Llopis, J., Tsien, R.Y., Harper, J.F., and Schroeder, J.I. (1999b). Cameleon calcium indicator reports cytoplasmic calcium dynamics in *Arabidopsis* guard cells. Plant J. *19*, 735–747.

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., et al. (2000). Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in *Arabidopsis det3* mutant. Science *289*, 2338–2342.

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

Armstrong, F., Leung, J., Grabov, A., Brearley, J., Giraudat, J., and Blatt, M.R. (1995). Sensitivity to abscisic acid of guard-cell K^+ channels is suppressed by *abi1–1*, a mutant *Arabidopsis* gene encoding a putative protein phosphatase. Proc. Natl. Acad. Sci. USA *92*, 9520–9524.

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

Bethke, P.C., and Jones, R.L. (1998). Gibberellin signaling. Curr. Opin. Plant. Biol. *1*, 440–446.

Bonetta, D., Bayliss, P., Sun, S., Sage, T., and McCourt, P. (2000). Farnesylation is involved in meristem organization in *Arabidopsis*. Planta *211*, 182–190.

Bueno, P., Piqueras, A., Kurepa, J., Savoure, A., Verbruggen, N., Van Montagu, M., and Inze, D. (1998). Expression of antioxidant enzymes in response to abscisic acid and high osmoticum in tobacco BY-2 cell cultures. Plant Sci. *138*, 27–34.

Cary, A.J., Liu, W., and Howell, S.H. (1995). Cytokinin action is coupled to ethylene in its effects on the inhibition of root and hypocotyl elongation in *Arabidopsis thaliana* seedlings. Plant Physiol. *107*, 1075–1082.

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.

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.

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 APETALA 2 domain protein. Plant Cell *10*, 1043–1054.

Finkelstein, R.R., and Lynch, T.J. (2000). The *Arabidopsis* abscisic

acid response gene *ABI5* encodes a basic leucine zipper transcription factor. Plant Cell *12*, 599–609.

Flaherty, S.M., Fortes, P., Izaurralde, E., Mattaj, I.W., and Gilmartin, G.M. (1997). Participation of the nuclear cap binding complex in pre-mRNA 3′ processing. Proc. Natl. Acad. Sci. USA *94*, 11893–11898.

Fortes, P., Kufel, J., Fornerod, M., Polycarpou-Schwarz, M., Lafontaine, D., Tollervey, D., and Mattaj, I.W. (1999). Genetic and physical interactions involving the yeast nuclear cap-binding complex. Mol. Cell. Biol. *19*, 6543–6553.

Fortes, P., Inada, T., Preiss, T., Hentze, M.W., Mattaj, I.W., and Sachs, A.B. (2000). The yeast nuclear cap binding complex can interact with translation factor eIF4G and mediate translation initiation. Mol. Cell *6*, 191–196.

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.

Furuichi, Y., LaFiandra, A., and Shatkin, A.J. (1977). 5′-terminal structure and mRNA stability. Nature *266*, 235–239.

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.

Gilmour, S.J., Artus, N.N., and Thomashow, M.F. (1992). cDNA sequence analysis and expression of two cold-regulated genes of *Arabidopsis thaliana*. Plant Mol. Biol. *18*, 13–21.

Gilroy, S., Read, N.D., and Trewavas, A.J. (1990). Elevation of cytoplasmic calcium by caged calcium or caged inositol triphosphate initiates stomatal closure. Nature *346*, 769–771.

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

Grabov, A., and Blatt, M.R. (1998). 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.

Guan, L.M., Zhao, J., and Scandalios, J.G. (2000). *Cis*-elements and *trans*-factors that regulate expression of the maize *CAT1* antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signaling molecule for the response. Plant J. *22*, 87–95.

Gutierrez, R.A., MacIntosh, G.C., and Green, P.J. (1999). Current perspectives on mRNA stability in plants: multiple levels and mechanisms of control. Trends Plant Sci. *4*, 429–438.

Guzman, P., and Ecker, J.R. (1990). Exploiting the triple response of *Arabidopsis* to identify ethylene- related mutants. Plant Cell *2*, 513–523.

