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THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 280, No. 2, Issue of January 14, pp. 1465–1473, 2005

Printed in U.S.A.

Arabidopsis HAF2 Gene Encoding TATA-binding Protein (TBP)-associated Factor TAF1, Is Required to Integrate Light Signals to Regulate Gene Expression and Growth*[S]

Received for publication, August 6, 2004, and in revised form, November 2, 2004
Published, JBC Papers in Press, November 3, 2004, DOI 10.1074/jbc.M409000200

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Plant growth and development are sensitive to light. Light-responsive DNA-binding transcription factors have been functionally identified. However, how transcription initiation complex integrates light signals from enhancer-bound transcription factors remains unknown. In this work, we characterized mutations within the *Arabidopsis HAF2* gene encoding TATA-binding protein-associated factor TAF1 (or $TAF_{II}250$). The mutation of *HAF2* induced decreases on chlorophyll accumulation, light-induced mRNA levels, and promoter activity. Genetic analysis indicated that *HAF2* is involved in the pathways of both red/far-red and blue light signals. Double mutants between *haf2-1* and *hy5-1*, a mutation of a light signaling positive DNA-binding transcription factor gene, had a synergistic effect on photomorphogenic traits and light-activated gene expression under different light wavelengths, suggesting that *HAF2* is required for interaction with additional light-responsive DNA-binding transcription factors to fully respond to light induction. Chromatin immunoprecipitation assays showed that the mutation of *HAF2* reduced acetylation of histone H3 in light-responsive promoters. In addition, transcriptome analysis showed that the mutation altered the expression of about 9% of genes in young leaves. These data indicate that TAF1 encoded by the *Arabidopsis HAF2* gene functions as a coactivator capable of integrating light signals and acetylating histones to activate light-induced gene transcription.

Light-regulated gene expression has been a paradigm to study transcriptional regulatory mechanism in plants. Light signals are perceived by a set of photoreceptors to regulate plant gene expression and growth. Seedlings grown in the dark are etiolated, with a long hypocotyl and closed cotyledons. In contrast, seedlings grown in the light have a short hypocotyl and open cotyledons that become photosynthetically competent. To affect developmental processes such as de-etiolation, changes in gene expression must occur. The expression of many genes is regulated by light (1). Ultimately, light signals are integrated by specific transcription factors that bind to various light responsive elements (LRE)[1] within the promoters (2). Recent results indicate that changes in gene expression were the result of a transcriptional cascade (3). Several repeated LRE have been found to be necessary for light induction of promoter activity. However, no single LRE is sufficient for light responsiveness. This suggests that interaction between LRE or between their cognate binding factors is required for light regulation of a promoter. DNA sequence-specific transcription factors involved in light-regulated gene expression have been identified from mutants with reduced ability to respond to light signals. These factors include the bZIP proteins HY5 (4) and its homologue HYH (5), the basic helix-loop-helix proteins HFR1/REP1/RSF1 (6–8) and PIF3 (9, 10), and the MYB proteins CCA1, LHY, and LAF1 (11–13).

The phytochrome photoreceptor family members essentially track the red and far-red light wavelengths through their capacity for switching between two light-induced and reversible forms: the red-absorbing, biologically inactive Pr form and the far red-absorbing, biologically active Pfr form (14). The cryptochromes (CRY1/2) are involved in the blue light perception (15). The mutations affecting either the protein or the synthesis of chromophores of phytochromes and cryptochromes as well as positive light signaling regulators lead to a constitutive long hypocotyl phenotype (reviewed in Ref. 16). Phytochrome-mediated gene expression is likely to be achieved through different pathways. First, light-activated phytochromes are imported into the nucleus and interact directly with the DNA-bound basic helix-loop-helix factor PIF3 to activate a subclass of light inducible genes (17). Second, genetic screens have led to the identification of mutants with a short hypocotyl and open cotyledons in the dark, referred to as *de-etiolated* (*det*), *constitutive photomorphogenic* (*cop*), or *fusca* (*fus*) (reviewed in Ref. 18). This *det/cop/fus* class of mutants exhibit ectopic expression of light-regulated genes and plastid development in the dark. The *det/cop/fus* mutants are recessive and epistatic to the photoreceptor mutants. The corresponding wild-type genes thus are thought to act as downstream negative regulators of the pho-

* This work was supported by French plant genomic program GENOPLANTE II Grant AF2001019 (to D.-X. Z.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[S] The on-line version of this article (available at http://www.jbc.org) contains Table 2.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY579213 for the HAF2 cDNA sequence.

The microarray data for *haf2* has been submitted to ArrayExpress (ebi.ac.uk/arrayexpress/) with accession number E-MEXP-178.

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[1] The abbreviations used are: LRE, light responsive element; HAT, histone acetyltransferase; TBP, TATA-binding protein; TAF_{II}, TATA-binding protein-associated factor; RT, reverse transcription; Ws, ecotype Wassilewskija plants; CHIP, chromatin immunoprecipitation.

tomorphogenic program. The *COP* genes appear to regulate photomorphogenesis at least in part via targeted protein degradation in the dark of transcription factors such as the bZIP factors HY5 and HYH (5, 19). The mutation of the *HY5* gene induces a constitutive long hypocotyl phenotype in the light (4). It has been shown that HY5 binds to the G-box element commonly found in the promoters of light-regulated genes including chlorophyll *a*/*b*-binding protein gene *CAB2* (lhcb1*1) and the ribulose bisphosphate carboxylase/oxygenase (Rubisco) small subunit gene *RBCS-1A* (20). The *hy5* mutation induces a decrease of light-regulated expression of these genes (20, 21). However, the mechanism by which HY5 and other sequence-specific factors activate light-induced transcription is unknown. Recent reports have suggested that histone acetylation on gene promoters may function as a regulatory mechanism of light activation of gene transcription (22–24).

The regulation of gene transcription involves transcription cofactors (co-activator or co-repressor) capable of transducing signals from enhancer-bound specific transcription factors to the RNA polymerase initiation complex. *In vitro* studies have shown that transcription cofactors are usually associated with chromatin remodeling and modification activities such as histone acetyltransferases (HATs) and histone deacetylases, which control the level of acetylation of specific lysine residues within the core histone tails (reviewed in Ref. 25). Transcription-associated proteins with HAT activity include GCN5, cAMP-response element-binding protein, and TAF1 (26). TAF1 (also called $TAF_{II}250$) is one of the 10–12 TATA-binding protein (TBP)-associated factors (TAF_{II}) that form with TBP, the TFIID complex in human and *Drosophila* cells (reviewed in Ref. 27). Specific cellular and developmental function of TAF1 has been demonstrated in yeast and animal systems (reviewed in Ref. 28). In this paper, we report characterization of mutations within one of the two genes (*i.e. HAF2*, Ref. 26) encoding *Arabidopsis* TAF1 and molecular and genetic evidence that this gene is required for light-dependent gene transcription and growth as well as for many other genes.

