

Histone Modifications and Expression of Light-Regulated Genes in Arabidopsis Are Cooperatively Influenced by Changing Light Conditions^{[W][OA]}

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Here, we analyzed the effects of light regulation on four selected histone modifications (H3K4me3, H3K9ac, H3K9me2, and H3K27me3) and the relationship of these histone modifications with the expression of representative light-regulated genes. We observed that the histone modifications examined and gene transcription were cooperatively regulated in response to changing light environments. Using H3K9ac as an example, our analysis indicated that histone modification patterns are set up very early and are relatively stable during Arabidopsis (*Arabidopsis thaliana*) seedling development. Distinct photoreceptor systems are responsible for mediating the effects of different light qualities on histone modifications. Moreover, we found that light regulation of gene-specific histone modifications involved the known photomorphogenesis-related proteolytic system defined by the pleiotropic CONSTITUTIVE PHOTOMORPHOGENIC/DE-ETOLIATED proteins and histone modification enzymes (such as HD1). Furthermore, our data suggest that light-regulated changes in histone modifications might be an intricate part of light-controlled gene transcription. Thus, it is possible that variations in histone modifications are an important physiological component of plant responses to changing light environments.

Light is the single most important environmental parameter for plant development. Arabidopsis (*Arabidopsis thaliana*) can detect all facets of light, such as direction, duration, quantity, and wavelength, and adapt its development to changes in different light parameters (Fankhauser and Chory, 1997; Whitelam et al., 1998; Thomas, 2006). Previous work revealed that light causes a large-scale reorganization of chromatin during the floral transition in Arabidopsis (Tessadori et al., 2007) and that the presence or absence of light results in distinct gene expression profiles during the development of Arabidopsis seedlings (Ma et al., 2001; Jiao et al., 2005). The transition of growth from skotomorphogenesis to photomorphogenesis is one example of a light-regulated developmental process in Arabidopsis. Although many protein factors involved in light signaling pathways have been identified by genetic screens (Somers et al., 1991; Wei et al.,

1994; Devlin et al., 1998), the detailed mechanisms of light-regulated gene transcription remain obscure. The discovery of light-regulated transcription factors (such as HY5, HYH, and PIF3) and their binding sites (light-responsive cis-elements) provided the first clues about its molecular mechanisms (Chattopadhyay et al., 1998; Ni et al., 1998; Holm et al., 2002). In the dark, pleiotropic CONSTITUTIVE PHOTOMORPHOGENIC/DE-ETOLIATED/FUSCA (COP/DET/FUS) proteins work cooperatively to inhibit light-induced plant development by ubiquitin-proteasome-mediated degradation of photomorphogenesis-promoting proteins, including transcription factors (Chory and Peto, 1990; Deng et al., 1991; Osterlund et al., 2000; Saijo et al., 2003; Jiao et al. 2007). Collectively, these data suggest the involvement of transcription factors in regulating light-responsive gene transcription and plant development.

Eukaryotic chromatin has a unique structure. Its basic units are nucleosomes, which consist of a histone core around which the DNA helix is wrapped. In this assembly state, the DNA is very compact and the histone proteins block gene expression by hampering the association of transcription factors with their binding sites and preventing the transcription machinery from moving along the DNA strands. A diverse array of posttranslational covalent modifications (e.g. methylation, acetylation, and phosphorylation) of the histone "tails" as well as some histone globular domains can influence nucleosome compaction and access to the DNA (Lee et al., 1993; Wolffe and Hayes, 1999;

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Jenuwein and Allis, 2001; Kouzarides 2007; Li et al., 2007; Zhang et al., 2007a). An additional layer of complexity is added to these modifications through their temporal and spatial distributions, their ability for cross talk, and the actions of coactivators (Jenuwein and Allis, 2001; Loidl, 2004; Li et al., 2007). Histone modifications also function as molecular signals to recruit transcription cofactors that enable the assembly and disassembly of histone complexes (Ahmad and Henikoff, 2002; Li et al., 2007). These rearrangements in the nucleosomal architecture provide freely accessible DNA such that transcription complexes can bind. In fact, histone modifications have been shown to be strongly correlated with gene expression in eukaryotes (Bernstein et al., 2000; Kurdiani et al., 2002, 2004; Barski et al., 2007; Zhang et al., 2007b), and recent studies in yeast and mammalian cells suggested that these modifications play a critical role in transcription (Li et al., 2007).

While the effects of temperature on changes in histone modifications in plants are well known (Sung and Amasino, 2004; Chen and Tian, 2007), analyzing the effects of light on histone modifications has only just begun. Histone modifications are important for plant development (Wagner, 2003; Loidl, 2004) through transcriptional regulation (Chua et al., 2001, 2004; Zhang et al., 2007b). For example, light-regulated expression of the pea (*Pisum sativum*) plastocyanin gene (*PetE*) was reported to specifically associate with the acetylation of histones H3 and H4 (Chua et al., 2001, 2003). Furthermore, results from genetic analyses of histone acetyltransferase (HAT) and histone deacetylase (HDAC) mutants in *Arabidopsis* suggest a role of histone acetylation in light-activated gene expression (Bertrand et al., 2005; Benhamed et al., 2006). However, a systematic analysis of the regulation of histone modifications during photomorphogenesis is not available to date.

In this study, we found that four select histone modifications and the expression levels of representative genes were cooperatively regulated by light. Furthermore, our detailed analysis of changes in the acetylation of H3K9 in seedlings grown under different light conditions suggests that light-regulated changes of histone modifications may be an important component of light-controlled gene transcription during seedling development in *Arabidopsis*.

RESULTS

Seedlings Grown in Continuous White Light or Darkness Show Different Histone Modification Levels in Representative Light-Regulated Genes

Previous studies (Ma et al., 2001; Jiao et al., 2005) estimated that light affects gene expression in 20% to 30% of the *Arabidopsis* genome. To analyze the relationships between light and histone modifications, we chose six representative genes (Table I) that showed

distinct changes in their mRNA abundance in dark- and white light-grown *Arabidopsis* seedlings based on microarray data from Ma et al. (2001): At2g34430 (PSII light-harvesting complex gene 1.4), At3g54890 (LHCA1), At5g38430 (ribulose biphosphate carboxylase small chain 1B), and At5g62300 (40S ribosomal protein S20) are light-up-regulated genes, while At2g37130 (peroxidase 21) and At2g42790 (citrate synthase 3) are light-down-regulated genes. For each gene, specific primers were designed (Supplemental Table S1) to determine the mRNA steady-state levels in seedlings grown in white light or darkness by reverse transcription and then quantitative real-time PCR (RT-qPCR). The RT-qPCR products were sequenced to verify that only the desired target gene was amplified. As shown in Table I, we found qualitative agreement for all six genes between the microarray data of Ma et al. (2001) and the PCRs conducted here.

