

***Arabidopsis* GCN5, HD1, and TAF1/HAF2 Interact to Regulate Histone Acetylation Required for Light-Responsive Gene Expression**

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We previously showed that *Arabidopsis thaliana* histone acetyltransferase TAF1/HAF2 is required for the light regulation of growth and gene expression, and we show here that histone acetyltransferase GCN5 and histone deacetylase HD1/HDA19 are also involved in such regulation. Mutation of *GCN5* resulted in a long-hypocotyl phenotype and reduced light-inducible gene expression, whereas mutation of *HD1* induced opposite effects. The double mutant *gcn5 hd1* restored a normal photomorphogenic phenotype. By contrast, the double mutant *gcn5 taf1* resulted in further loss of light-regulated gene expression. *gcn5* reduced acetylation of histones H3 and H4, mostly on the core promoter regions, whereas *hd1* increased acetylation on both core and more upstream promoter regions. *GCN5* and *TAF1* were both required for H3K9, H3K27, and H4K12 acetylation on the target promoters, but H3K14 acetylation was dependent only on *GCN5*. Interestingly, *gcn5 taf1* had a cumulative effect mainly on H3K9 acetylation. On the other hand, *hd1* induced increased acetylation on H3K9, H3K27, H4K5, and H4K8. *GCN5* was also shown to be directly associated with the light-responsive promoters. These results suggest that acetylation of specific histone Lys residues, regulated by *GCN5*, *TAF1*, and *HD1*, is required for light-regulated gene expression.

INTRODUCTION

Many physiological modifications induced by light signaling are caused by changes in gene expression. Although recent studies have led to the identification of a large number of regulators involved in light-signaling pathways, the molecular mechanisms of light activation of gene transcription remain obscure. Promoter analyses have defined a number of light-responsive elements. For instance, the ACGT core-containing G-box identified in the light-responsive genes *CAB2* (or *LHCB1.1*, hereafter referred to as *CAB2*) and *RBCS-1A* is bound by the basic domain/Leu zipper transcription factor HY5 in vitro (Chattopadhyay et al., 1998), which acts downstream of both phytochromes and cryptochromes (Oyama et al., 1997). Further studies have shown that a combination of several light-responsive elements is required for light activation, suggesting that interaction between the cognate DNA binding transcription factors is necessary for light-induced gene activation (Martinez-Hernandez et al., 2002; Yadav et al., 2002, 2005; Maxwell et al., 2003). However, it is not known how DNA binding proteins function to activate light-responsive gene transcription.

Chromatin structure plays a critical role in gene transcription. The basic structural unit of chromatin is the nucleosome. The

positioning of nucleosomes on promoters is inhibitory to transcription in that they prevent the transcriptional initiation complex or transcription factors from binding to a promoter (Horn and Peterson, 2002). Covalent modifications of the N-terminal tails of the core histones affect nucleosome positioning and compaction; thus, they play important roles in gene regulation (Lusser, 2002; Loidl, 2004). Histone acetylation involves the transfer of acetyl groups from acetyl-CoA to the N-terminal Lys residues of histones H3 and H4, as well as H2A and H2B. Hyperacetylation of histones relaxes chromatin structure and is associated with transcriptional activation, whereas hypoacetylation of histones induces chromatin compaction and gene repression (Sterner and Berger, 2000; Marmorstein and Roth, 2001; Carrozza et al., 2003).

Histone acetylation is catalyzed by histone acetyltransferases (HATs), whereas histone deacetylation is catalyzed by histone deacetylases (HDACs). Recent work has shown that several HATs are associated with transcription coactivators and that HDACs are associated with corepressor complexes in yeast and animal cells, indicating that histone acetylation is an integral part of transcriptional regulatory systems (Marmorstein and Roth, 2001; Berger, 2002; Carrozza et al., 2003). The HATs found in *Arabidopsis thaliana* are grouped into four types based on primary homology with yeast and mammalian HATs: GNAT, MYST, CBP, and TAF1 (Sterner and Berger, 2000; Pandey et al., 2002). The mutation of *Arabidopsis* HAT genes induces defects in many aspects of plant development and growth (Bertrand et al., 2003, 2005; Vlachonasios et al., 2003).

Plant HDACs can be grouped into four subclasses. Three of them have primary homology with three yeast HDACs: RDP3, HDA1, and SIR2 (Pandey et al., 2002). In addition, a specific class

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of HDAC (known as the HD2 class) is found only in plants (Lusser et al., 1997; Pandey et al., 2002). The RDP3-like protein is the major HDAC in yeast and mammals. Several RDP3-type HDACs have been found in *Arabidopsis* and maize (*Zea mays*) (Pandey et al., 2002; Varotto et al., 2003). *Arabidopsis* RDP3-type HDACs include HDA6, HDA7, HDA9, and HDA19 (also known as HD1; hereafter, we use HD1). Inactivation of HD1 in transgenic plants either expressing an antisense *At HD1* construct or containing a T-DNA insertion mutation (*athd1-t1*) induces pleiotropic developmental abnormalities (Wu et al., 2000; Tian and Chen, 2001; Tian et al., 2003).

We have previously shown that an *Arabidopsis* gene encoding the HAT-containing TAF1 was required for leaf greening and light-activated gene transcription, and its absence led to additional developmental abnormalities (Bertrand et al., 2005). In this work, we show that GCN5 and HD1 are also required for light-regulated gene expression and growth. Genetic and molecular analyses suggest that GCN5 and HD1 function antagonistically, whereas GCN5 and TAF1 act agonistically to control the histone acetylation balance required to switch on light-regulated gene transcription.

RESULTS

Mutations in *GCN5* and *HD1* Genes Induce Opposite Hypocotyl Phenotypes

To examine whether HAT- and HDAC-coding genes are involved in light-regulated plant developmental processes, we used previously characterized mutant alleles of *GCN5* (*dlx8*; hereafter called *gcn5*) and *HD1* (*athd1-t1*; hereafter called *hd1*) (all in a Wassilewskija [Ws] background) (Bertrand et al., 2003; Tian et al., 2003). Seeds of the wild type (Ws) and the mutants were germinated under different light conditions including complete darkness, white light (16 h/d at 120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and continuous blue (18 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), far-red (5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and red (10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) light. At day 7 after germination, hypocotyl lengths (a characteristic photomorphogenic trait) were measured and statistically analyzed (see Methods) to determine significant differences. In the dark, hypocotyl lengths of the different genotypes were similar. Under different light conditions, *gcn5* seedlings showed a light-hyposensitive phenotype with longer hypocotyls compared with those of wild-type plants. By contrast, hypocotyls of *hd1* seedlings were shorter than wild-type hypocotyls, showing an enhanced photomorphogenic phenotype. The phenotypes for both *gcn5* and *hd1* were most pronounced when grown under continuous far-red light (Figure 1A).