Izaurralde, E., Lewis, J., McGuigan, C., Jankowska, M., Darzynkiewicz, E., and Mattaj, I.W. (1994). A nuclear cap binding protein complex involved in pre-mRNA splicing. Cell *78*, 657–668.

Izaurralde, E., Lewis, J., Gamberi, C., Jarmolowski, A., McGuigan, C., and Mattaj, I.W. (1995). A cap-binding protein complex mediating U snRNA export. Nature *376*, 709–712.

Karssen, C.M., Zagorski, S., Kepczynski, J., and Groot, S.P.C. (1989). Key role for endogenous gibberellins in the control of seed germination. Ann. Botany *63*, 71–80.

Kataoka, N., Ohno, M., Moda, I., and Shimura, Y. (1995). Identification of the factors that interact with NCBP, an 80 kDa nuclear cap binding protein. Nucleic Acids Res. *23*, 3638–3641.

Koornneef, M., and Karssen, C.M. (1994). Seed dormancy and germination. In Arabidopsis, C.R. Somerville, and E. M. Meyerowitz, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 313–334.

Koornneef, M., Elgersma, A., Hanhart, C.J., Van Loenen-Martinet, E.P., Van Rijn, L., and Zeevaart, J.A.D. (1985). A gibberellin insensitive mutant of *Arabidopsis thaliana*. Physiol. Plant. *65*, 33–39.

Koornneef, M., Leon-Kloosterziel, K.M., Schwartz, S.H., and Zeevaart, J.A.D. (1998). The genetic and molecular dissection of abscisic acid biosynthesis and signal transduction in *Arabidopsis*. Plant Physiol. Biochem. *36*, 83–89.

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

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.

Lewis, J.D., and Izaurralde, E. (1997). The role of the cap structure in RNA processing and nuclear export. Euro. J. Biochem. *247*, 461–469.

Lewis, J.D., Goerlich, D., and Mattaj, I.W. (1996). A yeast cap binding protein complex (yCBC) acts at an early step in pre-mRNA splicing. Nucleic Acids Res. *24*, 3332–3336.

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.

Lin, X., Kaul, S., Rounsley, S., Shea, T.P., Benito, M.-I., Town, C.D., Fujii, C.Y., Mason, T., Bowman, C.L., Barnstead, M., et al. (1999). Sequence and analysis of chromosome 2 of the plant *Arabidopsis thaliana*. Nature *402*, 761–768.

Lincoln, C., Britton, J.H., and Estelle, M. (1990). Growth and development of the *axr1* mutants of *Arabidopsis*. Plant Cell *2*, 1071–1080.

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.

MacIntosh, G.C., Bariola, P.A., Newbigin, E., and Green, P.J. (2001). Characterization of Rny1, the *Saccharomyces cerevisiae* member of the T2 RNase family of RNases: unexpected functions for ancient enzymes? Proc. Natl. Acad. Sci. USA *98*, 1018–1023.

MacRobbie, E.A.C. (1998). Signal transduction and ion channels in guard cells. Philos. Trans. R. Soc. London *353*, 1475–1488.

Marcotte, W.R., Guiltinan, M.J., and Quatrano, R.S. (1992). ABA-regulated gene expression: *cis*-acting sequences and trans-acting factors. Biochem. Soc. Trans. *20*, 93–97.

McAinsh, M.R., Brownlee, C., and Hetherington, A.M. (1990). Abscisic acid-Induced elevation of guard cell cytosolic calcium precedes stomatal closure. Nature *343*, 186–188.

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.

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.

Pei, Z.-M., Ghassemian, M., Kwak, C.M., McCourt, P., and Schroeder, J.I. (1998). Role of farnesyltransferase in ABA regulation of guard cell anion channels and plant water loss. Science *282*, 287–290.

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

Rodriguez, P.L., Leube, M.P., and Grill, E. (1998). Molecular cloning in Arabidopsis thaliana of a new protein phosphatase 2C (PP2C) with homology to ABI1 and ABI2. Plant. Mol. Biol. *38*, 879–883.