In order to examine whether light influences histone modifications and whether these light-induced alterations affect gene expression, we performed chromatin immunoprecipitation (ChIP) assays to determine the relative enrichment of four well-studied histone modifications (H3K4me3, H3K9ac, H3K9me2, and H3K27me3) at these six representative light-regulated loci. Previous studies showed that H3K4me3 is mainly localized in the coding sequence of active genes (Millar and Grunstein, 2006; Barski et al., 2007; Li et al., 2007), that H3K9ac tends to have a peak at the promoter region of active genes and extends to the whole coding region (Millar and Grunstein, 2006), and that H3K9me2 and H3K27me3 mostly accumulate in the promoter regions of silent genes (Bastow et al., 2004; Barski et al., 2007; Li et al., 2007). Therefore, we designed the primers for ChIP-qPCR accordingly and selected an amplicon region for H3K4me3 primers at the target genes' 5' coding regions (immediately downstream of the start codon); for H3K9ac, H3K9me2, and H3K27me3-related primers, the amplicon regions were mostly in the promoter regions (Supplemental Table S2). Chromatin fragments were isolated from 6-d-old Columbia-0 (Col-0) wild-type seedlings grown in the dark or in continuous white light. After incubating with specific antibodies against H3K4me3, H3K9ac, H3K9me2, and H3K27me3 individually, the precipitated DNA was analyzed by qPCR (Table II). The enrichment of ChIP fragments relative to non-precipitated (input) DNA was calculated according to Frank et al. (2001). We found that the histone modifications studied differed for most genes when white light and dark growing conditions were compared. When comparing RT-qPCR data (Table I) with ChIP-qPCR data (Table II), we found that five genes showed an increase in H3K4me3 or H3K9ac under white light or darkness in parallel to increased transcript levels in the same light environment. In contrast, H3K9me2 and H3K27me3 were enriched in darkness for four genes, while the transcript levels of the respective genes were up-regulated in white light. This means that changes in histone modifications and gene

Table I. Representative light-regulated genes

Relative quantification of transcript ratios of representative genes in 6-d-old wild-type seedlings grown in white light and darkness (the expression level of seedlings grown in darkness was defined as 1). Shown are the results from RT-qPCR conducted in this study and microarray data obtained in a previous analysis (Ma et al., 2001).

Locus	Function	Light-Regulated Gene Expression Ratio	
		Microarray	RT-qPCR
At2g34430	Chlorophyll <i>a/b</i> -binding protein	30.211	89.337
At2g37130	Peroxidase	0.111	0.140
At2g42790	Citrate synthase	0.498	0.657
At3g54890	Chlorophyll <i>a/b</i> -binding protein	20.148	44.757
At5g38430	Ribulose biphosphate carboxylase small chain	14.628	558.572
At5g62300	Ribosomal protein	12.458	3.352

transcript levels are cooperatively regulated by light during *Arabidopsis* development. Interestingly, the degree of alterations of histone modifications was less pronounced than the gene expression level changes. This suggests that relatively small alterations in histone modifications might cause large changes in gene expression.

H3K9ac Correlates with mRNA Levels and Is Set Up in a Developmentally Dependent Manner

Based on our RT-qPCR and ChIP-qPCR data detailed above, we selected four representative genes for which we found strong indications for a correlation of histone modifications and transcript levels. Here, we examined a possible light-regulated relationship between H3K9ac enrichment and mRNA steady-state levels in detail by conducting a time course experiment in which wild-type seedlings (Col-0) were grown in continuous darkness for 6 d and then moved to white light for 0, 2, 4, 6, or 24 h. If a correlation exists between histone modifications and light-regulated gene expression, alterations in modification status and gene expression should follow similar patterns. RT-qPCR data shown in Figure 1A demonstrate that two of the genes, PSII light-harvesting complex gene 1.4 (At2g34430) and ribulose biphosphate carboxylase small chain 1B (At5g38430), were light up-regulated,

while the other two, peroxidase 21 (At2g37130) and citrate synthase 3 (At2g42790), were light down-regulated. Furthermore, we performed ChIP assays followed by qPCR to determine the enrichment of H3K9ac using the same biological material as for the RT-qPCRs to determine the mRNA levels. We found that for genes that were up-regulated by light, the relative abundance of H3K9ac also increased with extending exposure to white light. For PSII light-harvesting complex gene 1.4 (At2g34430), the correlation coefficient between ChIP-qPCR and RT-qPCR data was 0.97; for ribulose biphosphate carboxylase small chain 1B (At5g38430), the correlation coefficient was 0.85. This means that gene transcription and H3K9ac are highly correlated for these two genes. Conversely, relative H3K9ac enrichment decreased in light-down-regulated peroxidase 21 (At2g37130) the longer the plants were exposed to white light, although the expression level was up-regulated 2 h after the seedlings were transferred to light (Fig. 1B). At later time points, histone modifications were correlated with the gene expression. H3K9ac of citrate synthase 3 (At2g42790) did not show a dramatic down-regulation; however, its overall pattern corresponded well to our RT-qPCR data for this gene, especially after 2 h of light exposure. These results suggest that H3K9ac is regulated by light and that the mRNA levels of the four representative genes chosen

Table II. Histone modification changes in representative light-regulated genes

Two-tailed Student's *t* tests, resulting in *P* values, were performed to analyze the probability that wild-type seedlings grown in white light (WT/W) and in darkness (WT/D) belonged to the same group. If there was a difference between the two groups of values ($P < 0.1$), the ratio of the mean values between WT/W and WT/D was further computed. Ratio values greater than 1 indicate that histone modification signals were more enhanced in white light conditions, and ratio values less than 1 indicate that histone modification signals tended to be enriched in darkness. W/D, White light-dark ratio.

Locus	H3K4me3		H3K9ac		H3K9me2		H3K27me3	
	<i>P</i>	W/D	<i>P</i>	W/D	<i>P</i>	W/D	<i>P</i>	W/D
At2g34430	0.070	1.932	0.039	3.475	0.055	0.751	0.002	0.513
At2g37130	0.032	0.580	0.045	0.491	0.132	–	0.117	–
At2g42790	0.127	–	0.046	0.507	0.019	0.352	0.108	–
At3g54890	0.041	1.632	0.383	–	0.001	0.363	0.007	0.365
At5g38430	0.006	2.598	0.005	5.718	0.013	0.490	0.025	0.481
At5g62300	0.924	–	0.190	–	0.014	0.486	0.001	0.657

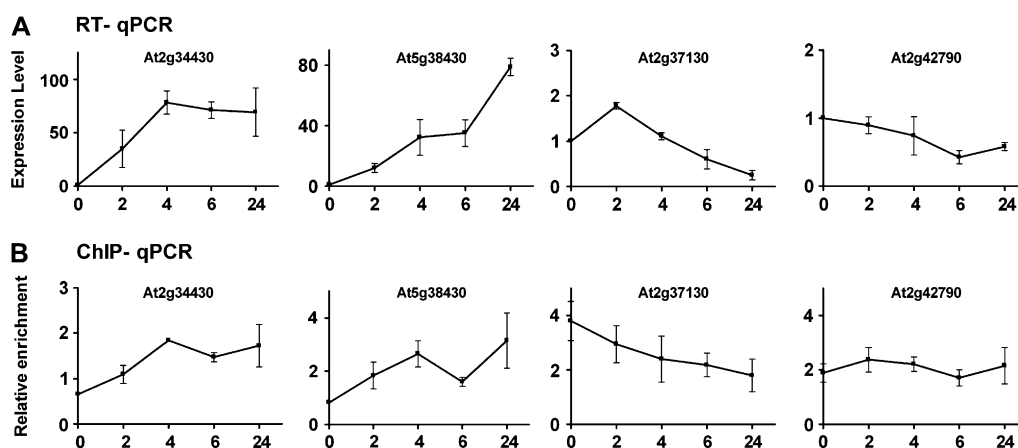


Figure 1. Time course analysis showing the relative enrichment of gene transcription and H3K9ac alterations at representative light-regulated loci. A, mRNA RT-qPCR data showing transcript changes at representative loci. RNA was extracted from Col-0 wild-type seedlings that were grown in the dark for 6 d and then exposed to continuous white light ($130 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 0, 2, 4, 6, or 24 h. Actin (At5g09810) was used as an endogenous control. All data were normalized with cDNA from Col-0 wild-type seedlings grown in the dark for 6 d (defined as 1). The x axis represents the light exposure time, and the y axis represents the relative transcript level, the ratio of light sample to dark sample. Error bars represent SE values computed based on at least three independently performed RT real-time experiments with independent tissue samples. B, Relative enrichment changes of H3K9ac at representative gene loci. The experiments were performed with the same samples as for the RNA isolation. The x axis represents the light exposure time, and the y axis represents the relative enrichment of H3K9ac, the percentage of immunoprecipitates compared with the corresponding input samples (defined as 100). Error bars represent SE values computed based on at least three independently performed ChIP experiments with independent tissue samples.

here might be correlated with the relative enrichment of H3K9ac.