To confirm these observations, we analyzed another *GCN5* T-DNA insertion allele, called *gcn5-1* (in a Ws background) (Vlachonasios et al., 2003). The T-DNA insertion is located in the last exon of the gene. The *gcn5-1* mutant also showed a long-hypocotyl phenotype (Figure 1B). In addition, complementation of the *gcn5* mutation with the *GCN5* cDNA restored a normal-hypocotyl phenotype (Bertrand et al., 2003) (Figure 1B). Furthermore, *hd1 gcn5* double mutants were made using *gcn5* pollen to fertilize *hd1*. The hypocotyl length of the double mutant was similar to that of the wild type, suggesting that both

mutations could mutually compensate for the respective phenotypes.

gcn5 and *hd1* Have Opposite Effects on Light-Inducible Gene Expression

Light-inducible gene expression in *gcn5* and *hd1* mutants was analyzed by RNA gel hybridization. Total RNA isolated from either dark- or light-grown seedlings was hybridized with probes corresponding to the *CAB2* and *RBCS-1A* genes. As shown in Figure 2A, the transcripts of *CAB2* and *RBCS-1A* in light-grown *gcn5* seedlings were seriously reduced compared with those of the wild type. Decreases were also observed in dark-grown plants (Figure 2A). By contrast, the *hd1* mutation induced a clear increase of *CAB2* expression in both light- and dark-grown plants (Figure 2B). Thus, *GCN5* and *HD1* also have opposite functions in light-inducible gene expression. The decrease of *CAB2* and *RBCS-1A* expression in *gcn5* mutants could be restored by complementation with the *GCN5* cDNA or by the *hd1 gcn5* double mutation (Figure 2C). Together, these data indicated that the *gcn5* mutation induced the downregulation of light-activated gene expression and that the *gcn5* and *hd1* mutations could compensate mutually with respect to light-induced gene expression.

Effects of the Double Mutation of *gcn5* and *taf1*

We have shown previously that the mutation of *TAF1* (*haf2*; hereafter called *taf1*) also impairs *CAB2* and *RBCS-1A* gene expression (Bertrand et al., 2005). To assess any functional relationship between *TAF1* and *GCN5*, we made *taf1 gcn5* double mutants by genetic crosses. The double mutant exhibited a severe growth phenotype but managed to yield mature plants (Figure 3A). RNA gel blot analyses showed that the transcripts of *CAB2* and *RBCS-1A* were further decreased to background levels in the double mutant (Figure 3B), suggesting that *gcn5* and *taf1* mutations had cumulative effects on both plant growth and light-responsive gene expression. However, the expression of the chalcone synthase gene (*CHS*), another light-responsive gene, was not affected significantly by either mutation, except for a slight decrease in the double mutant (Figure 3B), suggesting that the two HAT genes are involved only in the expression of a subset of light-inducible genes. By contrast, the expression of a highly light-activated gene, *IAA3* (Tian et al., 2002), was repressed dramatically in *gcn5* and *gcn5-1* (Figure 3B) (Vlachonasios et al., 2003) but not in *taf1* (Figure 3B), indicating that the two HAT genes have distinct light-inducible target genes. This finding is in agreement with the different photomorphogenic phenotypes observed in the respective mutants (Figure 3C) (Bertrand et al., 2005). The *hd1* mutation also induced the expression of *IAA3* but did not affect that of *CHS* (Figure 3B).

The mutation of the light-responsive positive transcription factor *HY5* affects the plant perception of light signals and induces constitutive long hypocotyls (Oyama et al., 1997). We have shown previously that the *taf1* mutation could enhance the *hy5* long-hypocotyl phenotype irrespective of light conditions (Bertrand et al., 2005), suggesting that the *TAF1* pathway may interact with that of *HY5*. In this study, we obtained *gcn5 hy5*

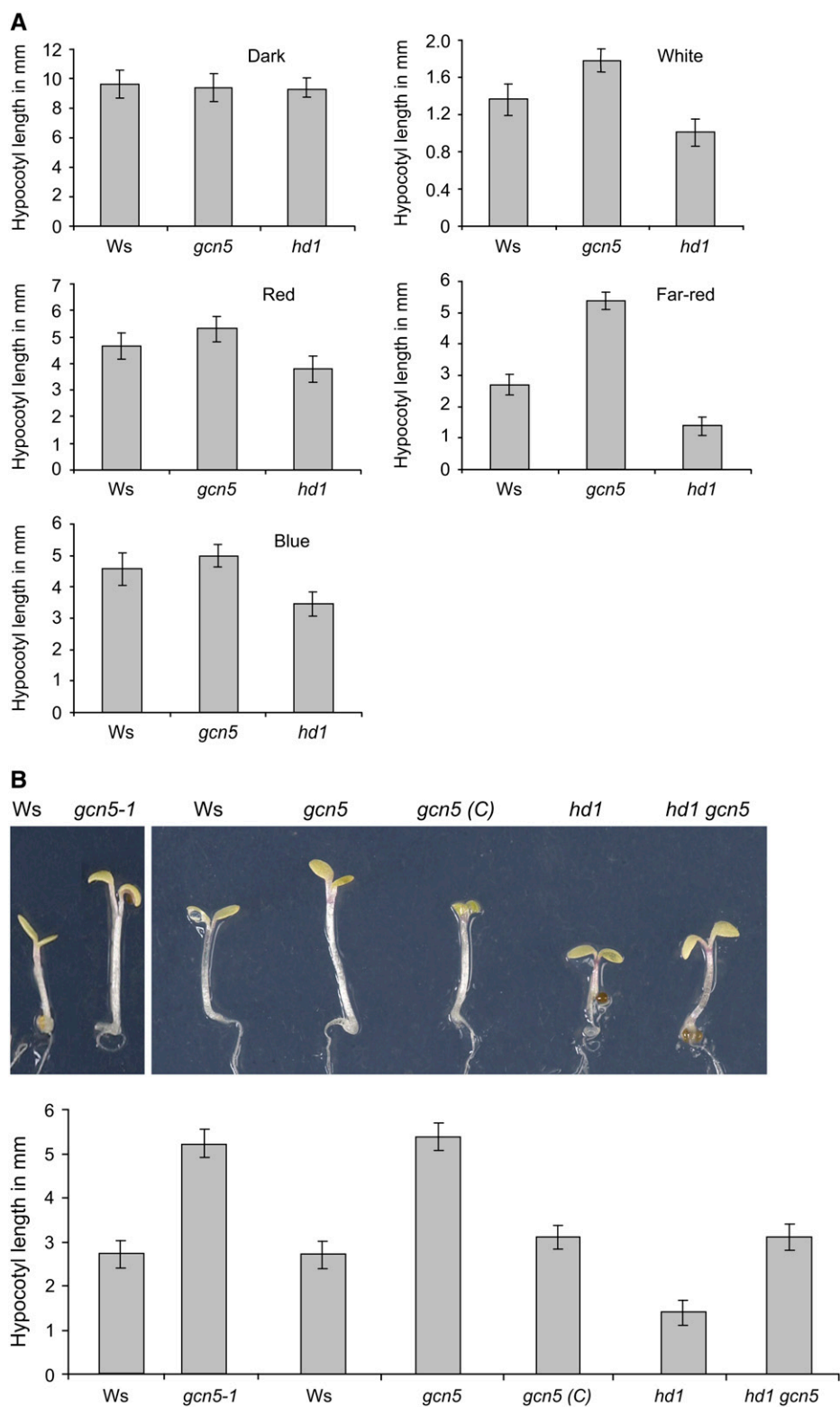


Figure 1. *gcn5* and *hd1* Mutations Affect the Light Repression of Hypocotyl Elongation.