EXPERIMENTAL PROCEDURES

Plant Materials—Arabidopsis T-DNA insertion mutant plants were in the Wassilewskija (Ws, *haf2-1*) and Columbia-0 (Col-0, *haf2–2*and *haf2–3*) backgrounds. *haf2-1* was obtained from the Versailles data base, *haf2-2*, *haf2-3*, and the *hy* mutants (in Lansberg background) were obtained from the Nottingham Arabidopsis Stock Centre (NASC, Nottingham, UK). To obtain double mutants, F2 plants of genetic crosses were genotyped with PCR by using primers that could distinguish the wild-type from the mutant DNA sequences. *Arabidopsis* plants grown in a greenhouse were under long day conditions (16 h of light) at 19.5 °C (day) and 17.5 °C (night). *In vitro* cultures of mutant plants were performed in 0.5 × Murashige Skoog media at 20 °C under either white light (16 h light/day at 120 μmol m^{-2} s^{-1}), or under continuous red (10 μmol m^{-2} s^{-1}), far-red (5 μmol m^{-2} s^{-1}), or blue (18 μmol m^{-2} s^{-1}) light.

Genomic DNA and Total RNA Extraction, PCR, RT-PCR, and Northern Blots—Arabidopsis leaves were used for genomic DNA extraction. PCR were carried out by using the Promega TfiI polymerase. The primers used to check the T-DNA insertion indicated in Fig. 1 were: Primer 1, 5′-ATGGGAGCAATGATGAAGAG-3′; Primer 2, 5′-AAAGGCTCGAGCATGTTGTT-3′; Primer 3, 5′-CTACAAATTGCCTTTTCTTATCGA-3′; Primer 4, 5′-TCTGTGGCTCTTGTATAGC-3′; Primer 5, 5′-ACTCAAGATGAGACGGTGG-3′; Primer 6, 5′-TCATCGTAAGCTCTTCTCACC-3′.

Total RNA was isolated with TRIzol reagents (Invitrogen). First strand cDNA was synthesized from 5 μg of total RNA using Superscript II reverse transcriptase (Invitrogen). For semi-quantitative PCR analysis, 2 μl of the reverse transcription reactions were used per PCR in a final volume of 20 μl.

For Northern blot analysis, 5 μg of total RNA were separated by 1% denaturing agarose gels, blotted onto nylon membrane, and hybridized with ^{32}P-labeled gene-specific probes that were prepared from cDNA clones. Hybridization signals were scanned with Molecular Image FX Pro (Bio-Rad), and normalized to actin mRNA signals by using Bio-Rad Quantity One 1-D Analysis software.

Chlorophyll Contents Determination—Total chlorophyll contents were determined for an average of 20 mg of plantlets (fresh weight) in 80% acetone. The absorbance of chlorophyll *a* and *b* was measured at 662.2 and 645.8 nm, respectively, and their concentration was calculated as described by Lichtenthaler (29).

Cloning of HAF2 cDNA and Complementation of haf2-1 Mutants—HAF2 cDNA was amplified sequentially by RT-PCR from Ws apex mRNA. The PCR fragments were cloned into pGEM-T (Promega) and sequenced. The fragments were assembled using restriction enzyme sites present in the overlapping region of the fragments. The full-length cDNA was further cloned under the control of the cauliflower mosaic virus 35S promoter (*35S/HAF2*) and introduced into *Agrobacterium tumefaciens* (HBA10S). *haf2-1* plants were transformed using the floral dip method. Seeds from the T1 plants were selected on 0.5× Murashige and Skoog medium containing 50 mg/liter gentamycin. Resistant plantlets were transferred to soil and grown in the greenhouse under long day conditions. The presence of the *35S/HAF2* insertion was checked by PCR.

CAB2/udiA Trangenic Plants and GUS Activity Measure—The promoter region from −201 to −37 relative to the initiation of the ATG codon was inserted upstream the *uidA* gene in the plant transformation vector pBI101. The construct was used to transform Ws plant by the floral dip method. Homozygous single copy transgenic plants were characterized from the T3 population by a combination of Southern blot and analysis of offspring segregation. One homozygous single copy line was used to cross with *haf2-1* and Ws. Plants homozygous for the transgene in both background identified similarly from the F3 populations were used for GUS activity assays as described previously (30).

Chromatin Immunoprecipitation—Immunoprecipitations were performed as described previously (31). Ws and *haf2* seeds were sterilized, kept 2 days at 4 °C, and grown *in vitro* under long day conditions. Chromatin was extracted from seedlings 5 days after germination and fixed with formaldehyde. Cross-linked chromatin was immunoprecipitated with antibodies against histone H3, acetylated H3, or hyperacetylated H4 (Upstate Biotechnology). Immunoprecipitated DNA was analyzed by semi-quantitative PCR using primers designed on *CAB2*, *RBCS1-A*, and *ACTIN-2* promoters: CAB2 −260, 5′-CATTCTTGTCACGAGGGTGT-3′; CAB2 −20, 5′-AAAACTGGTTCGATAGTGTTG-3′; RBCS −237, 5′-CAAGCCGATAAGGGTCTCAA-3′; RBCS −38, 5′-GTGACTGAGGTTTGGTCTAG-3′; ACT-FP, 5′-CTAAGCTCTCAAGATCAAAGGCTT-3′; ACT-RP, 5′-TTAACATTGCAAAGAGTTTCAAGG-3′.

Transcriptome Studies—The microarray analysis was performed with the CATMA array containing 24,576 gene specific tags from *Arabidopsis thaliana* (catma.org). Plants were grown *in vitro* under white light for 14 days. RNA was extracted from the first two pairs of leaves using the TRIzol extraction kit (Invitrogen) followed by two ethanol precipitations, then checked for RNA integrity with the Bioanalyzer from Agilent (Waldbroon, Germany). cRNAs were produced from 2 μg of total RNA from each sample with the "Message Amp aRNA" kit (Ambion, Austin, TX). Then 5 μg of cRNAs were reverse transcribed in the presence of 300 units of SuperScript II (Invitrogen), cy3-dUTP and cy5-dUTP (PerkinElmer Life Sciences) for each slide.

RESULTS

Characterization of a T-DNA Insertion Mutation within the HAF2 Gene—To study the developmental function of plant HAT genes, we isolated the cDNA of *HAF2* by RT-PCR from *A. thaliana* ecotype Ws plants. The *HAF2* cDNA sequence (AY579213) had a few differences compared with the previous annotation: 17 exons were found and some of them were produced from a splicing model slightly different from the previous annotation that predicts 18 exons (26). The characteristic modules homologous to ubiquitin, HAT, and bromodomain were preserved in the deduced protein sequence (Fig. 1*A*). The *HAF2* mRNA levels in different organs or tissues were too low to be detected by Northern blots. RT-PCR experiments showed that *HAF2* seemed to be more expressed in growing organs such as shoots and flower buds (Fig. 1*B*). A search of T-DNA insertion mutant collections identified three *Arabidopsis HAF2* alleles (*haf2-1*, *haf2-2*, and *haf2-3*). *haf2-1* was in the Ws background, whereas the others were in Columbia-0 (Col-0) background. The T-DNA insertions disrupted the 5′-half of the gene (Fig. 1*A*). Homozygous insertion plants were identified by PCR, only the results on *haf2-1* are shown (Fig. 1*C*). RT-PCR experiments