At comparative time points, H3K9ac and gene expression changes did not show obvious alterations compared with dark-grown seedlings transferred to light (Fig. 1). We also conducted experiments in which white light-grown seedlings were transferred to darkness (data not shown). This result suggests that effects due to the decline of light signals are much slower than those due to light activation.

To investigate when during seedling development the observed H3K9ac patterns are set up for all four genes and whether these patterns are stable, we examined the relative enrichment of H3K9ac in 3-, 6-, and 9-d-old Col-0 wild-type seedlings grown in continuous white light or darkness with ChIP-qPCR experiments. We found that H3K9ac was set up already at the earliest time point studied for both light-up-regulated genes: PSII light-harvesting complex gene 1.4 (At2g34430) and ribulose biphosphate carboxylase small chain 1B (At5g38430; Fig. 2). While the histone acetylation level remained stable for At5g38430, it fell slightly for At2g34430 once the seedlings were 6 d old. Interestingly, the H3K9ac enrichment took longer in the light-down-regulated than in the light-up-regulated genes. For both peroxidase 21 (At2g37130) and citrate synthase 3 (At2g42790), the levels of H3K9ac were almost identical for seedlings grown in white light or darkness after 3 d and showed significantly higher levels only at the 6- and 9-d time points (Fig. 2). These data indicate that light-controlled H3K9ac patterns are set up within the first week of

seedling development, but at different time points and in a gene-specific manner depending on whether the respective genes are up- or down-regulated by light.

H3K9ac Enrichment Is Affected by Light Intensity and Light Wavelength

Previous studies (Ma et al., 2003) showed that light intensity influences the expression of light-regulated genes. Here, we analyzed the effects of light intensity on H3K9ac and mRNA abundance of selected genes in 6-d-old seedlings that were grown under different light intensities. As expected, we found that high light intensity led to a higher mRNA level of the light-up-regulated ribulose biphosphate carboxylase small chain 1B gene (At5g38430) and decreased the mRNA levels of peroxidase 21 (At2g37130), which is down-regulated by light. Citrate synthase 3 (At2g42790) was clearly down-regulated with increased light intensity, even though its transcript level initially rose when complete darkness and 1% intensity were compared. The transcript level of PSII light-harvesting complex gene 1.4 (At2g34430), which is light-up-regulated, showed a higher transcript level in every light intensity than in darkness but was more strongly expressed in low light intensities than in high ones (Fig. 3A). Similar observations have been made for other genes, and it has been found that increasing light intensity does not always lead to increased expression for certain light-up-regulated genes (Ma et al., 2003). Furthermore, we observed that the H3K9ac levels for these genes responded to changing light intensities

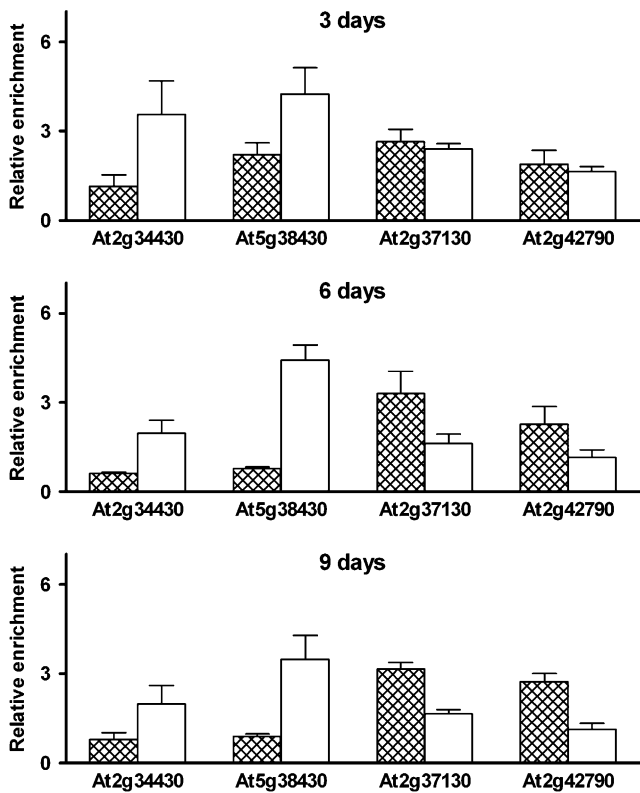


Figure 2. Comparison of light-regulated H3K9ac patterns in 3-, 6-, and 9-d-old seedlings. Col-0 wild-type seedlings grown in continuous dark (cross-hatched columns) were compared with Col-0 wild-type seedlings grown in continuous white light (white columns) at representative gene loci.

and rose or fell together with the respective mRNA levels (Fig. 3B). Together, these results suggest that H3K9ac and the expression level of representative genes may be influenced cooperatively by light intensity.

Second, we determined the effects of different light wavelengths on the relative enrichment of H3K9ac. We found that H3K9ac was up-regulated in PSII light-harvesting complex gene 1.4 (At2g34430) and ribulose biphosphate carboxylase small chain 1B (At5g38340) when Col-0 wild-type seedlings were grown in continuous color lights, as in white light. In general, seedlings did not show significant differences in H3K9ac in light-down-regulated peroxidase 21 (At2g37130) and citrate synthase 3 (At2g42790) when continuously exposed to different light wavelengths. The only exception to this was a pronounced decline in H3K9ac in At2g37130 when seedlings were grown in blue light (Fig. 4A). Collectively, these results suggest that H3K9ac responds gene specifically to changes in the light spectrum.

H3K9ac in *phy* and *cry* Photoreceptor Mutants Depends on Light Wavelength

To dissect the relationship between H3K9ac and light color in more detail, we examined H3K9ac en-

richment in 6-d-old wild-type seedlings and photoreceptor mutants that were continuously exposed to different light wavelengths (or darkness) during their growth. We found that for the light-up-regulated PSII light-harvesting complex gene 1.4 (At2g34430) and ribulose biphosphate carboxylase small chain 1B (At5g38430), the H3K9ac enrichment levels were higher in wild-type seedlings compared with *phyB phyD phyE*, *cry1 cry2*, and *phyA phyB phyE* photoreceptor mutants in all three light colors studied (red, blue, and far red). Genes that were down-regulated by light, peroxidase 21 (At2g37130) and citrate synthase 3 (At2g42790), showed the opposite (i.e. wild-type seedlings had a lower level of H3K9ac for these genes than mutant lines grown in the same light conditions, with the exception of far red light in At2g37130; Fig. 4B). The *phyB phyD phyE*, *phyA phyB phyE*, and *cry1 cry2* mutants used here are in different genetic backgrounds (Landsberg *erecta* [Ler] and Col, respectively, as detailed in "Materials and Methods"). However, an analysis of H3K9ac enrichment and mRNA levels for the genes under study in various Arabidopsis ecotypes showed that all genetic backgrounds behaved in a very similar fashion (Supplemental Fig. S1). Therefore, it can be reasonably assumed that the differences in H3K9ac enrichment observed here are based on the differences in light color and are gene specific rather than based on the genetic background of the wild-type and mutant plants used.