(A) Seven-day-old seedling hypocotyl lengths of wild-type *Ws*, *gcn5*, and *hd1* plantlets grown in darkness or under white light (16 h/d at $120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or continuous red ($10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) far-red ($5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), or blue ($18 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) light.

(B) Seven-day-old far-red light-grown seedling phenotypes. Left, comparison of the *gcn5-1* allele with the wild type (*Ws*); right, comparison of *Ws*, *gcn5*, *gcn5* complemented with *GCN5* cDNA [*gcn5* (C)], *hd1*, and the double mutant *gcn5 hd1-1*. Means of 20 plantlets are given. Error bars represent SD values. The measures were analyzed by Student's *t* test at $\alpha = 0.05$.

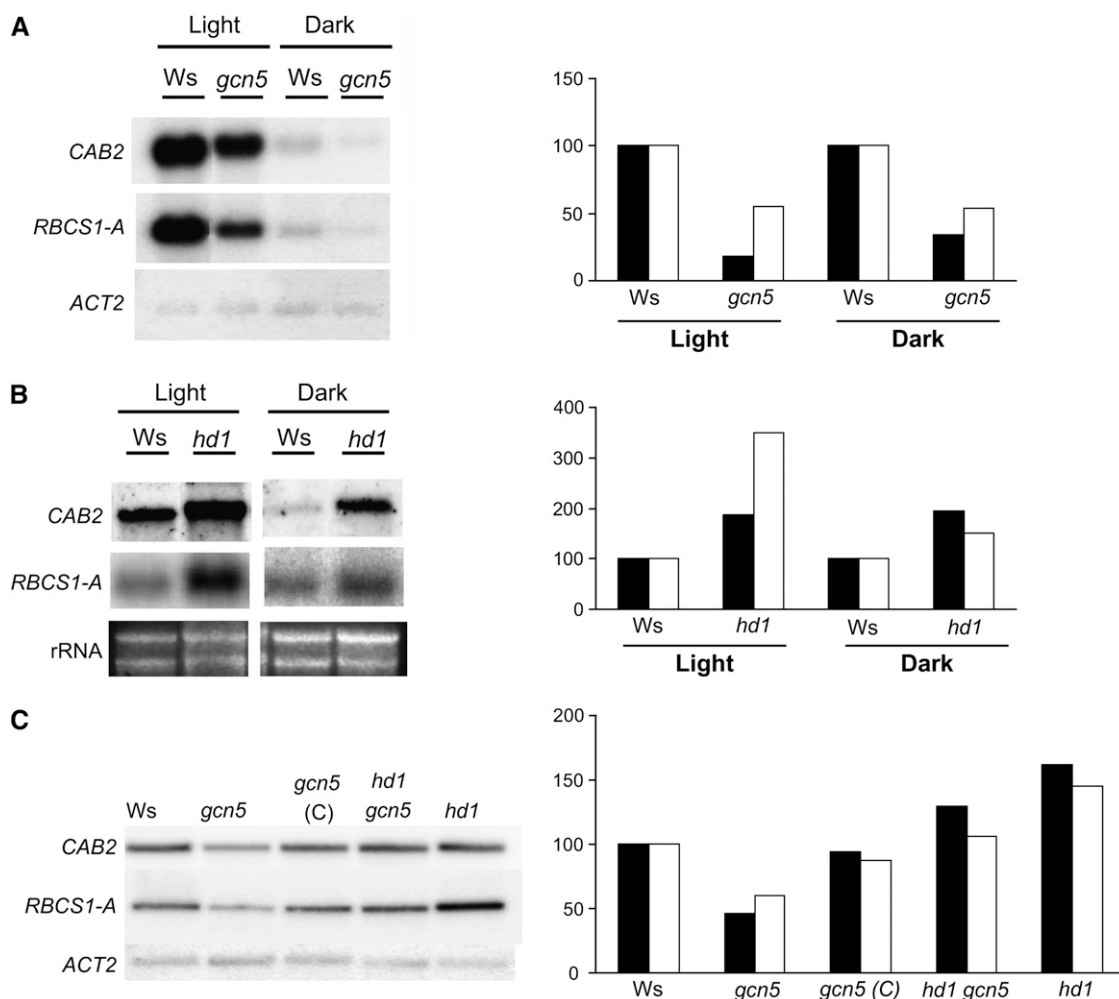


Figure 2. The *gcn5* and *hd1* Mutations Have Opposite Effects on Light-Inducible Gene Expression.

(A) RNA gel analysis of *CAB2* and *RBCS1-A* gene expression in both light- and dark-grown *gcn5* and *Ws* seedlings. Actin mRNA levels were analyzed to normalize gel loading.

(B) RNA gel analysis of *CAB2* and *RBCS1-A* gene expression in both light- and dark-grown *hd1* and *Ws* seedlings. rRNA transferred to the membranes was used to normalize gel loading.

(C) RNA gel analysis of *CAB2* and *RBCS1-A* gene expression in light-grown *Ws*, *gcn5*, *gcn5* complemented (C), the *gcn5-2 hd1* double mutant, and *hd1* seedlings.

Signal quantifications relative to the wild type (arbitrarily given as 100) are presented at right of the gels. Black bars, *RBCS1-A*; white bars, *CAB2*.

double mutants and, in contrast with the enhanced hypocotyl elongation observed in *hy5 taf1* plants, the hypocotyl lengths of *gcn5 hy5* were similar to those of the *gcn5* single mutants. Such data suggest that *GCN5* and *TAF1* might interact differently with *HY5*.

***gcn5* and *hd1* Affect Histone Acetylation on Light-Inducible Promoters**

We have shown previously that the *taf1* mutation reduces the acetylation of histones H3 and H4 on the promoters of *CAB2* and *RBCS1-A* (Bertrand et al., 2005). To study whether the deregulation of light-inducible gene expression observed in *gcn5* and *hd1* mutants was also linked to alterations in histone acetylation on the genes, we performed chromatin immunoprecipitation

(ChIP) assays. Chromatin fragments isolated from 12-d-old light-grown seedlings of the wild type and *gcn5* or *hd1* mutants were immunoprecipitated with antibodies raised against acetylated histone H3 or acetylated histone H4 (Upstate Biotechnology; see Methods). The precipitated chromatin DNA was analyzed by real-time PCR to test for enrichment relative to nonprecipitated (input) genomic DNA according to Frank et al. (2001). The enrichment of promoter fragments relative to input chromatin DNA in the wild type (arbitrarily given as 100%) was compared with that found for each mutant. Primer sets used corresponded to upstream promoter regions (~1.5 to 2 kb from the initiation ATG codon), TATA box proximal or core promoters, coding regions, and 3' untranslated sequences of *CAB2*, *RBCS1-A*, and *IAA3* (Figure 4). Because the intergenic space between *CAB2*