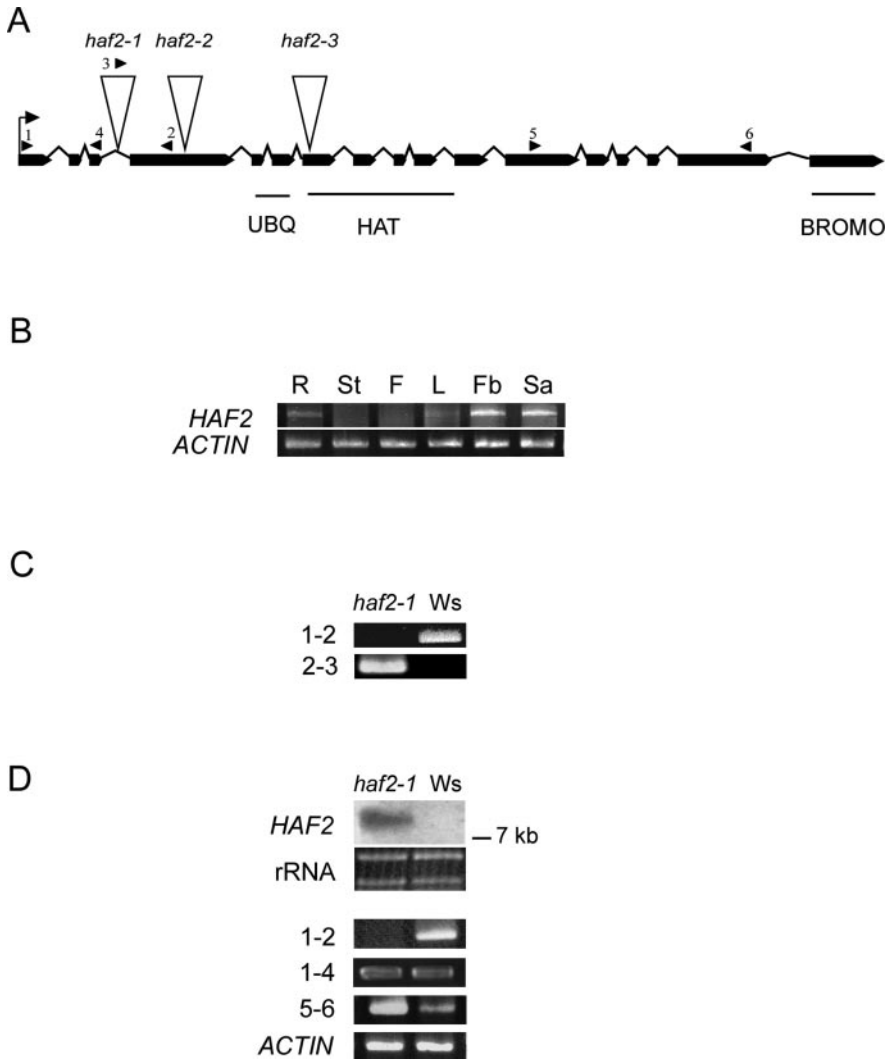

FIG. 1. **Isolation of *Arabidopsis* T-DNA insertion mutants in the *HAF2* gene (At3g19040).** *A*, diagram of the *HAF2* gene structure and the T-DNA insertion positions of the *haf2-1*, *haf2-2*, and *haf2-3* alleles. The exons and introns were deduced based on the cDNA sequence (AY579213). The regions corresponding to ubiquitin (*UBQ*), HAT, and bromodomain (*BROMO*) are indicated. *Numbered chevrons* represent primers used in *B–D*. *B*, RT-PCR detection of HAF2 mRNA in roots (*R*), stem (*St*), flowers (F), rosette leaves (*L*), flower buds (*Fb*), and shoot (*Sa*) of wild-type plants (*Ws*). Actin mRNA levels detected by RT-PCR are shown as controls. *C*, genotyping of the *haf2-1* mutation by PCR with primer sets as indicated in *A*. *D*, the T-DNA insertion in *haf2-1* interrupted the full-length mRNA of *HAF2*, but induced overexpression of the downstream region of the gene. *Upper parts*, Northern blot analysis of RNAs isolated from the mutant and the wild-type seedlings with the *HAF2* cDNA as probe. The rRNAs are shown as loading control. *Lower parts*, RT-PCR analysis of the *haf2-1* and the wild-type seedling RNA with primer sets as indicated in *A*. Actin mRNA levels are shown as controls.

with primers that span the insertion sites were not able to detect any *HAF2* mRNA in the mutant plants (results on *haf2-1* are shown Fig. 1*D*). However, PCR analysis with primers corresponding to the regions flanking the insertion site detected *HAF2* expression in *haf2-1* plants. The 5′ primer set (primers 1 and 4) detected a transcript at a comparable level in both the wild-type and mutant. But the 3′ primer set (primers 5 and 6) detected a transcript at a higher level in the mutant than in the wild type. Consistent with this observation, Northern blots detected a larger than expected transcript in *haf2-1*, but was not sensitive enough to reveal any signal in the wild-type sample (Fig. 1*D*). These data suggest that the *HAF2* transcript was interrupted by T-DNA insertion and that the 3′ transcript was likely to be induced by a promoter in the T-DNA construct. It is therefore likely that the *haf2-1* was a null mutation.

Phenotype Characterization of the haf2 Mutants—To evaluate phenotypes of the mutants, *haf2-1*, *haf2-2*, and *haf2-3* seeds were germinated along with the wild-type Ws and Col-0 cultured under white light (16 h/day at 120 μmol m^{-2} s^{-1}). Ws and *haf2-1* plants at 4, 7, and 21 days after germination were photographed (Fig. 2*A*). The cotyledons of *haf2-1* plants were paler than the wild-type ones. The newly produced young leaves and shoots were yellowish (Fig. 2*A*). However, during expansion, the leaves became gradually greener from the base to the distal end (Fig. 2*A*). In the dark, the etiolated *haf2* seedlings were indistinguishable from wild-type ones (not shown). The progeny of heterozygous plants showed a 3/1 segregation ratio for the *haf2* phenotype, indicating that the *haf2-1* mutation was a recessive loss-of-function mutation that segregated as a single nuclear locus. Seven-day-old plants of Col-0, *haf2-2*, and *haf2-3* are shown in Fig. 2*B*. Cotyledons of *haf2-2* and *haf2-3* were paler than that of Col-0, whereas the leaf phenotype of these mutants seemed to be less pronounced than *haf2-1*. Quantification of chlorophyll contents in cotyledons at day 7 after germination revealed that there was about 35–40 and 50% of reduction of chlorophyll *a* and chlorophyll *b* in *haf2-1* compared with Ws, and in *haf2-2* and *haf2-3* compared with Col-0, respectively (Table I).

Light-regulated gene expression in the mutants was analyzed by Northern blots. Total RNA samples isolated at the same hour of the day (to avoid circadian variations) from germinating wild-type and mutant seedling were hybridized with probes corresponding to *RBCS-1A* and *CAB2*. As shown in Fig. 2*C*, the induction of expression of the two genes during germination was seriously impaired in *haf2-1*. Similar results were obtained in *haf2-2* and *haf2-3* (not shown). These data suggest that *HAF2* is required for light-induced gene expression.