H3K9ac Enrichment Is Higher in the *hd1* Mutant Than in Wild-Type Seedlings

To investigate the relationships between H3K9ac and gene expression in detail, we analyzed an Arabidopsis line with a T-DNA insertion in the *HD1* gene (Supplemental Fig. S2). HD1 is one of 18 known HDACs in Arabidopsis (Pandey et al., 2002). Mutations in *HD1* have been shown to cause pleiotropic developmental abnormalities (Wu et al., 2000; Tian and Chen, 2001; Tian et al., 2003; Benhamed et al., 2006). Furthermore, previous studies demonstrated that mutations in HDACs can dramatically increase their target genes' acetylation levels, including H3K9ac (Tian et al., 2003, 2005; Benhamed et al., 2006). Here, we found that the H3K9ac level for four representative genes was higher in *hd1* mutant lines compared with the wild type in both light- and dark-grown seedlings, regardless of whether the respective genes were up- or down-regulated by light (Fig. 5A). This suggests that HD1 is involved in the maintenance of H3K9ac in a light-dependent manner.

Furthermore, we determined the transcription level of these representative genes. Standard controls routinely used in RT-qPCR assays to analyze the mRNA steady-state level of genes, such as actin and tubulin, cannot be chosen for HDAC mutant lines because the acetylation level of such control genes is affected by *hd1* mutations as well. This means that the transcript levels of these standard control genes do not remain

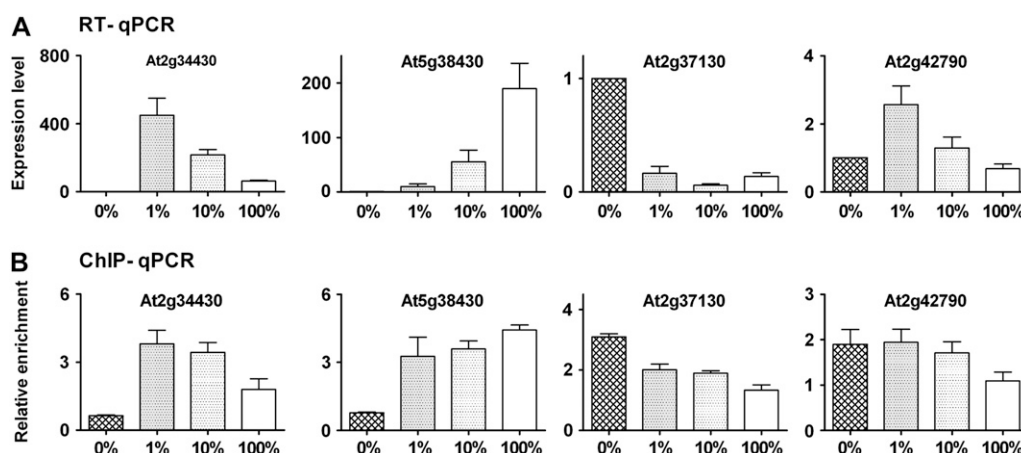


Figure 3. Analysis of light intensity-regulated gene transcription and H3K9ac patterns. A, Transcript levels of select genes in 6-d-old Col-0 wild-type seedlings grown at different light intensities. The x axis represents light intensity, where $130 \mu\text{mol m}^{-2} \text{s}^{-1}$ is defined as 100%. The y axis represents the transcript levels of the respective genes. B, H3K9ac enrichment patterns at representative gene loci in 6-d-old Col-0 wild-type seedlings grown continuously at different light intensities. The same samples as for the RNA isolation were used. The x axis represents light intensity as in A. The y axis represents the relative enrichment of H3K9ac.

constant in HDAC mutant lines, which prevents reliable quantification of the target genes. Previous reports circumvented this problem by using rRNA as normalization and loading equal amounts of total rRNA in RNA gel-blot assays instead (Benhamed et al., 2006). For RT-qPCR approaches, however, this issue remains an obstacle. Since the net transcript accumulation in RNA gel-blot assays depends on the rate of transcription and the kinetics of degradation (Folta and Kaufman, 2006), we took the binding capability of RNA polymerase II (Pol II) as a more direct index to determine the transcript level of the representative genes under study in our *hd1* mutant analysis. In other species (e.g. human), this index has been used successfully (Barski et al., 2007; Garcia-Bassets et al., 2007). We performed a ChIP assay with an antibody against Arabidopsis Pol II and then analyzed the relative enrichment of the genes under study in Pol II ChIP samples relative to input samples using qPCR (see Supplemental Table S4 for primers). We found that the transcript levels of all four genes under study were significantly higher in *hd1* mutants compared with wild-type plants in light- and dark-grown seedlings (Fig. 5B). The Pol II ChIP-qPCR results were very similar to our findings for H3K9ac (Fig. 5A) in that a higher relative enrichment for H3K9ac and Pol II binding was observed in *hd1* mutants. This means that a correlation between HD1-regulated H3K9ac levels and Pol II binding capabilities exists at the loci surveyed here. Furthermore, our data suggest that HD1-involved H3K9ac regulation of gene expression might function through an alteration of Pol II binding capacity.

H3K9ac Is Increased in *det1-1* and *cop1-4* Mutants

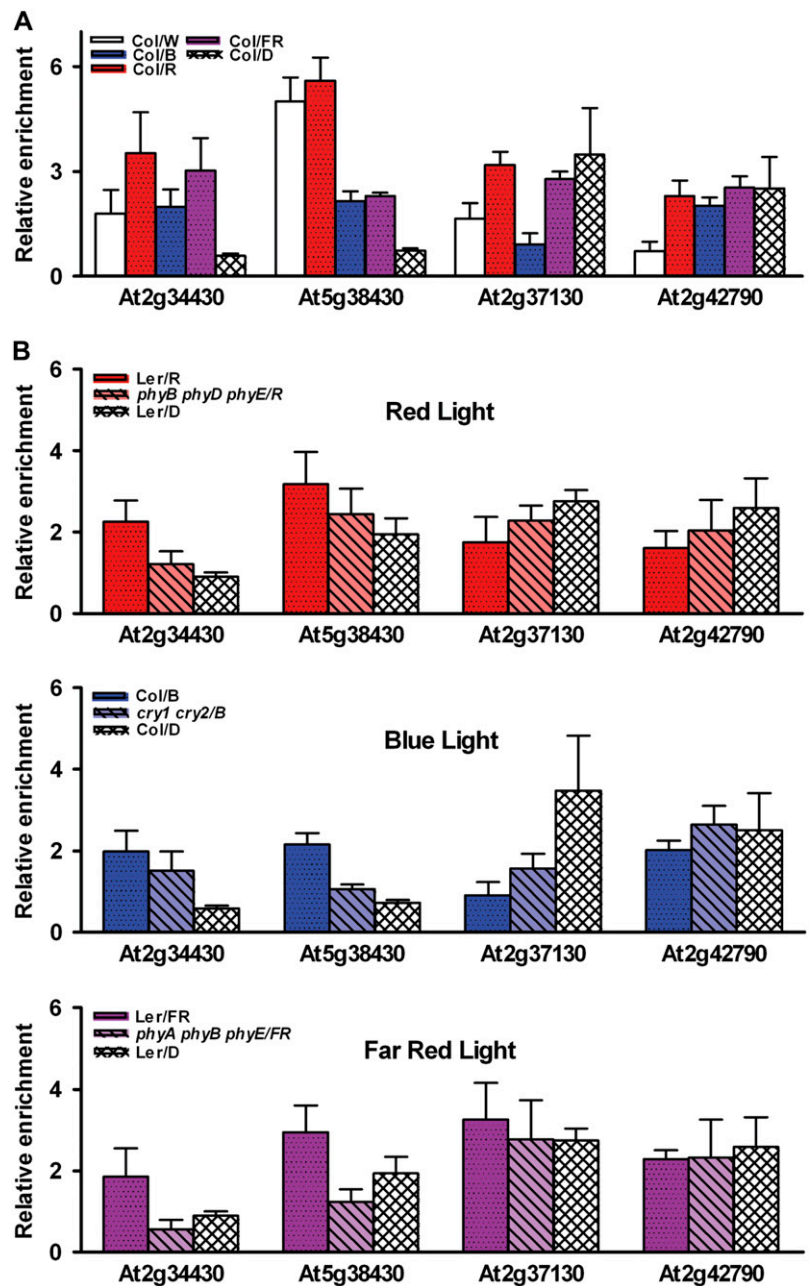
As one of the key factors in the photomorphogenesis pathway, DET1 has been shown to regulate gene expression in response to light signals through protein