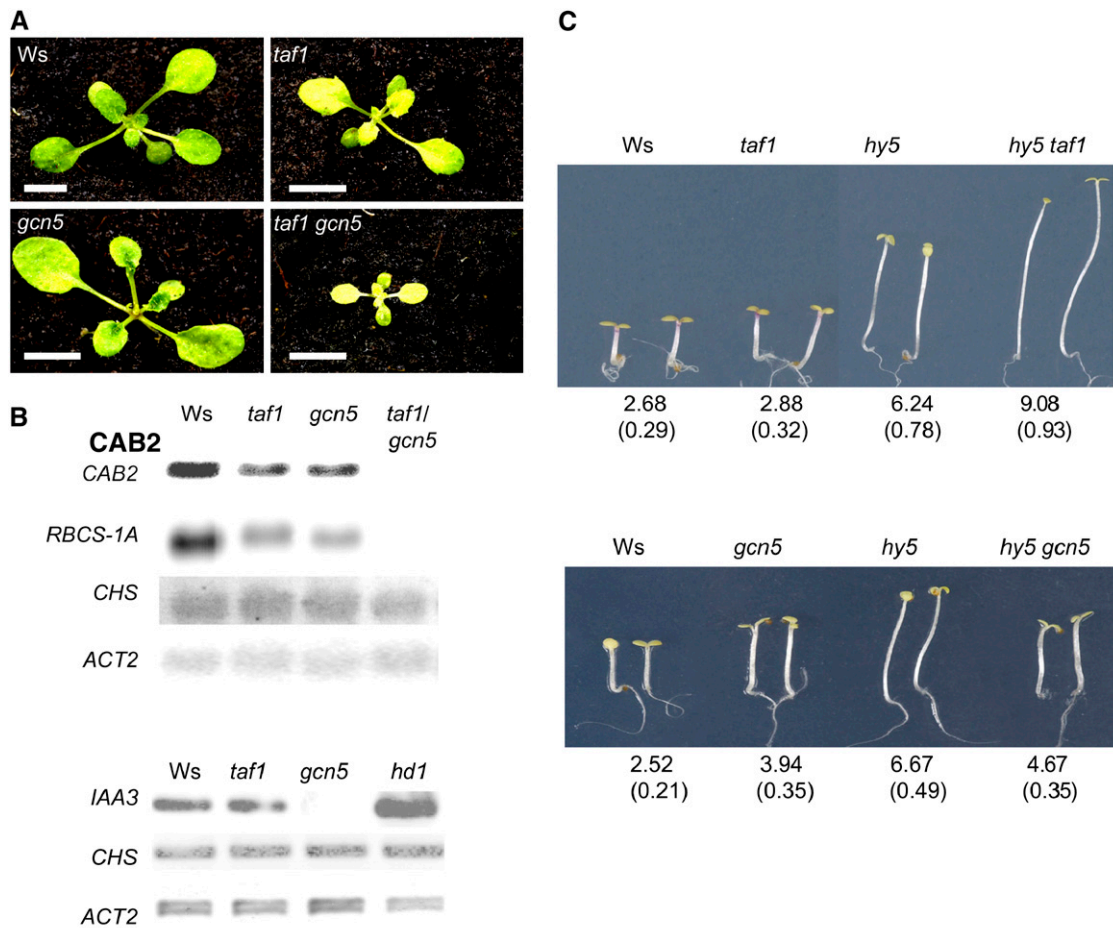


Figure 3. Genetic Relationship between *gcn5* and *taf1* Mutations.

(A) Phenotypes of *taf1 gcn5* double mutants with rosette leaves (3 weeks) grown in the greenhouse. Bars = 0.5 cm.

(B) Top, expression of the light-inducible genes *CAB2*, *RBCS-1A*, and *CHS* in the double mutant compared with that in Ws and the single mutants. Bottom, comparison of expression levels of *IAA3* and *CHS* in Ws, *gcn5*, *taf1*, and *hd1*.

(C) Comparison of 7-d-old seedling hypocotyl lengths of *taf1 hy5* (top) and *gcn5 hy5* (bottom) double mutants grown under continuous far-red light ($5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Average lengths (in millimeters) are indicated at bottom with SD values from at least 20 plantlets (in parentheses).

and the upstream gene *CAB1* constitutes <1.5 kb, no upstream promoter primer set was designed for *CAB2*. Because the expression of *CHS* was not affected by the different mutations, a primer set corresponding only to the core promoter region of *CHS* was used as a control. From the results of these experiments (Figure 4), several observations could be made. (1) The *gcn5* mutation induced a clear decrease of histone H3 acetylation on the core promoter region of *CAB2*, *RBCS-1A*, and *IAA3*. Histone H4 acetylation was decreased on the core promoter of *RBCS-1A*. However, the mutation had no or much less effect on the other analyzed regions of the three genes. (2) By contrast, the *hd1* mutation increased the acetylation levels of both histones H3 and H4, not only on the core promoter of the three genes but also on the upstream region of *IAA3* and, to a lesser extent, *RBCS-1A*. (3) Acetylation of histones H3 and H4 on the core promoter of *CHS* was not significantly affected by the mutations. These data suggested that the *gcn5* and *hd1* mutations had opposite effects

on the acetylation of histones H3 and H4 on the promoters of a subset of light-inducible genes.

Requirement of *GCN5*, *TAF1*, and *HD1* for the Acetylation/Deacetylation of Specific Histone Lys Residues

To know which Lys residues in the N-terminal end of histones H3 and H4 were the respective targets of *GCN5*, *TAF1*, and *HD1*, we performed ChIP assays with antibodies raised against specific acetylated Lys residues of histones H3 and H4. The N-terminal tail of histone H3 has five acetylated lysines, K9, K14, K18, K23, and K27, whereas that of H4 has four (K5, K8, K12, and K16). Specific antibodies against H3K9, H3K14, H3K27, H4K5, H4K8, H4K12, and H4K16 (Upstate Biotechnology) were used in this set of ChIP assays. The core promoter regions of three representative genes (*RBCS-1A*, *IAA3*, and *CHS*) were quantified by real-time PCR using the primer sets indicated in Figure 4. The light