To determine whether the mutant phenotype was caused solely by the T-DNA insertion in the *HAF2* gene, genetic complementation was performed by transformation of *haf2-1* plants with the full-length cDNA that was driven by the cauliflower mosaic virus 35S promoter. Transformed seedlings produced normal green leaves (Fig. 2*D*) indicating that the transgene expression complemented the *haf2-1* phenotype. Therefore, no other mutations in *haf2-1* were responsible for the phenotype.

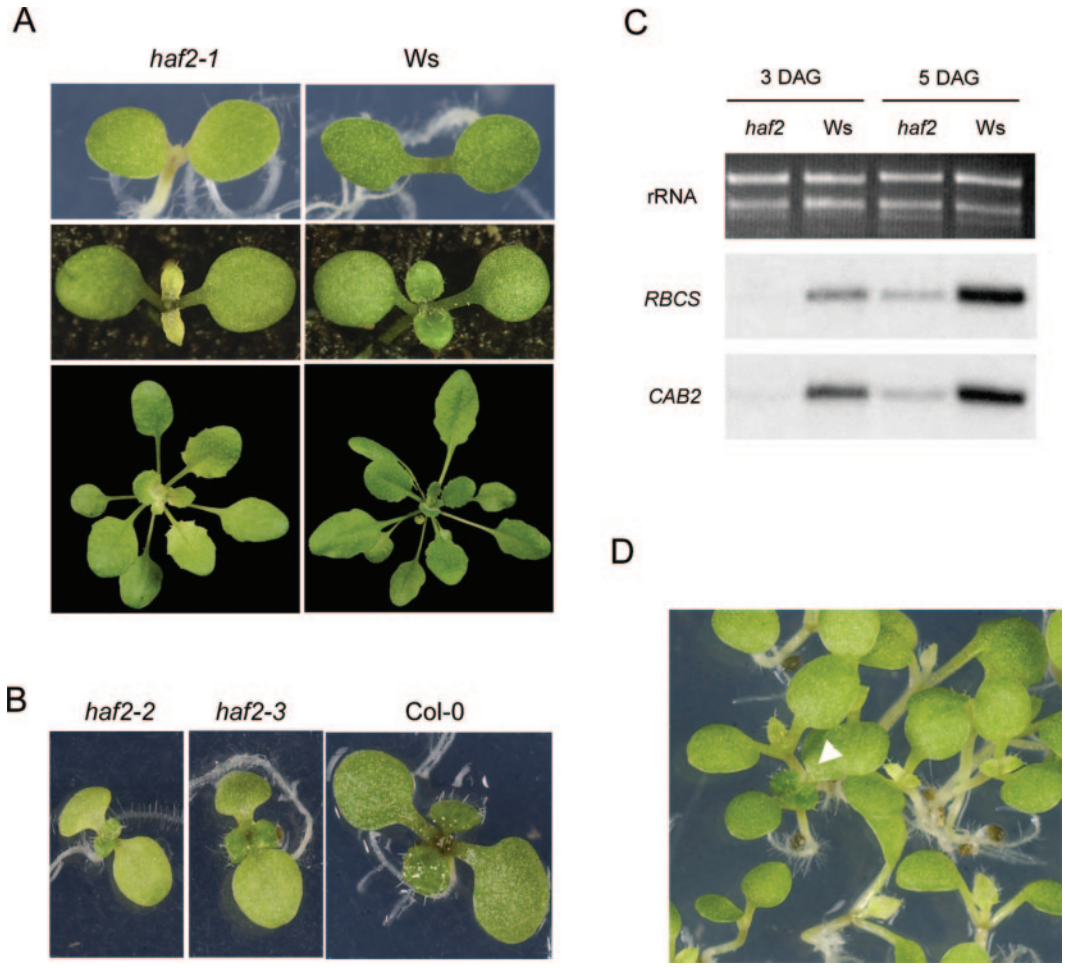

FIG. 2. **Phenotypes of the *haf2* alleles and complementation of *haf2-1*.** *A*, the *haf2-1* mutation affected cotyledon and leaf greening. Comparison of *haf2-1* (*left*) and wild-type (*right*) plants at cotyledon, young leaf, and rosette stages grown *in vitro* in white light (16 h/day at 120 μmol m^{-2} s^{-1}) is shown. *B*, comparison of *haf2-2* and *haf2-3* with Col-0 7 days after germination *in vitro* in white light as in *A*. *C*, *CAB2* and *RBCS-1A* expression in *haf2-1* was lower than in wild-type plants. Total RNA isolated from seedlings at 3 or 5 days after germination (*DAG*) were hybridized with the probes indicated on the *left*. rRNA bands are shown as loading control. *D*, the HAF2 cDNA under the control of 35S promoter (*35S/HAF2*) complemented the *haf2-1* phenotype. Shown are T1 seedlings of *35S/HAF2*-transformed *haf2-1* plants. *Arrows* indicate complemented seedlings with green leaves.

TABLE I

Cotyledon chlorophyll accumulation in haf2 alleles and double mutants with hy1, hy4, and hy5 in comparison with the wild-type Ws and Col-0

Chlorophyll contents were determined from cotyledons of about 40 seedlings per sample. Average values from two measures were presented (FW, fresh weight).

Genotype	*Ws*	*haf2-1*	*Col-0*	*haf2-2*	*haf2-3*	*hy1-1*	*haf2-1/hy1-1*	*hy5-1*	*haf2-1/hy5-1*
Chlorophyll *a* (μg/mg FW)	1.11	0.73	0.77	0.38	0.37	0.37	0.21	0.82	0.40
Chlorophyll *b* (μg/mg FW)	0.37	0.22	0.31	0.15	0.14	0.03	0.02	0.25	0.11
Chlorophyll *a* + chlorophyll *b* (μg/mg), FW	1.49	0.95	1.08	0.53	0.51	0.40	0.23	1.07	0.57

haf2-1 Affected the CAB2 Promoter Activity—To examine whether the down-regulation of the light-inducible genes was at the transcription level, the promoter region from −147 to +17 (relative to the transcription start site) or −201 to −37 relative to the initiation ATG codon of *CAB2*, which contains sufficient elements to recapitulate most aspects of *CAB2* regulation (32), was used to drive the GUS-coding *uidA* reporter gene expression in transgenic plants. A transgenic line homozygous for the *CAB2/uidA* fusion was used to cross with *haf2-1* and Ws plants. GUS activities were measured from light grown shoots of F3 plants that were homozygous for the transgene. The GUS activities in *haf2-1* were decreased about 3-fold when compared with that in Ws plants (Fig. 3) in the light, but had no difference in the dark. These data suggest that *HAF2* is required for the full activity of the *CAB2* promoter in the light.