degradation (Yanagawa et al., 2004). Furthermore, other studies suggested that DET1 might recruit HATs through binding to DDB1 and cause chromatin remodeling (Benvenuto et al., 2002; Schroeder et al., 2002). COP1 is another important protein factor during Arabidopsis photomorphogenesis. COP1 is a component of the COP/DET/FUS machinery, which is involved in the degradation of photomorphogenesis-promoting factors (Ang et al., 1998; Holm and Deng, 1999). However, to date, direct evidence of a relationship between DET1 or COP1 and histone acetylation in plants is lacking. Here, we used an H3K9ac-specific antibody for a ChIP assay using both *cop1-4* and *det1-1* mutants as well as Col-0 wild-type Arabidopsis seedlings (grown in white light or darkness) and studied the relative enrichment of H3K9ac at representative loci. We found a dramatic increase in H3K9ac in the light-up-regulated PSII light-harvesting complex gene 1.4 (At2g34430) and ribulose biphosphate carboxylase small chain 1B (At5g38430) in both *cop1-4* and *det1-1* mutant lines when the seedlings were grown in the dark (Fig. 6). These results further support a model in which light regulates histone modifications.

H3K9ac Is Suppressed in *hy5* Mutants

HY5 is a well-known transcription factor that can mediate light-induced gene expression (Chattopadhyay et al., 1998; Lee et al., 2007). In darkness, it is quickly degraded by the COP/DET/FUS machinery (Osterlund et al., 2000; Saijo et al., 2003). To analyze the H3K9ac enrichment in *hy5* mutant seedlings, we used a *hy5-215* Arabidopsis mutant line and used ChIP to study the relative enrichment of H3K9ac as well as RT-qPCR to determine the mRNA levels of representative light-regulated genes. We observed that the relative enrichment of H3K9ac was suppressed in 6-d-old white light-grown *hy5-215* mutant seedlings compared with

Figure 4. Analysis of H3K9ac patterns in wild-type seedlings and photoreceptor mutants grown at different light wavelengths. A, Comparison of H3K9ac patterns in 6-d-old Col-0 wild-type seedlings grown continuously at different light wavelengths. B, Blue light; D, darkness; FR, far-red light; R, red light; W, white light. B, Comparison of H3K9ac patterns in 6-d-old wild-type seedlings and photoreceptor mutants grown continuously at different light wavelengths and in the dark. Wild-type *Ler* seedlings were used for direct comparison with both *phyB phyD phyE* mutant and *phyA phyB phyE* mutant seedlings in their respective ecotypes. Col-0 wild-type seedlings were used for comparison with *cry1-304 cry2-1* mutants.



Col-0 wild-type seedlings at light-up-regulated loci (At2g34430 and At5g38430). Similarly, H3K9ac enrichment was reduced in *hy5-215* mutants for the two light-down-regulated loci under study (At2g37130 and At2g42790) when the plants were grown in continuous darkness (Fig. 7A). These results contrast with our findings for *cop1-4* and *det1-1* mutants, in which H3K9ac was increased, particularly for light-up-regulated genes, when the seedlings were grown in the dark (Fig. 6).

Moreover, we analyzed the mRNA steady-state levels of the four representative genes under study in *hy5-215* mutant and wild-type lines and found a significant reduction in the transcripts of light-up-regulated genes

in a *hy5-215* mutant background in white light-grown plants (Fig. 7B). Little difference was found at light-down-regulated loci in dark-grown samples, which is consistent with our results for H3K9ac and previous reports that HY5 promotes photomorphogenesis but is only responsible for limited contributions in the dark due to degradation (Ang et al., 1998; Chattopadhyay et al., 1998).

Light-Dependent HY5-Induced Transcription Is Influenced by H3K9ac in Light-Regulated Genes

Distinct histone modifications are not distributed equally over the entire gene territory but have specific

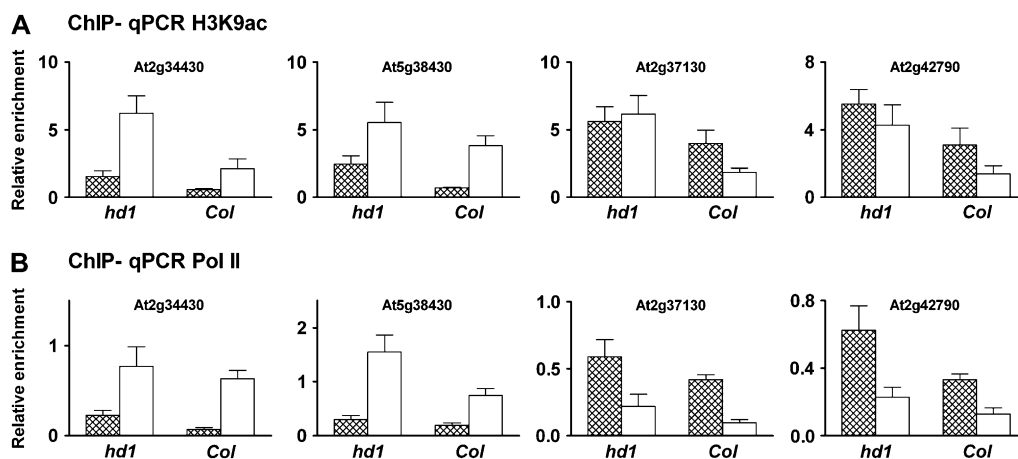


Figure 5. Comparison of H3K9ac and gene transcription changes between *hd1* mutant and Col-0 wild-type seedlings grown for 6 d in continuous darkness (cross-hatched columns) or white light (white columns). A, Comparison of H3K9ac alterations at representative gene loci between *hd1* mutant and Col-0 wild-type seedlings. B, Comparison of transcription changes between *hd1* mutant and Col-0 wild-type seedlings. The gene transcription level is shown as relative enrichment of RNA Pol II at representative loci.

spatial patterns that are tightly regulated and essential for their effects on transcription (Millar and Grunstein, 2006; Barski et al., 2007; Li et al., 2007). To further analyze the role of HY5 in histone modifications and gene transcription alterations, we determined putative HY5 binding sites in two representative genes, PSII light-harvesting complex gene 1.5 (At2g34420) and PSII light-harvesting complex gene 1.4 (At2g34430), using the PlantCARE database (Lescot et al., 2002). At2g34420 and At2g34430 are two PSII type I chlorophyll *a/b*-binding protein-coding genes that are located adjacent to each other in the Arabidopsis genome. Both genes share the same promoter but are transcribed in opposite directions. Two of the putative binding sites we obtained with this method were located in a HY5-enriched region in At2g34420 and At2g34430 that had been identified previously by a ChIP-chip assay (Lee et al., 2007; Fig. 8A). It has been reported that many light-responsive cis-elements, which are putative HY5 binding sites, are localized around transcription start sites (Lee et al., 2007).

Second, we surveyed the spatial distributions of H3K9ac and Pol II in different regions of At2g34420 and At2g34430 in both 6-d-old *hy5-215* mutant and wild-type seedlings using 15 primer pairs (Supplemental Table S5). The primers used here spanned the entire territories of these two genes, including their putative promoter region (Fig. 8A). The amplified products were sequenced to confirm the specificity of the primers. We found that H3K9ac was enriched in the coding region of both genes as well as their

promoter in seedlings grown in white light. The relative abundance of H3K9ac was lower in dark-grown seedlings compared with those grown in white light and showed a relatively uniform spatial distribution. H3K9ac enrichment in white light-grown *hy5-215* mutants decreased throughout the entire gene territory relative to wild-type seedlings (Fig. 8B). However, no significant differences could be detected between *hy5* and wild-type lines cultivated in the dark.