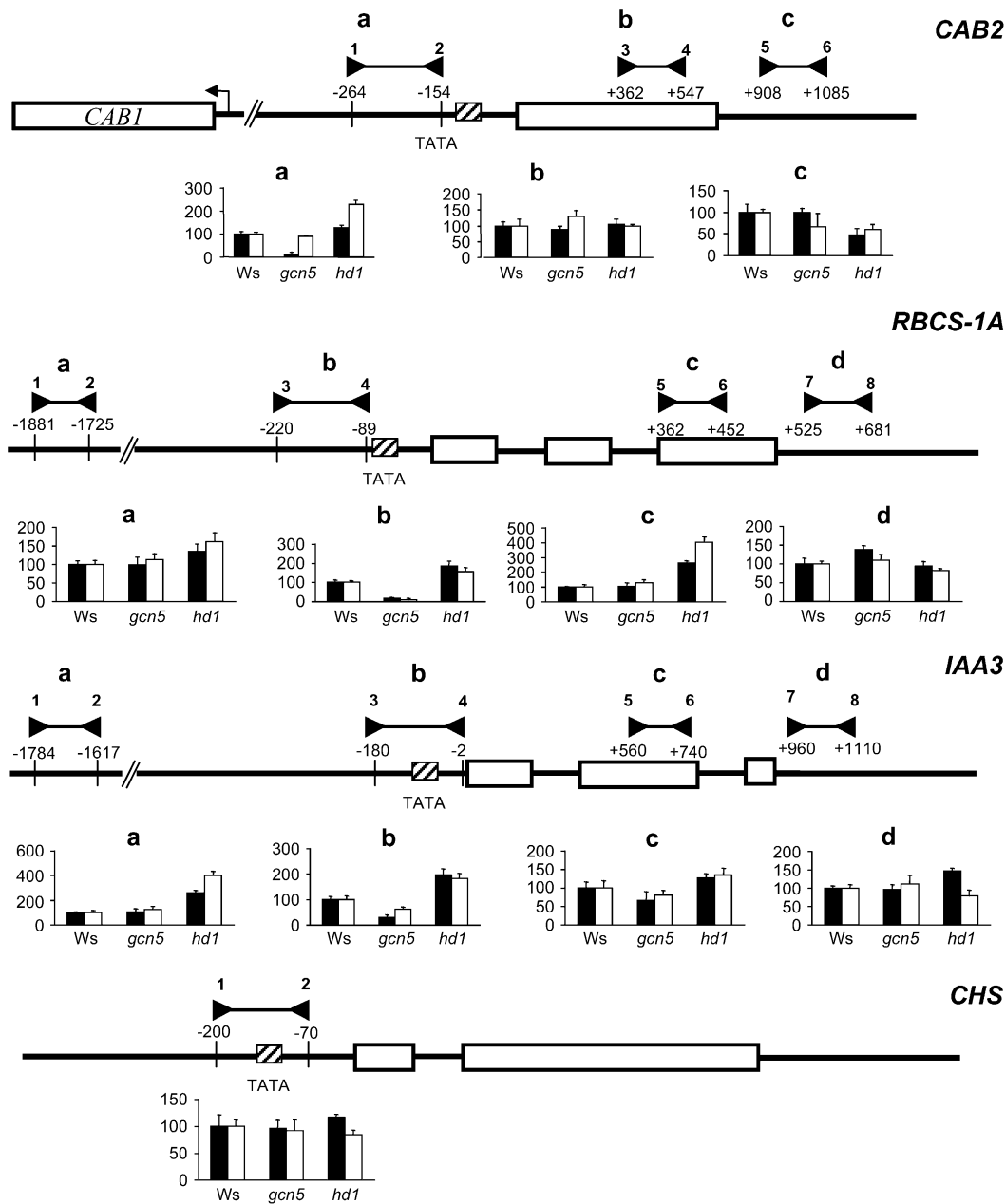


Figure 4. Acetylation State of Histones H3 and H4 of *CAB2*, *RBCS-1A*, *IAA3*, and *CHS* in *Ws*, *gcn5*, and *hd1* Seedlings.

Nuclei were extracted from cross-linked 12-d-old light-grown seedlings, sonicated, and immunoprecipitated with antibodies specific to total histone H3, acetylated histone H3, and acetylated histone H4. The immunoprecipitates were analyzed by real-time PCR. The primer sets (arrowheads) are numbered for each gene (open boxes represent the translated regions), and the positions of the primers relative to the initiation ATG codon are indicated. The relative amounts of the PCR products compared with input chromatin from wild-type extracts (arbitrarily given as 100) are shown below the genes. Black bars, acetylated histone H3; white bars, acetylated histone H4. Error bars represent SD values from at least three repetitions.

activation of *RBCS-1A* was shown to be regulated by both *GCN5* and *TAF1*, whereas that of *IAA3* was dependent only on *GCN5*. The mutation of *HD1* increased the expression from both promoters. The expression of *CHS* was not affected by the three mutations. The enrichment of the promoter fragments relative to input chromatin DNA in the wild type (arbitrarily given as 100%)

was compared with that found in each mutant. As shown in Figure 5, the *gcn5* mutation significantly decreased the acetylation of H3K9, H3K14, H3K27, and H4K12 on both promoters, with the decreases most pronounced on the H3 Lys residues. The *taf1* mutation significantly impaired the acetylation of H3K9, H3K27, and H4K12 on the *RBCS-1A*, but not on the *IAA3*,

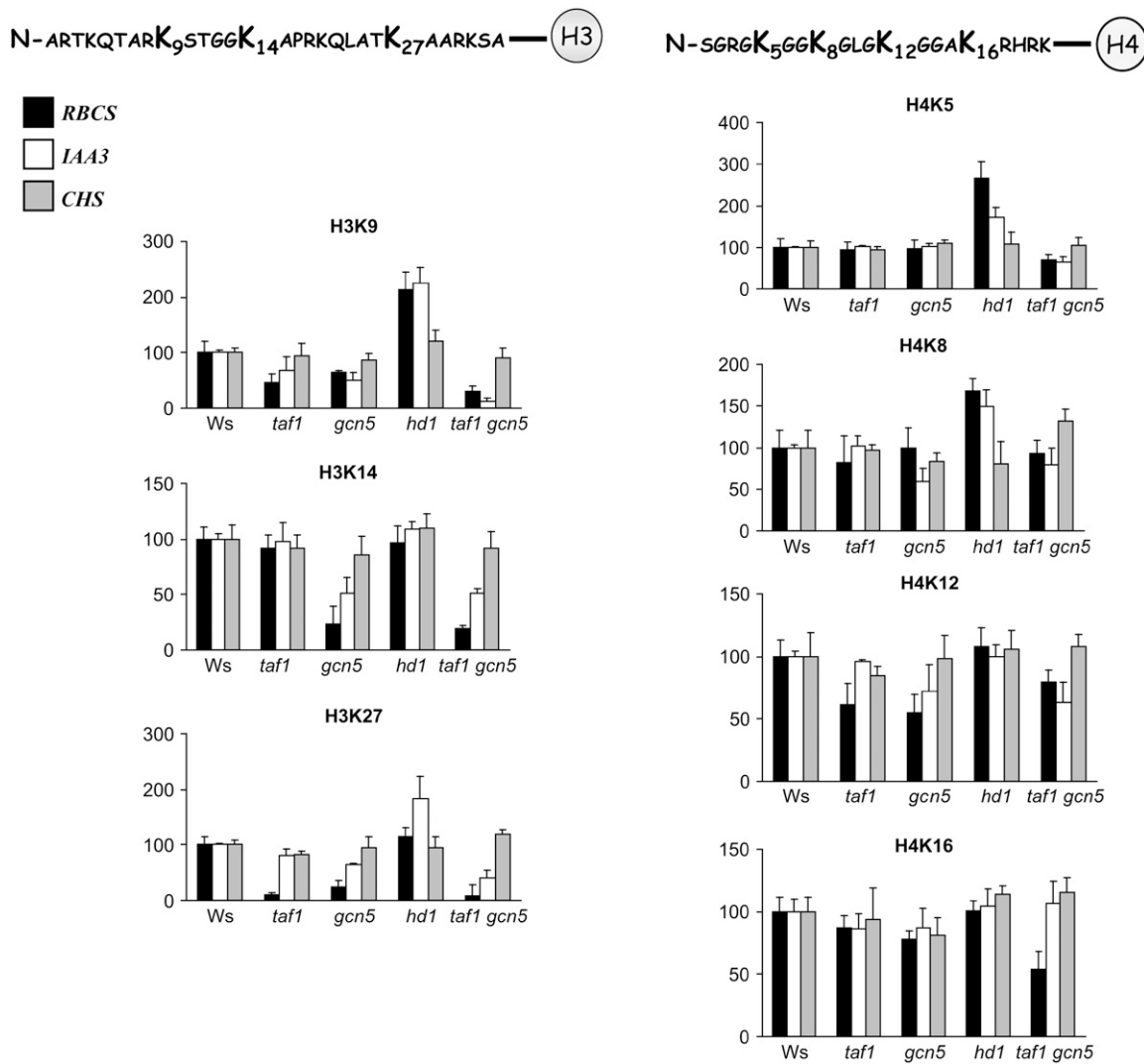


Figure 5. Acetylation Profiles of the N-Terminal Tails of Histones H3 and H4 on Light-Responsive Promoters in *taf1*, *gcn5*, *hd1*, and *taf1 gcn5* Compared with Wild Type Ws.