Phenotype of haf2/hy1-1 Double Mutants—The defects in light-activated gene expression and *CAB2* promoter activity induced by the *haf2-1* mutation suggested that *HAF2* might function as a positive regulator in the light-regulated pathways. Genetic screens for mutant plants that are impaired in their ability to perceive light, and thus are constitutively with long hypocotyls (*hy*) have identified positive light signaling components such as the *hy5* mutations. The *hy1* mutations affect the gene of the phytochrome chromophore biosynthetic enzyme, heme oxygenase (33), whereas the *hy4* mutations affect the blue light photoreceptor cryptochrome 1 (*CRY1*) gene (34). However, the *haf2* mutations did not alter significantly the hypocotyl length of seedlings grown in the light (Fig. 4*A*). To know if there was any genetic relationship between *hy* mutants and *haf2–1*, double mutants *haf2-1/hy1-1*, *haf2-1/hy5-1*, and *haf2-1/hy4-1* were made by genetic crosses.

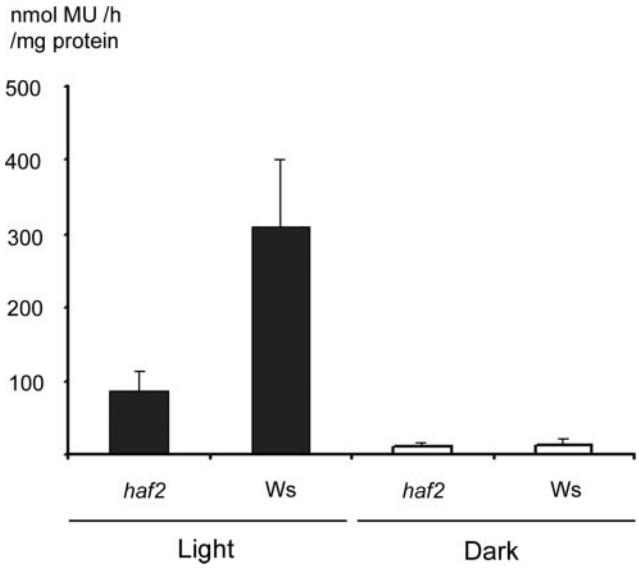

FIG. 3. **The *CAB2* promoter activity was impaired by the *haf2-1* mutation.** The promoter region from −201 to −37 relative to the initiation ATG codon controlling the *uidA* gene was introduced into *haf2-1* and Ws by genetic crosses. Shoots of light and dark-grown F3 plants homozygous for the transgene in each background were used for GUS activity assays. The activities are the average of 8 F3 plants and the *error bars* indicate S.D.

In white light, *haf2-1/hy1-1* double mutants were found to be even paler than *hy1-1*, but still could complete the life cycle. In fact, the *haf2-1/hy1-1* double mutants showed a greater reduction of total chlorophyll contents than either single mutant, with only about 19% of that from the wild-type (Ws) plants (Table I). These observations indicated that there was an additive effect between *hy1-1* and *haf2-1* on chlorophyll accumulation in white light. The average hypocotyl length of *haf2-1/hy1-1* seemed to be comparable with, if not slightly higher than, *hy1-1* in white light (Fig. 4*A*). In far-red light, the *haf2-1/hy1-1* hypocotyl length was also comparable with that of *hy1-1* (Fig. 4*B*). The red light condition was less effective to suppress hypocotyl elongation. There was no significant change of hypocotyl length in the double mutant compared with the single mutants in red light (Fig. 4*C*). These data suggest that *hy1-1* is epistatic to *haf2-1* with respect to this phenotype.

Phenotype of haf2/hy4-1 Double Mutants—The *hy4* mutations that affect the blue light receptor *CRY1* gene showed a long hypocotyl in blue light. The hypocotyl length of *haf2-1/hy4-1* was comparable with that of *hy4-1* in blue light, suggesting that *hy4-1* was epistatic to *haf2-1* with respect to the hypocotyl length in blue light (Fig. 4*D*). In contrast, in white light the *haf2-1/hy4-1* double mutants showed a clear enhancement of the hypocotyl length that was about twice of that in the *hy4-1* (Fig. 4*A*). These observations indicate that there existed genetic interactions between *HAF2* and both *HY1* and *HY4* to control hypocotyl length in white light.

Phenotype of haf2/hy5-1 Double Mutants—The *hy5-1* and *haf2-1* single mutants had comparable levels of cotyledon chlorophyll contents, whereas the *haf2-1/hy5-1* double mutants showed a further reduction of chlorophyll accumulation with only about 50% of that in the single mutants (Table I). *haf2-1/hy5-1* double mutants in the white light exhibited also a spectacular enhancement of the hypocotyl length that was about twice the length of the *hy5-1* single mutants (Fig. 4*A*). The increase of hypocotyl length in *haf2-1/hy5-1* was also observed in seedlings grown in continuous far-red, red, and blue lights (Fig. 4, *B–D*), although the increase was less important than in white light. These data show that *haf2-1* and *hy5-1* had a synergetic and an additive effect to control hypocotyl growth and chlorophyll accumulation in white light, respectively, indicating a genetic interaction between the two loci.