Schroeder, J.I., and Hagiwara, S. (1989). Cytosolic calcium regulates ion channels in the plasma membrane of *Vicia faba* guard cells. Nature *338*, 427–430.

Schroeder, J.I., and Hagiwara, S. (1990). Repetitive increases in cytosolic Ca^{2+} of guard cells by abscisic acid activation of nonselective Ca^{2+} permeable channels. Proc. Natl. Acad. Sci. USA *87*, 9305–9309.

Schroeder, J.I., Kwak, J.M., and Allen, G.J. (2001). Guard cell abscisic acid signalling and engineering drought hardiness in plants. Nature *410*, 327–330.

Schwartz, S.H., Leon-Kloosterziel, K.M., Koornneef, M., and Zeevaart, J.A.D. (1997). Biochemical characterization of the *aba2* and *aba3* mutants in *Arabidopsis thaliana*. Plant Physiol. *114*, 161–166.

Seki, M., Narusaka, M., Abe, H., Kasuga, M., Yamaguchi-Shinozaki, K., Carninci, P., Hayashizaki, Y., and Shinozaki, K. (2001). Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell *13*, 61–72.

Shatkin, A.J. (1976). Capping of eucaryotic mRNAs. Cell *9*, 645–653.

Shatkin, A.J. (1985). mRNA cap binding proteins: essential factors for initiating translation. Cell *40*, 223–224.

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.

Shimotohno, K., Kodama, Y., Hashimoto, J., and Miura, K.I. (1977). Importance of 5′-terminal blocking structure to stabilize mRNA in eukaryotic protein synthesis. Proc. Natl. Acad. Sci. USA *74*, 2734–2738.

Sonenberg, N., and Gingras, A.C. (1998). The mRNA 5′ cap-binding protein eIF4E and control of cell growth. Curr. Opin. Cell Biol. *10*, 268–275.

Staswick, P.E., Su, W., and Howell, S.H. (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.

Staxen, I., Pical, C., Montgomery, L.T., Gray, J.E., Hetherington, A.M., and McAinsh, M.R. (1999). Abscisic acid induces oscillations in guard-cell cytosolic free calcium that involve phosphoinositide-specific phospholipase C. Proc. Natl. Acad. Sci. USA *96*, 1779–1784.

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.

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.

Uemura, H., and Jigami, Y. (1992). GCR3 encodes an acidic protein that is required for expression of glycolytic genes in *Saccharomyces cerevisiae*. J. Bacteriol. *174*, 5526–5532.

Vranova, E., Langebartels, C., Van Montagu, M., Inze, D., and Van Camp, W. (2000). Oxidative stress, heat shock and drought differentially affect expression of a tobacco protein phosphatase 2C. J. Exp. Bot. *51*, 1763–1764.

Weigel, D., Ahn, J.H., Blazquez, M.A., Borevitz, J.O., Christensen, S.K., Fankhauser, C., Ferrandiz, C., Kardailsky, I., Malancharuvil, E.J., Neff, M.M., et al. (2000). Activation tagging in *Arabidopsis*. Plant Physiol. *122*, 1003–1013.

Wilhelm, K.S., and Thomashow, M.F. (1993). *Arabidopsis thaliana cor15b*, an apparent homologue of *cor15a*, is strongly responsive to cold and ABA, but not drought. Plant Mol. Biol. *23*, 1073–1077.

Wilson, K.F., Fortes, P., Singh, U.S., Ohno, M., Mattaj, I.W., and Cerione, R.A. (1999). The nuclear cap-binding complex is a novel target of growth factor receptor-coupled signal transduction. J. Biol. Chem. *274*, 4166–4173.

Yalovsky, S., Rodriguez-Concepcion, M., Bracha, K., Toledo-Ortiz, G., and Gruissem, W. (2000). Prenylation of the floral transcription factor APETALA1 modulates its function. Plant Cell *12*, 1257–1266.

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.

Accession Numbers

The *ABH1* cDNA described in this paper has been deposited in GenBank with accession number AF272891.