As indicated, ChIP with specific antibodies against histones H3K9, H3K14, and H3K27 and H4K5, H4K8, H4K12, and H4K16 were used. The immunoprecipitated promoter fragments of *RBCS-1A* (black boxes), *IAA3* (white boxes), and *CHS* (gray boxes) were quantified by real-time PCR. The relative amounts of the promoter fragments compared with input chromatin from wild-type extracts were arbitrarily given as 100. Error bars represent SD values from at least three repetitions.

promoter, in agreement with its nonrequirement for the activation of this gene. Thus, acetylation of H3K9, H3K27, and H4K12 depended on both GCN5 and TAF1, whereas acetylation of H3K14 depended only on GCN5. By contrast, the *hd1* mutation highly increased the acetylation levels of H3K9, H4K5, and H4K8 on both promoters, whereas a significantly increased acetylation on H3K27 was detected on the *IAA3* promoter. Interestingly, compared with the single mutations, the *taf1 gcn5* double mutation induced a further loss of acetylation of H3K9, and to a lesser extent of H3K27 and H4K5, of both *RBCS-1A* and *IAA3* core promoters. With the *CHS* core promoter primer set, no significant changes were detected in the mutants with respect to

the analyzed Lys residues, except for the *taf1 gcn5* double mutant that showed some increase of H4K8 acetylation (Figure 5).

GCN5 Is Recruited to the Light-Inducible Promoters

The results described above suggested that GCN5 might act directly on the light-responsive promoters. We tested this hypothesis by ChIP assays using a polyclonal antibody raised against GCN5 (made using two peptide epitopes located between amino acids 85 and 99 and between amino acids 136 to 150, corresponding to the N-terminal region of the protein; see Methods). On protein gel blots, the antibody recognized the

recombinant GCN5 protein produced in *Escherichia coli* (Figure 6A), which showed a molecular mass close to 60 kD (the calculated molecular mass being 62 kD). In wild-type nuclear protein extracts, a protein band was detected that migrated to a position similar to that of the recombinant protein. In the *gcn5* mutant, a protein band with a lower molecular mass was detected, suggesting that the T-DNA insertion (located in the 3' end of the gene) might have produced a truncated or hybrid GCN5 protein (Bertrand et al., 2003). The precipitated chromatin fragments were analyzed by real-time PCR to test for enrichment relative to input chromatin. For *CAB2* and *RBCS-1A*, the GCN5 antibody precipitated ~0.3 and 0.5% of the input in the wild type, whereas <0.1% was precipitated in *gcn5* (Figure 6B). The *CHS* promoter was not precipitated significantly by the GCN5 antibody in either the wild type or *gcn5*. These data indicate that GCN5 was recruited specifically to the target light-responsive promoters.

DISCUSSION

Arabidopsis has at least 12 HAT and 18 HDAC genes (Pandey et al., 2002), and the members may have either redundant or specific functions. Our data have shown that at least three of them are required for normal photomorphogenesis and light-regulated gene expression. Thus, the control of histone acetylation levels is an important mechanism for light-regulatory processes in plants.

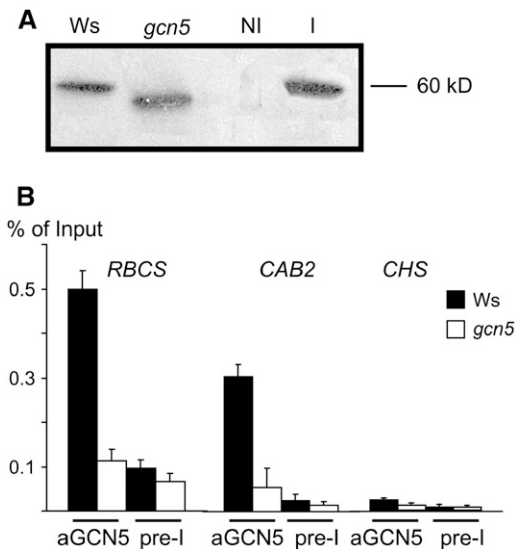


Figure 6. GCN5 Was Associated with the Target Light-Responsive Promoters.

(A) Protein gel blot analysis of nuclear extracts from wild-type (Ws) and *gcn5* rosette leaves and protein extracts of *E. coli* transformed by a plasmid expressing GCN5 (I, induced by isopropylthio- β -galactoside; NI, noninduced).

(B) Real-time PCR quantification of *RBCS-1A*, *CAB2*, and *CHS* core promoter regions (as in Figure 4) immunoprecipitated by the GCN5 antibodies or by the preimmune serum. The signals are given as percentage of the input chromatin. Error bars represent SD values from at least three repetitions.

Function of GCN5 and HD1 in the Light Regulation of Gene Expression

The light-hyposensitive phenotype and the decreases in gene expression and histone acetylation in *gcn5* mutants (Figures 1 to 3) indicate that GCN5 is a positive regulator of a subset of light-inducible genes. Two lines of evidence suggest that GCN5 is involved directly in the transcriptional regulation of the light-inducible genes. (1) Decreases of histone acetylation induced by the *gcn5* mutation were detected essentially on the core promoter regions (Figure 4). (2) ChIP assays using a GCN5 antibody revealed direct association of the protein with the analyzed photosynthetic gene promoters (Figure 6). The recruitment of GCN5 to the promoters may be mediated by interactions with specific DNA binding proteins, although it could also bind to promoter-associated acetylated histone Lys residues through its C-terminal bromodomain. Association of GCN5 with the promoters would induce either higher levels of overall histone acetylation or specific histone Lys acetylation required for light-activated transcription. These two hypotheses may not be mutually exclusive, because the *gcn5* mutation reduced the acetylation of at least four Lys residues in the N-terminal tails of histones H3 and H4, with H3K14 and H3K27 being the most affected (Figure 5). This acetylation profile of histone Lys residues is in agreement with the function of yeast and animal GCN5 proteins that preferentially acetylate histone H3 in vitro (Grant et al., 1997).

Our data show that HD1 negatively regulates light-responsive gene transcription by reducing histone acetylation levels. The *hd1* mutation induced histone acetylation on both upstream and core promoter regions, suggesting that HD1 might operate on a relatively larger range of the promoters. Tian et al. (2005) have shown that the HD1 mutation induces the acetylation levels of H3K9 and H4K12 on different target promoters. However, our data show that the mutation induced increases of acetylation levels of H3K9, H3K27, H4K5, and H4K8 on the light-responsive promoters studied (Figure 5). Therefore, the regulation of histone acetylation pattern by HD1 might be promoter-specific.