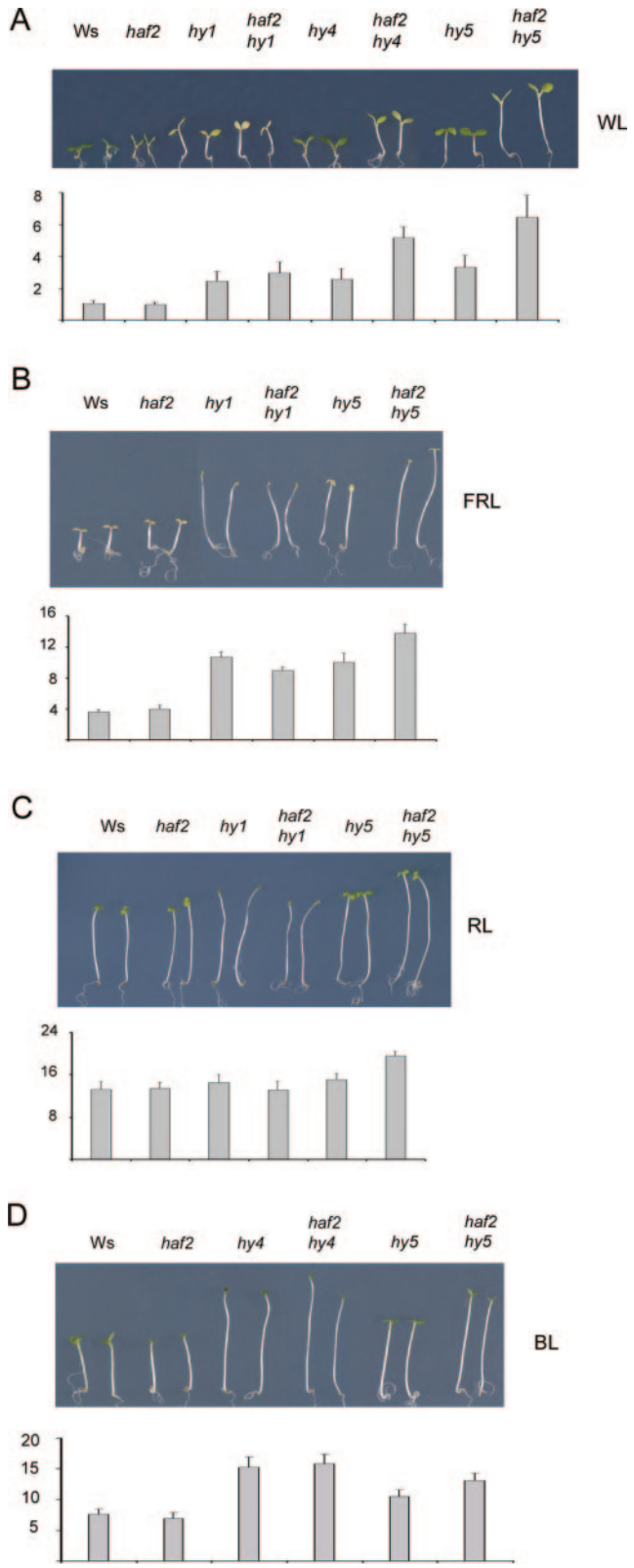

FIG. 4. **Phenotypes and hypocotyl lengths of wild-type (*WS*), *haf2-1*, *hy1*, *hy5-1*, *hy4-1*, and the double mutants as indicated on the *top* of the panels.** *A*, white light; *B*, far-red light (5 μmol m^{-2} s^{-1}); *C*, red light (10 μmol m^{-2} s^{-1}); *D*, blue light (18 μmol m^{-2} s^{-1}). Average hypocotyl lengths (in mm) were determined from measures of more than 30 plantlets. *Bars* = S.D.

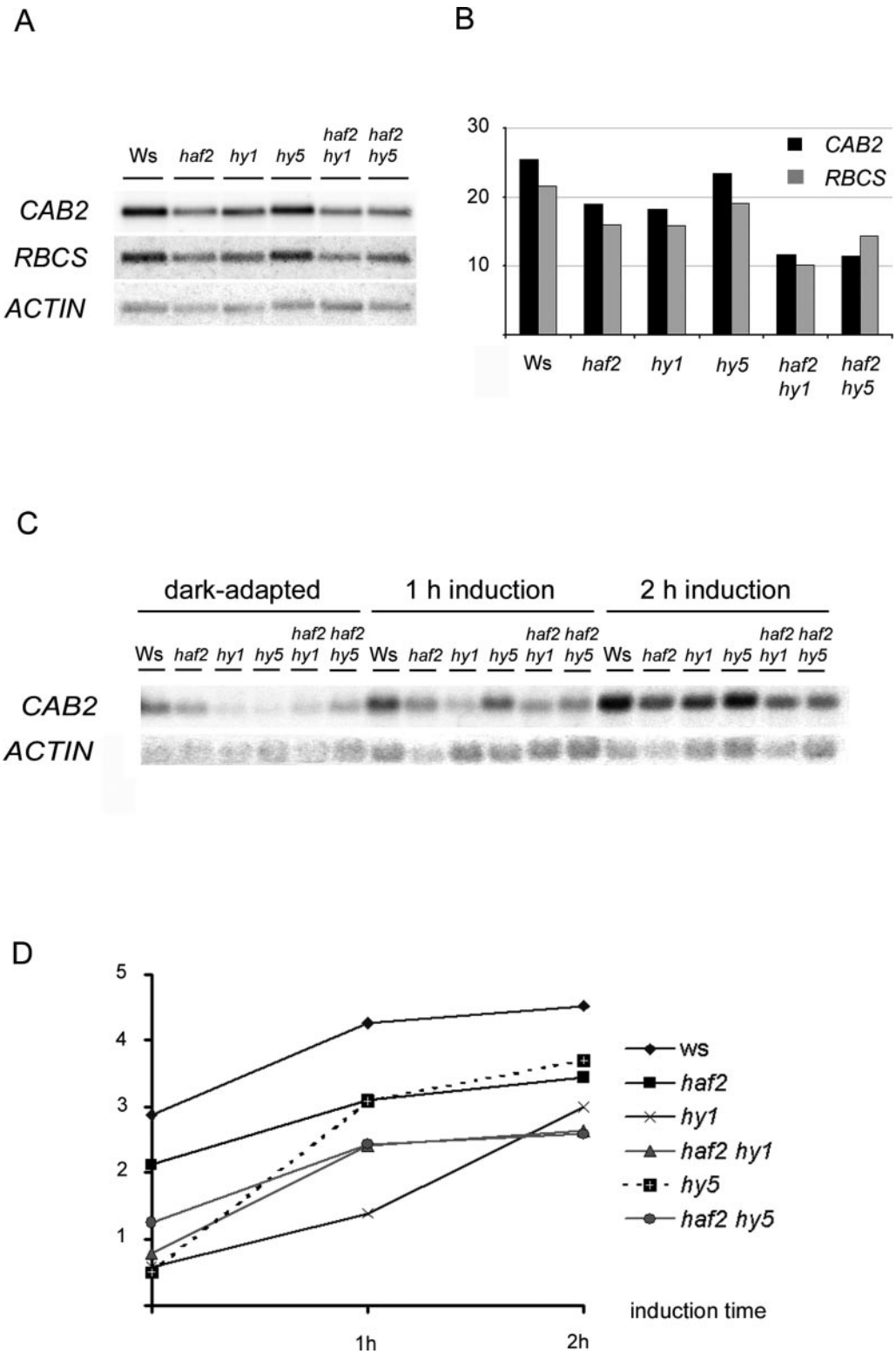

FIG. 5. **Light-regulated gene expression in the double mutants *haf2-1/hy1-1* and *haf2-1/hy5-1*.** *A*, total RNA isolated 5-day-old seedlings of different genotypes as indicated were hybridized with probes corresponding to *CAB2* and *RBCS-1A*. *B*, quantification data of the hybridization bands in *A* after normalization with the actin mRNA signals. *C*, Northern blot analysis of *CAB2* expression in 17-day-old leaves harvested from 72-h dark-adapted and dark-adapted then re-illuminated plants of the indicated genotypes. *D*, relative quantification data of the hybridization bands in *C* after normalization with the actin mRNA signals.

Light-regulated Gene Expression in the Double Mutants—The mRNA levels of *CAB2* and *RBCS-1A* of the single and the double mutants grown under white light were compared by Northern blots (Fig. 5*A*). The quantification results showed that the mRNA levels of *CAB2* in *haf2-1* were lower than in *hy5-1*, but comparable with that in *hy1-1* (Fig. 5*B*). *haf2-1/hy1-1* and *haf2-1/hy5-1* had lower expression levels of *CAB2* than the single mutants (Fig. 5*B*). Similar results were obtained for *RBCS-1A*. In dark-adapted rosette leaves, the *haf2-1 CAB2* mRNA level was above that of *hy1-1* and *hy5-1*, whereas the double mutants had intermediate levels. After 2 h re-illumination, the *CAB2* mRNA levels in the double mutants were the