Third, we observed that Pol II accumulated in the coding regions of both genes under study, but not in the promoter region (Fig. 8C). Furthermore, similar to our findings for H3K9ac, the level of Pol II was higher in white light-grown wild-type seedlings than in *hy5* mutants, while no differences could be detected in dark-grown plants. Results obtained from RT-qPCR confirmed the Pol II-ChIP data and showed that the steady-state mRNA levels of At2g34420 and At2g34430 were reduced in *hy5* mutants grown in white light (data not shown). Together, these results suggest that light-dependent HY5-induced transcription is influenced by H3K9ac in light-regulated genes.

H3K4me3 Shows a Spatial and White Light-Dependent Distribution Similar to H3K9ac, But H3K9me2 and H3K27me3 Are Dissimilar and Are Up-Regulated in Darkness

It has been reported that different histone modifications have individual distribution patterns, which might hint at specific functions during gene transcription (Li

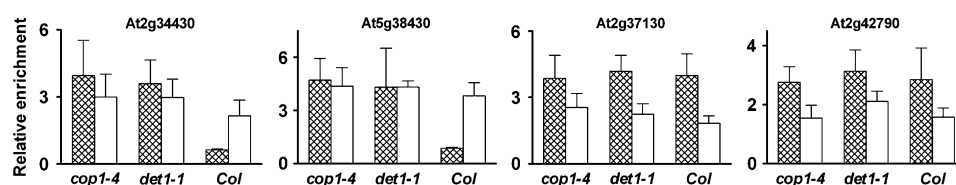


Figure 6. Comparison of H3K9ac changes in *cop1-4* and *det1-1* mutants and Col-0 wild-type seedlings grown in continuous darkness (cross-hatched columns) or white light (white columns) for 6 d.

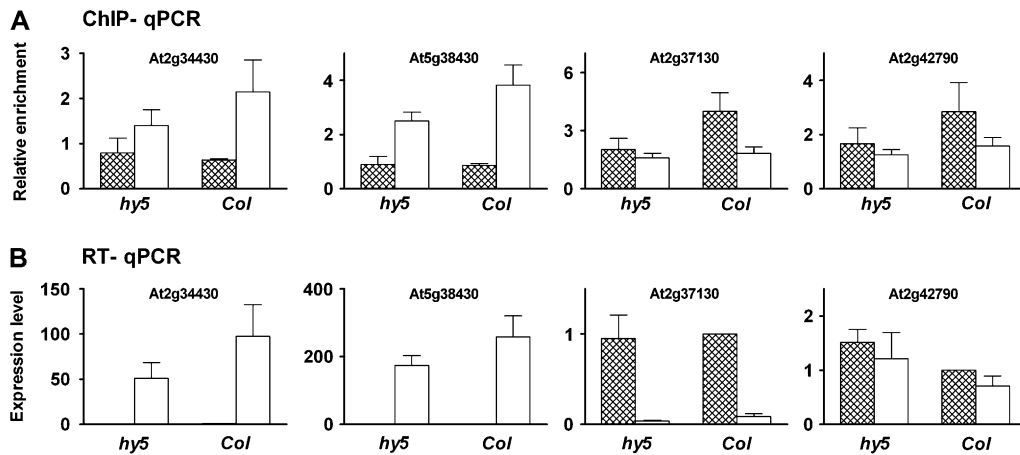


Figure 7. Comparison of H3K9ac and gene transcription changes between *hy5* mutant and Col-0 wild-type seedlings grown for 6 d in continuous darkness (cross-hatched columns) or white light (white columns). A, Comparison of H3K9ac changes between *hy5* mutant and wild-type seedlings. B, Comparison of gene transcription changes between *hy5* mutant and wild-type seedlings.

et al., 2007). To further analyze possible relationships between histone modifications and transcription of PSII light-harvesting complex gene 1.5 (At2g34420) and PSII light-harvesting complex gene 1.4 (At2g34430), we determined the relative spatial enrichment of three additional histone modifications. Specifically, we performed ChIP assays using H3K4me3, H3K9me2, and H3K27me3 antibodies in extracts obtained from 6-d-old Col-0 wild-type seedlings grown in white light and darkness. As shown in Figure 8D, H3K4me3 revealed a similar spatial distribution as H3K9ac (Fig. 8B), except that H3K4me3 signal was not enriched in the promoter region in white light-grown plants. In contrast, the relative enrichment of H3K9me2 and H3K27me3 was about 2-fold higher in dark-grown compared with white light-grown seedlings. It was more obvious in the promoter region of these two genes. These results are consistent with previous observations (Bastow et al., 2004; Millar and Grunstein, 2006; Barski et al., 2007; Li et al., 2007). In summary, this means that light clearly influences histone acetylation and methylation in a gene-specific and gene region-specific (coding region versus promoter) manner.

DISCUSSION

Using a ChIP-qPCR approach, we showed that light cooperatively regulates histone modifications and transcript levels of representative genes during seedling development in Arabidopsis. Collectively, our data suggest that changes in histone modifications might be a key process in plant responses to different light environments.

Light as a Regulator of Histone Modifications during Arabidopsis Photomorphogenesis

Our experiments delivered multiple lines of evidence to support a model in which histone modifica-

tions are influenced by light during Arabidopsis seedling development. First, we found that the enrichment of select histone modifications (H3K4me3, H3K9ac, H3K9me2, and H3K27me3) showed marked differences at representative loci in the presence or absence of light (Table II). Second, transferring dark-grown 6-d-old seedlings into white light was accompanied by changes in H3K9ac patterns (Fig. 1B). Third, we demonstrated that H3K9ac was affected by changes in light intensity and wavelength (Figs. 3B and 4), which could mean that different photoreceptors are involved in altering histone modifications. For this study, only partial photoreceptor mutants were available, such that not the entire light signaling pathway was blocked. The diversity and functional redundancy of photoreceptors might explain why the levels of H3K9ac in mutants were higher than in dark-grown wild-type seedlings. Previous studies suggested that PHYA is the primary photoreceptor for the far-red signaling pathway (Wang et al., 2002; Rockwell et al., 2006). Correspondingly, in PSII light-harvesting complex gene 1.4 (At2g34430) and ribulose biphosphate carboxylase small chain 1B (At5g38430), the H3K9ac enrichment was decreased most obviously in the *phyA phyB phyE* mutant compared with other mutants, which further supports our conclusions. Finally, we provided evidence for the involvement of the light-dependent transcription factor HY5 and of two key components of the photomorphogenesis pathway, COP1 and DET1, in the regulation of H3K9ac (Figs. 6 and 7). Together, these data demonstrate in detail that light specifically regulates histone modifications in Arabidopsis, complementing previous studies that showed that vernalization and flowering processes affect histone modifications (He et al., 2003; Bastow et al., 2004) and that light causes a large-scale reorganization of chromatin during floral transition in Arabidopsis (Tessadori et al., 2007). Furthermore, our data for several representative genes suggest that light-