The normal photomorphogenic phenotype and light-responsive gene expression observed in the *hd1 gcn5* double mutant (Figures 1B and 2C) suggest that GCN5 and HD1 might form an antagonistic HAT/HDAC couple to control the reversible histone acetylation required for a light-regulated switch of gene expression. However, we have observed that the histone Lys residues, of which the acetylation levels were reduced by the *gcn5* mutation, did not correspond completely with those hyperacetylated in the *hd1* mutant (Figure 5). For instance, H3K14 acetylation was reduced in *gcn5*, but it was not altered in *hd1*. Conversely, H4K5 acetylation was enhanced in *hd1*, but it was not affected by the *gcn5* mutation. However, H3K9 acetylation was increased in *hd1* and decreased in *gcn5* mutants. It is known that H3K9 acetylation is an essential marker of gene activation (Jenuwein and Allis, 2001). Hypoacetylation of H3K9 induces its methylation, leading to the formation of repressive chromatin. The antagonist function of the GCN5/HD1 couple may operate on the acetylation levels of this Lys residue. Synergistic effects of acetylation exist between neighboring Lys residues (Jenuwein and Allis, 2001). The regulation of light-inducible gene expression by GCN5 and HD1 also could be interpreted by a cumulative

mechanism of histone acetylation. We noted that both *gcn5* and *athd-1* mutations also affected *CAB2* and *RBCS-1A* mRNA levels in the dark (Figure 2). This finding suggests that the balance of histone acetylation and deacetylation maintained by GCN5 and HD1 is also required for the basal transcription of the light-inducible genes. Furthermore, GCN5 has been shown to be involved in the expression of cold-inducible genes (Stockinger et al., 2001; Vlachonasios et al., 2003). Recently, the *Arabidopsis* *FVE* gene, which encodes a homolog of a mammalian HDAC complex component, was found to be a negative regulator of the CBF/DREB-dependent cold-responsive pathways (Kim et al., 2004). Thus, distinct HAT/HDAC couples might be formed to carry out reversible histone acetylation in response to different environmental signals.

Functional Relationship between Histone Acetyltransferases in Light Regulation

The regulation of gene transcription involves transcription coactivators and corepressors capable of transmitting signals from enhancer-bound specific transcription factors to the RNA polymerase initiation complex (Sternier and Berger, 2000). We have shown that the disruption of both *GCN5* and *TAF1* reduced light-responsiveness. In yeast and mammalian cells, GCN5 is a key component of several transcription cofactors, including the SAGA complex (Carozza et al., 2003). TAF1 within the TFIID complex also functions as a transcriptional coactivator (Sternier and Berger, 2000). Transcriptional cofactors are recruited to promoters by interacting with enhancer-bound transcription factors. A combination of several light-responsive elements is required to construct a light-activating enhancer module, suggesting the possible involvement of transcription coactivators (Martinez-Hernandez et al., 2002; Yadav et al., 2002, 2005; Maxwell et al., 2003). Recent results have shown that the *PetE* enhancer activates transcription by mediating histone acetylation on the promoter (Chua et al., 2003), suggesting that light-responsive enhancers may interact, via the cognate DNA binding factors, with a HAT-containing transcriptional coactivator. Our results suggest that GCN5 and TAF1 might be the bona fide light-responsive coactivators.

In contrast with the *gcn5* mutants, the *taf1* mutants did not show the typical (light-hyposensitive) long-hypocotyl phenotype under different light conditions. However, this mutation could dramatically enhance the long-hypocotyl phenotype of *hy5* mutants, irrespective of light quality (Bertrand et al., 2005) (Figure 3C). Differences between the two mutants also existed at the level of the target genes. For instance, *gcn5*, but not *taf1*, severely affected the expression of the light-activated *IAA3* gene. This difference suggests that the recruitment of the proteins may be dependent on promoter structure. TAF1 is believed to be recruited by the TATA binding protein TBP, whereas GCN5 proteins can be recruited by interaction with specific DNA binding transcription factors. The enhancement of the *hy5* phenotype by the *taf1* mutation suggests that TAF1 may function in a transcriptional pathway in parallel with that of the basic domain/Leu zipper protein HY5. The phenotype observed in the *gcn5 hy5* double mutant suggests that GCN5 has a different relationship with HY5 compared with TAF1.

The analysis of the *taf1 gcn5* double mutant suggests that GCN5 and TAF1 have a cumulative function in light regulation (Figure 3). This hypothesis is supported by the ChIP data showing that the two genes have redundant and distinct requirements for the acetylation of both histones H3 and H4 on the targeted promoters (Figure 5). Therefore, it is likely that both the cumulative and specific mechanisms of acetylation of histone Lys residues proposed by Dion et al. (2005) are involved in light regulation. These observations are in agreement with the redundant and distinct roles of GCN5-containing SAGA and TAF1-containing TFIID complexes in global transcription in yeast (Lee et al., 2000; Huisinga and Pugh, 2004). In addition, both GCN5 and TAF1 proteins contain a bromodomain in their C-terminal ends, which is known to bind to acetylated histone Lys residues. Histone acetylation by one of the HAT proteins would induce the binding of the other to the chromatin, enhancing histone acetylation on the targeted promoters. Interestingly, the ChIP assays presented in Figure 5 showed that the cumulative effect of the *taf1 gcn5* double mutation was mostly observed on H3K9 acetylation, which, as mentioned previously, is critical for gene activation (Jenuwein and Allis, 2001).

METHODS

Plant Materials and Growth Conditions

The *Arabidopsis thaliana* T-DNA insertion mutant plants studied in this work were in the Ws background (for *gcn5*, see Bertrand et al., 2003; for *gcn5-1*, see Vlachonasios et al., 2003; for *athd-1*, see Tian et al., 2003; for *taf1*, see Bertrand et al., 2005). Three backcrosses were performed on *gcn5* and *taf1*. The *hy5-1* mutant (in the Landsberg background) was obtained from the Nottingham Arabidopsis Stock Centre. *Arabidopsis* plants were grown in a greenhouse under long-day conditions (16 h of light) at 19.5°C (day) and 17.5°C (night). For in vitro cultures, seeds were sown on 0.5× Murashige and Skoog medium, incubated at 4°C for 48 h, and then transferred to growth chambers for germination at 20°C under white light (16 h/d at 120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or continuous red (10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), far-red (5 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), or blue (18 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) light. Hypocotyl lengths were measured 7 d after germination. Student's *t* tests were performed to determine whether the difference between two sample means was significant at $\alpha = 0.05$.

Construction of Double Mutants

Double mutants were made from genetic crosses with *gcn5* as the male donor and *hd1-t*, *taf1*, or *hy5-1* as female. Putative double mutants were selected from the F2 generation, genotyped by PCR using a T-DNA-matching primer and gene-specific primers to distinguish the wild type from the mutant DNA sequences for *gcn5* or *hd1* or by sequencing the genomic region of the *hy5-1* locus, and confirmed in the F3 generation based on the mutant phenotypes. The primers used for genotyping were as follows: *gcn5*, 5'-GGTATCGGGGAGTTGTAAGTTCTAC-3' and 5'-TTGTGCTAGTCGCTCCATGA-3'; T-DNA in *gcn5*, 5'-CTACAAATTGCCTTTATCGAC-3'; *hd1*, 5'-ATGCTGGAGGATCTGTTGG-3' and 5'-CCAGA-C AATGAATCAGCACC-3'.