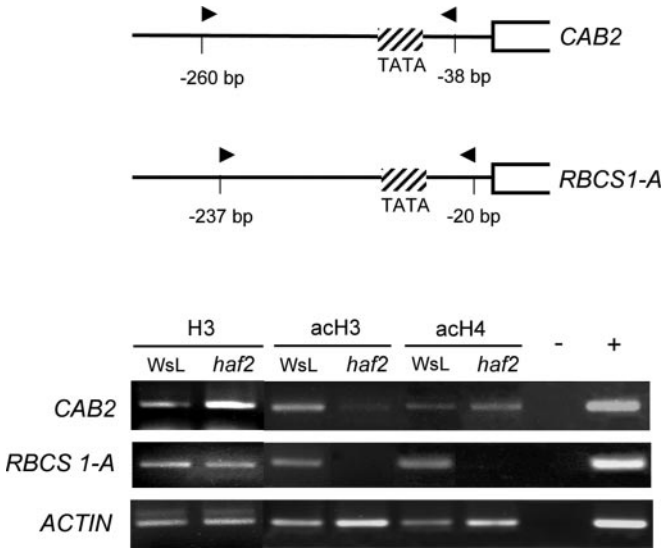

FIG. 6. **Acetylation state of histones H3 and H4 at the TATA-proximal promoter region of *CAB2* and *RBCS-1A* in *haf2-1* and Ws seedlings.** Nuclei were extracted from cross-linked 5-day-old light-grown seedlings, sonicated, and immunoprecipitated with antibodies specific to total histone H3, acetylated histones H3 (*AcH3*) and H4 (*AcH4*). The immunoprecipitates were analyzed for the presence of DNA by PCR. The primers used for PCR to detect the promoter region of *CAB2* and *RBCS-1A* as well as *ACTIN2* are indicated in the *upper part*. The PCR (25 cycles) results are shown in the *lower part*. The lane labeled with the *plus sign* (+) contains the products of PCR performed with chromatin solution before immunoprecipitation. The lane with the *minus sign* (−) corresponds to PCR performed with immunoprecipitations without antibodies. Similar results were obtained for three independent CHIP experiments.

lowest (Fig. 5, *C* and *D*), which was consistent with the data obtained from the light-grown leaves (Fig. 5*A*).

HAF2 Is Involved in Histone Acetylation of the Light Responsive Promoters—To investigate whether lower expression of *CAB2* and *RBCS-1A* was because of reduced histone acetylation in the promoter region induced by the *haf2-1* mutation, we performed chromatin immunoprecipitation (CHIP) by following the experimental procedures described by Gendrel *et al.* (31). Nuclei were extracted from 5-day-old seedlings of Ws and *haf2-1* grown in white light after fixation with formaldehyde treatment to cross-link chromatin proteins to DNA. The chromatin was sheared to an average of 500–1000 bp by sonication and immunoprecipitated with antibodies specific for histone H3 and acetylated histones H3 and H4. DNA was released from the immunoprecipitated chromatin fragments by heating and analyzed by PCR with primers specific to the TATA box proximal or core promoter region of *CAB2* and *RBCS-1A* (Fig. 6). Primers specific to the actin promoter region were used as internal control. To assess nonspecific binding, an immunoprecipitation reaction was also performed in the absence of antibody. A fraction of sonicated nuclei was used in the CHIP assays to allow quantification relative to input chromatin. In CHIP assays performed with antibody specific to acetylated H3, the levels of precipitated promoter fragments for both *CAB2* and *RBCS-1A* were much lower in *haf2-1* than in Ws, whereas the levels for the actin promoter were comparable between the two samples (Fig. 6), indicating that the *haf2* mutation affected H3 acetylation in the proximal promoter region of the light-responsive promoters. In CHIP assays performed with antibody specific to acetylated H4, a decrease of H4 acetylation of the *RBCS-1A* promoter in *haf2-1* was observed. H4 acetylation of the *CAB2* promoter seemed not to be affected by the mutation (Fig. 6). A control with antibodies against total histone H3 did not reveal any significant difference between *haf2* and the wild-type (Fig. 6).

Variations of Gene Expression Profiles in haf2 Leaves—To identify genes whose expression is affected by the *haf2* mutation, we used the Complete Arabidopsis Transcriptome MicroArray (CATMA) that contains 24,576 genes of the *Arabidopsis* genome (catma.org). Fluorescent cDNA probes were synthesized from total RNA harvested from 14-day-old leaves of wild-type and mutant plants in three separate experiments permitting three independent pairwise comparisons of mutant and wild-type leaves. Based on the statistical test (see "Experimental Procedures") 868 genes were found to be differentially expressed between *haf2* and the wild-type plants (p value $<$ 0.05). 9,960 genes of the 24,576 probes printed on the CATMA chips showed detectable expression, being defined as a median signal higher than the median background plus 2 standard deviations over background. Therefore, 8.7% of the genes considered as expressed in those samples were revealed as differentially expressed between the mutant and control. The affected genes are listed under Supplemental Materials. Complete data files were deposited to ArrayExpress (ebi.ac.uk/arrayexpress/) under accession number E-MEXP-178. Of the affected genes, 52% were induced and 48% were repressed. The affected genes belonged to different categories shown in Fig. 7. We noted that in consistence with the phenotype, down-regulation of a number of photosynthetic genes was observed in *haf2* (see Supplemental Materials).

DISCUSSION

Gene activation requires transcriptional cofactors to integrate signals carried by promoter-specific DNA-binding transcription factors to the RNA polymerase II initiation complex. So far little is known on the developmental function of plant transcription cofactors, although some pioneer work has been recently published (30, 35–37).

The multisubunit complex, TFIID, consists of the TBP and several TAFs, whose primary sequences are well conserved from yeast to humans. Data from reconstituted cell-free transcription systems and binary interaction assays suggest that the TAF subunits can function as promoter-recognition factors, as coactivators capable of transducing signals from enhancer-bound activators to the basal machinery, and even as enzymatic modifiers of other proteins. For instance, TAF1, in addition to serving as a scaffold within TFIID, possesses coactivator activities because of its ability to interact directly with transcriptional activators (reviewed in Ref. 28) or to acetylate nucleosomal histones (38, 39). Whether TAFs function similarly *in vivo*, however, has been an open question. In mammalian or yeast cells, temperature-sensitive alleles of the TAF1 gene induce G_1 arrest at the nonpermissive temperature (39, 40). Null alleles of *Drosophila* TAF1 are larval lethal (41), whereas weak loss-of-function alleles can survive to adulthood and reveal potential development pathways and genes regulated by TAF1. TAF1 in *Arabidopsis* is encoded by two genes (*HAF1*, *HAF2*). We have shown in this report that the mutation of *Arabidopsis HAF2* affected expression of light-regulated genes and acetylation of histone H3 in light-responsive promoter regions. This indicates that TAF1 encoded by the *Arabidopsis HAF2* gene functions as a specific coactivator capable of transducing light signals to the basal machinery, in which the HAT activity of the protein is involved. However, it is possible the *haf2* mutation characterized in this study may reveal only part of the developmental pathways regulated by *Arabidopsis* TAF1. A broad range of genes affected in *haf2* presented in Fig. 7 supports this hypothesis. As with any such microarray analysis, the results cannot distinguish between direct and indirect effects of the *haf2* mutation. The phenotypic effects induced by the *haf2* mutation suggest that the function of *HAF1* and *HAF2*