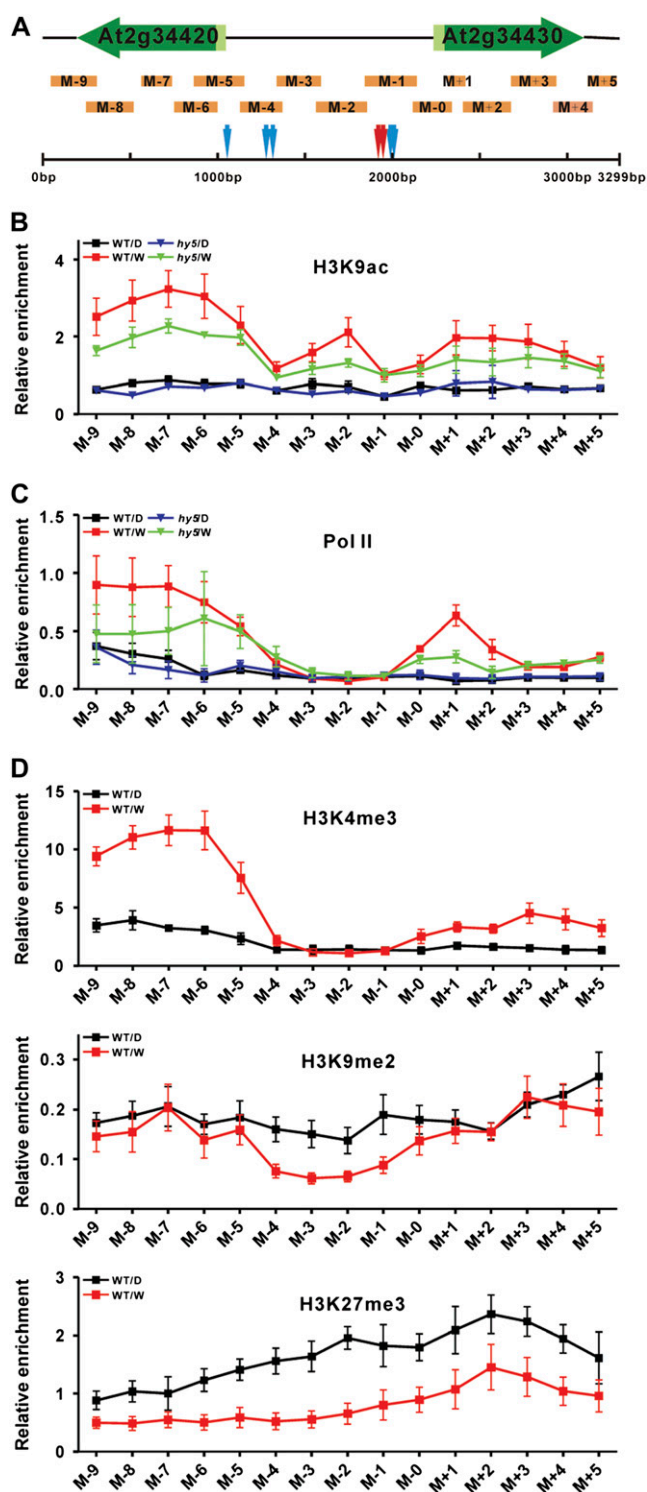


Figure 8. Analysis of histone modifications and gene transcription changes at loci At2g34420 and At2g34430 in *hy5* mutant and Col-0 wild-type seedlings. A, Schematic representation of two *CAB* genes (At2g34420, PSII type I chlorophyll *a/b*-binding protein, LHB1B2; At2g34430, PSII type I chlorophyll *a/b*-binding protein, LHB1B1). Dark green arrows represent the coding sequences of these two genes, which are transcribed in opposite directions. Pale green rectangles indicate 5' untranslated regions. The locations of primers (orange rectangles) and cis-elements (red arrowheads), confirmed HY5 binding sites; blue

induced histone modification might be a universal phenomenon during light-regulated development in Arabidopsis. However, we did observe that not all light-associated histone modification alterations took place at all representative loci, even though distinct gene expression changes were detected. One conceivable explanation is that alterations in histone modification are just one of several mechanisms for plants to respond to light and that additional strategies might be used to regulate gene expression. At the same time, it cannot be ruled out that changes in other histone modifications that we did not survey here are light responsive and affect gene expression.

Studies from yeast and mammalian cells showed that different histone modifications have different correlations with gene expression (Jenuwein and Allis, 2001; Millar and Grunstein, 2006; Li et al., 2007). This might hint at different roles of distinct histone modifications in the regulation of gene transcription (Li et al., 2007). Our data for light-regulated gene expression (Table I) and histone modifications (Table II) support these observations. Moreover, we found that increasing H3K9ac in *hd1* mutants was associated with elevated Pol II binding capabilities at the loci under study, which is a direct index of a gene's transcription level (Fig. 5). Furthermore, *hy5* mutants showed a decrease in both H3K9ac and transcript levels of representative light-up-regulated genes in light-grown seedlings (Figs. 7 and 8, B and C). In summary, these findings strongly suggest that light-responsive histone modifications are associated with light-regulated gene transcription.

In our work, we also noticed that not all histone modification signals were correlated with gene expression. Previous studies in other organisms have observed roles for alterations in histone modifications in almost all DNA-related metabolic processes (Jenuwein and Allis, 2001; Li et al., 2007). Furthermore, studies in yeast proposed some cumulative and specific mechanisms among different histone modifications (Dion et al., 2005). One modification alteration can only represent a part of the final results, which may be an integration of the effects of all histone modifications. Benhamed et al. (2006) described a similar phenomenon

arrowheads, putative light-responsive cis-elements predicted by Plant-CARE) are indicated. B, Relative enrichment of H3K9ac at different gene regions. Six-day-old *hy5* mutant and Col-0 wild-type seedlings grown in continuous dark or white light were used. The primer locations are shown in A. The black line represents Col-0 wild-type seedlings grown in the dark (WT/D), the red line represents Col-0 wild-type seedlings grown in white light (WT/W), the blue line represents *hy5* mutants grown in the dark (*hy5*/D), and the green line represents *hy5* mutants grown in white light (*hy5*/W). C, Relative enrichment changes of RNA Pol II at different gene regions. The samples are the same as in B, and the different lines represent the same light conditions and genotypes as in B. D, Relative enrichment regions of histone modifications in At2g34420 and At2g34430. Six-day-old Col-0 wild-type seedlings grown in the dark (WT/D, black line) and in white light (WT/W, red line) were compared.

in which no strict and predictable effect between histone modifications and the expression of light-regulated genes in *Arabidopsis* was found.

Histone Modifications Act in a Light-Dependent Manner as Transcription Modulators

Previous studies showed that H3K9me2 and H3K27me3 act as gene repressors by binding to promoter regions (Barski et al., 2007; Zhang et al., 2007b). Here, we found H3K9me2 and H3K27me3 in the promoter region of PSII light-harvesting complex genes At2g34420 and At2g34430 in dark-grown seedlings and a very low level of Pol II binding at these two loci in darkness. This is indicative of strongly decreased transcription rates and, therefore, supports previous observations (Fig. 8, C and D). Conversely, consistent with their role as potential gene expression activators, H3K9ac and H3K4me3 signals were increased in white light-grown plants, which showed a high relative enrichment of Pol II compared with dark-grown seedlings (Fig. 8). Recent models to explain the detailed mechanisms of transcription derived from studies in yeast and mammalian cells suggested that acetylated histones such as H3K9ac might be present at areas surrounding transcription start sites as a signal to recruit transcription initiation complexes or transcription factors and to facilitate an association with the promoter during the start of transcription (Li et al., 2007). During transcription elongation, both histone acetylation and methylation (such as H3K4me3) of coding regions are essential to maintain transcriptional activity and to reintegrate previously removed histones. Our data from *Arabidopsis* seedlings revealed distribution patterns of histone modifications that were similar to observations made in other organisms. This suggests that a wide range of histone modifications respond to light and regulate gene expression in plants in a manner that is similar to phenomena in other organisms.