Genomic DNA and Total RNA Extraction, PCR, and RNA Gel Blots

Arabidopsis leaves were used for genomic DNA extraction. PCR was performed using the Promega Taq polymerase. Total RNA was isolated from 12-d-old light-grown or dark-grown seedlings using TRIzol (Invitrogen).

For RNA gel blot analysis, 5 µg of total RNA were separated on 1% denaturing agarose gels, blotted onto a nylon membrane, and hybridized with ³²P-labeled gene-specific probes that were prepared from cDNA clones of *CAB2*, *RBCS-1A*, *CHS*, and *ACT2*. Hybridization signals were scanned with a Molecular Image FX Pro phosphor imager (Bio-Rad) and normalized to actin mRNA or rRNA signals using Bio-Rad Quantity One one-dimensional analysis software.

GCN5 Antibody and Recombinant Protein Production and Protein Gel Blots

Polyclonal antibodies were custom-made in rabbits (Eurogenetic) using two synthetic peptides (H₂N-CARGADTSDPDESED and H₂N-SSRNTKLKTESSTVKLC; both peptides are located on the N-terminal region of the protein) and affinity-purified. For recombinant GCN5 production, the cDNA was cloned into pQE30 (Qiagen) and introduced into *Escherichia coli* strain M15. The conditions used for induction and purification were described by Ayadi et al. (2004). For protein gel blots, nuclear proteins were extracted from the wild type or *gcn5* by grinding rosette leaves to a fine powder in liquid nitrogen. The powder was transferred to nuclear extraction buffer (0.4 M sucrose, 10 mM Tris-HCl, pH 8, 10 mM MgCl₂, 5 mM β-mercaptoethanol, and 0.1 mM phenylmethylsulfonyl fluoride [PMSF]), and the extracts were filtered twice through Miracloth (Calbiochem) and centrifuged at 4000g for 20 min at 4°C. The nuclei pellets were dissolved in 15% SDS-PAGE gel-loading buffer for electrophoretic analysis along with protein extracts from isopropylthio-β-galactoside-induced or noninduced *E. coli* cells transformed with the expression plasmid. The blots were incubated with the GCN5 antibody (1:500 dilution) overnight at 4°C and revealed by enhanced chemiluminescence (Amersham).

ChIP Assays

Immunoprecipitations were performed as described previously (Gendrel et al., 2002). Seeds of *Ws*, *gcn5*, *taf1*, *hd1*, and the double mutants were sterilized, kept for 2 d at 4°C, and grown in vitro under long-day conditions. Twelve-day-old seedlings were harvested and fixed in 1% formaldehyde for 15 min in a vacuum and then neutralized by 0.125 M Gly. After washing with sterilized water, the samples were ground in liquid nitrogen as described above. Nuclei pellets were suspended in a buffer containing 0.25 M sucrose, 10 mM Tris-HCl, pH 8, 10 mM MgCl₂, 1% Triton X-100, 5 mM β-mercaptoethanol, 0.1 mM PMSF, and protease inhibitors (one minitab per milliliter; Roche). The suspensions were transferred to microfuge tubes and centrifuged at 12,000g for 10 min. The pellets were suspended in 1.7 M sucrose, 10 mM Tris-HCl, pH 8, 2 mM MgCl₂, 0.15% Triton X-100, 5 mM β-mercaptoethanol, 0.1 mM PMSF, and protease inhibitors and centrifuged through a layer of the same buffer in microfuge tubes. The nuclear pellets were lysed in a buffer containing 50 mM Tris-HCl, pH 8, 10 mM EDTA, 1% SDS, and protease inhibitors. The lysed nuclei were sonicated four times for 15 s at 4°C followed by centrifugation. The supernatants containing chromatin fragments were diluted 10-fold with 1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8, and 167 mM NaCl. A 1 mL aliquot of the dilution was used for an immunoprecipitation assay.

The antibodies for histone acetylation tests were purchased from Upstate Biotechnology: anti-histone H3 (05-499), anti-acetylated H3 (06-599), anti-acetylated H4 (06-598), anti-acetyl-histone H3K9 (07-352), anti-acetyl-histone H3K14 (07-353), anti-acetyl-histone H3K27 (07-360), anti-acetyl-histone H4K5 (07-327), anti-acetyl-histone H4K8 (07-328), and anti-acetyl-histone H4K12 (07-323). Immunoprecipitated DNA was analyzed by real-time PCR (Light Cycler; Roche) using the following primers. For *CAB2*: 1, 5'-CATTCTTGTACAGAG-3'; 2, 5'-CGTTTAGTAGTCTTAC-3'; 3, 5'-GGAACGGAGTCAAGTTTGA-3'; 4, 5'-AACGGCTCCATCAAATAA-3'; 5, 5'-TTTGTGTTTGGTGGATGG-3'; 6, 5'-CAA-

TCACGTTGCTCGATTGT-3'. For *RBCS-1A*: 1, 5'-TGTTTACTGAGTCTCAAAGTGG-3'; 2, 5'-ACAATCTCGACCACGGAAAA-3'; 3, 5'-CAAGCCGATAAGGGTCTCA-3'; 4, 5'-TGATCGGAGGGTCTAGGATA-3'; 5, 5'-TGA-TGGACGGTACTGGACAA-3'; 6, 5'-TGGGGTACTCCTTCTTGAC-3'; 7, 5'-GGATCATCGATTTCGACAAC-3'; 8, 5'-CCGAAATTAACCCCAAGA-3'. For *IAA3*: 1, 5'-CTTACGTTCCACTGACGGATT-3'; 2, 5'-CTTGACAGAACAGGTCATAAGTTTG-3'; 3, 5'-GAAAACAGTTTCTCTCTCTACCA-3'; 4, 5'-TCTTCAAGAATTGCAGGAGAAG-3'; 5, 5'-TGGATGCTCATTGGT-GATGT-3'; 6, 5'-GCCTAACCTTTGGCTTCTG-3'; 7, 5'-GGTCTTAAGC-ATATGAACTGGAAC-3'; 8, 5'-GATCAATGAGAACGCAAAACAG-3'. For *CHS*: 1, 5'-CACAGAAAAGGGGGCTAACA-3'; 2, 5'-AGAGTTTGATGTT-GCTGTTGTG-3'.

Quantification of immunoprecipitated chromatin fragments was performed according to Frank et al. (2001).

Accession Numbers

Arabidopsis Genome Initiative locus identifiers for the genes mentioned in this article are as follows: *GCN5*, At3g54610; *HD1*, At4g38130; *TAF1*, At3g19040; *CAB2*, At1g29920; *RBCS-1A*, At1g67090; *CHS*, At5g13930; and *IAA3*, At1g04240.

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Arabidopsis GCN5, HD1, and TAF1/HAF2 Interact to Regulate Histone Acetylation Required for Light-Responsive Gene Expression

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