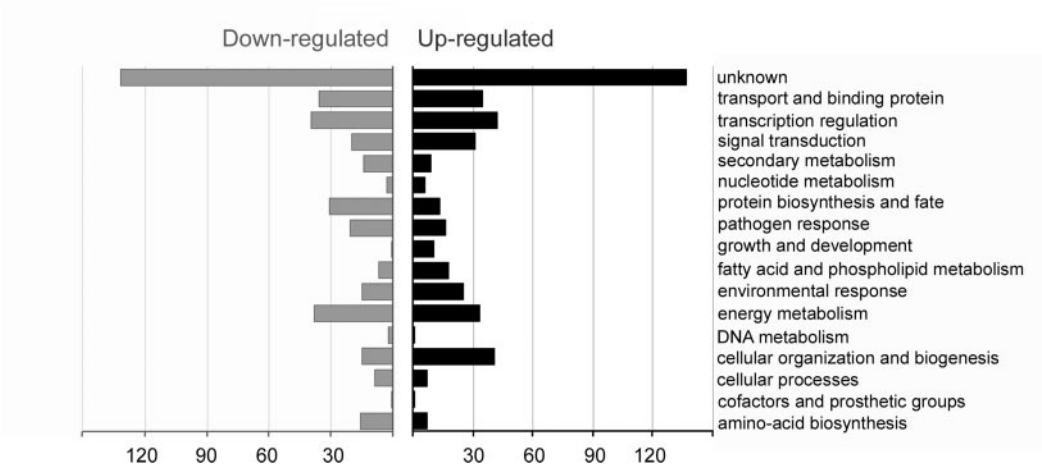

FIG. 7. **Gene expression profiles of *haf2* mutation.** Functional categories of genes with increased or decreased expression in rosette leaves of *haf2* mutants. Scales correspond to gene numbers. Gene function classification is based on the scheme of Schoof *et al.* (43).

is not fully redundant. The two genes may be expressed in different cells or tissues or may be expressed differentially in response to developmental or environmental cues. We have shown that *HAF2* was expressed preferentially in cell-dividing organs such as shoots. This expression profile of *HAF2* is consistent with the *haf2-1* phenotype that affects the greening process of shoots and young leaves. Alternatively, the proteins encoded by the two genes may be partially redundant, but *HAF2* may have a subset of different target genes that could be achieved through specific interaction with relevant DNA-binding transcription factors. This hypothesis is supported by the observations that a T-DNA insertion line within *HAF1* (line N610848 from the Nottingham Arabidopsis stock center) did not show only visible phenotype in greenhouse conditions (data not shown).

Mechanism of Light Regulation of Gene Transcription by HAF2—The epistatic relationship with respect to hypocotyl length between *haf2-1* and *hy1-1* or between *haf2-1* and *hy4-1* in red and far-red or in blue light, respectively, indicates that *HAF2* functions in the downstream of both the phytochrome and cryptochrome signaling pathways and that *HAF2* is a common regulator of different light signaling pathways. This hypothesis is reinforced by the observations that in white light *haf2-1/hy4-1*, and likely *haf2-1/hy1-1*, exhibited a severer hypocotyl phenotype than the corresponding single *hy* mutants (Fig. 5). These data support indirectly the hypothesis that TAF1 encoded by *HAF2* may act as a transcriptional cofactor capable of integrating signals from different lights.

The synergistic relationship between *haf2-1* and *hy5-1* with respect to hypocotyl elongation in different light conditions (Fig. 4) and to *CAB2* expression (Fig. 5), suggests that other light-responsive transcription factors may use TAF1 as cofactor to activate transcription. It has been shown that HY5 regulates the *CAB2* and *RBCS-1A* gene transcription by binding to the G-box element (or CGF1 box for *CAB2*) within the promoters (20, 21). HY5 is not the only G-box binding transcription factor that mediates the light-activated gene transcription. The HY5 homologous protein HYH also binds to G-Box and mediates light-dependent transcription and shows functional overlap with HY5 (5). In addition, PIF3, a basic helix-loop-helix DNA binding factor that interacts with phytochromes binds also to the G-box within light-responsive promoters. It is known that these proteins are likely interchangeable with different affinities for the promoters. TAF1 may be an adaptor in the transcription complex, which allows HY5, HYH, or PIF3 to associate with the RNA polymerase II. In *hy5*, TAF1 may allow HYH to partially compensate for the loss of HY5. This would account for the additive phenotype observed after loss of *HY5* and *HAF2*.

As mentioned in the Introduction, single LRE such as the G-box is a necessary but not a sufficient *cis*-element to modulate light induction of the promoter activity (2). Other LRE-binding proteins together with G-box binding HY5, HYH, or PIF3 are likely required to fully respond to light induction. Functional interaction must occur between DNA-bound transcription factors and/or transcription cofactors. However, our attempt to detect any direct interaction between HY5 and HAF2 in two-hybrid experiments turned out to be unsuccessful (not shown). One possibility is that HY5 may activate gene transcription through the mediation of a distinct cofactor that interacts in turn with TAF1. This hypothesis can be supported by the phenotype difference between the single mutants and by synergetic effect in the double mutant of *haf2-1* and *hy5-1*.

Histone Acetylation and Light Activation of Transcription—It has been shown recently that light-induced pea plastocyanin gene (*PetE*) transcription in tobacco green shoots was associated with histone hyperacetylation (22, 23). Hyperacetylation of both histones H3 and H4 was light-dependent and targeted to the *PetE* enhancer/promoter region in green shoots, suggesting that nucleosomal histone acetylation is used as a regulatory switch to integrate light signals to control gene transcription. A possible connection between the GCN5 type HAT and light activation of transcription has been suggested, because of physical interaction between the photomorphogenesis regulator DET1 and Damaged DNA-binding protein 1 (24). The latter has been shown to interact with a histone acetylation complex containing GCN5 in mammalian cells (42). The data shown in Fig. 6 suggest that *HAF2* was needed for nucleosomal histone H3 acetylation of the *CAB2* and *RBCS-1A* promoter regions that contain sufficient elements to mediate light response (20, 32). However, the *haf2-1* mutation seemed to only affect H4 acetylation of the *RBCS-1A*, but not the *CAB2*, promoter. It is not known whether this difference can be in part accounted for the TAF1 preference for histone H3 acetylation *in vitro* (38) and/or difference in promoter structure. Acetylation of nucleosomal histones induces changes in chromatin structure, therefore the HAT activity of TAF1 may provide a mechanism for TFIID to access the chromatin-structured promoter. The finding of Chua *et al.* (23) shows that the light-responsive enhancer of the *PetE* gene mediates the acetylation of histones on the promoter. This suggests a recruitment of HAT by the enhancer-binding transcription factors. The genetic interaction data presented in this paper suggest a possible recruitment of TAF1 to promoter by LRE binding factors.

Acknowledgments—We thank Dr. Jean-Pierre Bouly for providing red/far-red and blue light culture facilities, Dr. Bernard Pineau for help in chlorophyll quantifications, Séverine Domenichini for assistance in measuring hypocotyl lengths, Roland Boyer for taking photographs, and the Nottingham Arabodopsis Stock Center for seeds.

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