Light-Regulated H3K9ac Acts as a Transcriptional Regulator and Is Influenced by Photomorphogenesis-Promoting Components and HY5

In this study, we specifically analyzed the possibility of H3K9ac being a transcription regulator during light-controlled plant development. A potential connection between histone acetylation and light-activated transcription has been proposed before (Chua et al., 2001; Schroeder et al., 2002; Benhamed et al., 2006). Our data from *det1* and *cop1* mutants provided direct evidence that some key factors of the photomorphogenesis pathway also participate in the regulation of histone modifications (Fig. 6). Moreover, recent studies in *Arabidopsis* suggested that factors that are involved in the regulation of histone modifications, such as HATs and HDACs, are light responsive and that acetylated histones are required for light-controlled gene expression (Bertrand et al., 2005; Benhamed et al.,

2006). Our findings showing that H3K9ac- and Pol II binding capabilities in *hd1* mutants are related (Fig. 5) further support these observations. Further evidence comes from HY5, a well-studied transcription factor for which multiple binding sites, including light-responsive cis-element sequences such as G boxes and Z boxes (Chattopadhyay et al., 1998; Lee et al., 2007), have been identified. Here, we found potential HY5-binding cis-elements in the promoter region of PSII light-harvesting complex genes At2g34420 and At2g34430, two representative light-regulated loci. Additionally, our data suggest that HY5 influences the relative enrichment of H3K9ac and Pol II during gene transcription (Fig. 8, B and C). This means that transcription factors that bind to cis-elements, such as HY5, might be key players to relay environmental stimuli like light to gene transcription in *Arabidopsis*.

In mammals, gene transcription has been described as a cascade of recruitment events involving various coactivator complexes: transcription factors bind to cis-elements, which are located in an approximately 200-bp nucleosome-free region at the SST region of active genes. At the same time, they can interact with transcriptional cofactors. The resulting complexes have been shown to induce histone acetylation, chromatin remodeling, and other structural chromatin changes. Subsequently, RNA polymerase initiation complexes are recruited to start transcription (Agalioti et al., 2000; Pavri et al., 2006; Li et al., 2007). Recent studies in plants reported that histone acetylation states and chromatin remodeling are also involved in the regulation of transcription of the light-induced *PetE* gene in pea (Chua et al., 2001). Interestingly, two classes of cis-acting elements, transcriptional enhancers and matrix attachment regions, have been suggested to participate in this process (Chua et al., 2003). Here, we propose that a similar mechanism might exist to mediate light-induced gene expression in *Arabidopsis*. Interestingly, H3K9ac enrichment showed a decrease just at the SSTs (Fig. 8B), which might be because of the lower concentration of nucleosomes at this location.

Ubiquitin-Proteasome-Mediated Protein Degradation and Light-Regulated Histone Modifications Are Potentially Interrelated

Earlier genetic and biochemical studies have indicated that components of the photomorphogenesis pathway, such as COP10, DDB1a, and DET1, can form a so-called CDD complex (Yanagawa et al., 2004). This complex in turn cooperates with the COP1 complex to specifically mediate the degradation of photomorphogenesis-promoting factors (such as HY5) in darkness (Saijo et al., 2003; Yanagawa et al., 2004). We showed here that H3K9ac was increased at light-activated loci in dark-grown *det1* and *cop1* mutants (Fig. 6) and that a loss of functional HY5 in *hy5* mutants caused both H3K9ac and gene transcription levels to decrease at light-up-regulated loci in white light (Fig. 7). Mutations

in *DET1* or *COP1* might prevent the degradation of HY5 in darkness. As discussed above, HY5 binding caused an increase in H3K9ac. Thus, the presence of HY5 in dark-grown *det1* or *cop1* mutants might lead to an elevated level of H3K9ac. Previous observations that HY5 binding sites do not change in darkness and white light (Lee et al., 2007) further support this idea. Together, these results suggest that ubiquitin-proteasome-mediated protein degradation and light-regulated histone modifications might be connected during plant development.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The wild-type genotype used in this work was *Arabidopsis thaliana* ecotype Col-0 unless specified otherwise. Furthermore, we used the following mutants: *cry1-304 cry2-1* (Col-0 background; Mockler et al., 1999); *phyA phyB phyE* (Ler; Devlin et al., 1998); *phyB phyD phyE* (Ler; Devlin et al., 1999); *cop1-4* (Col-0; McNellis et al., 1994); *det1-1* (Col-0; Pepper et al., 1994); and *hy5-215* (Col-0; Oyama et al., 1997). The T-DNA insertion mutant *hd1* was obtained from the Salk T-DNA collection in a Col-0 background (SALK_139445.43.60.X). Using a PCR-based reverse genetic screen (for primers, see Supplemental Table S3), homozygous seeds were isolated and collected for further experiments (Supplemental Fig. S2B). *Arabidopsis* seedlings were grown on 1× Murashige and Skoog medium agar plates containing 0.3% Suc. Unless stated otherwise, the seedlings were incubated at 4°C for 2 to 4 d, after which they were exposed to continuous light conditions at 20°C for 6 d. The white light intensity was 130 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The colored light growth chambers (E-30LED; Percival Scientific) used had light intensities of 0.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for blue light, 16 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for red light, and 0.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for far-red light.

ChIP-qPCR Assays

ChIP was performed as described before (Gendrel et al., 2005). Briefly, seedlings grown under different light conditions were harvested and fixed in 1% formaldehyde for 15 min in a vacuum. The reaction was terminated by adding Gly to a final concentration of 0.125 M and incubated in a vacuum for another 5 min. After chromatin isolation, histone-bound DNA was fragmented into an average size of approximately 500 bp by sonication. After removing the cellular debris, a small aliquot of each sample was stored for reverse cross-linking as a direct total input DNA control. Other chromatin solutions were diluted 10-fold and precleared by incubating with protein A-Sepharose Fast Flow beads (Sigma) for 1 h at 4°C. Immunoprecipitation was performed overnight at 4°C under rotation. The antibodies we used are as follows: anti-H3K9ac (1328-1) from Epigenetics; anti-H3K9me2 (07-441), anti-H3K4me3 (05-745), and anti-H3K27me3 (07-449) from Upstate; and anti-RNA polymerase II (sc-33754) from Santa Cruz. An equal amount of sample without antibody was used as a mock control. The samples were exposed to protein A beads for 1.5 h at 4°C to pull down protein-DNA complexes. After washing with low-salt wash buffer (150 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA, and 20 mM Tris-HCl, pH 8.0), high-salt wash buffer (500 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA, and 20 mM Tris-HCl, pH 8.0), LiCl wash buffer (0.25 M LiCl, 1% Nonidet P-40, 1% sodium deoxycholate, 1 mM EDTA, and 10 mM Tris-HCl, pH 8.0), and TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA), the immunocomplexes were eluted from the protein A beads. The samples were reverse cross-linked by incubation at 65°C overnight and then treated with proteinase K at 45°C for 1 h. DNA was extracted with phenol-chloroform and precipitated with ethanol containing one-tenth volume of 3 M NaAc and 1 μg of glycogen. DNA from approximately 0.3 g of seedlings was resuspended in 20 μL of sterilized water, and 0.3 μL was used for each PCR (20 μL).

ChIP DNA was analyzed by qPCR (ABI 7500; ABI), in which TaKaRa SYBR Premix ExTaq was used as the reaction system. Specific primers (Supplemental Tables S1–S5) were designed, and the PCR products were sequenced to validate that only the desired target region was amplified.

Quantification of immunoprecipitated DNA was performed as described (Frank et al., 2001). Input chromatin DNA samples were defined as 100. Each

ChIP sample was compared with its input correspondingly. At least three independently performed ChIP experiments from independent tissue samples were analyzed.

Total RNA Extraction and RT-qPCR

Total RNA was extracted with TRIzol (Invitrogen) from the same seedling samples that were used for the ChIP assay. After DNase I (Promega) digestion, RNA was reverse transcribed with SuperScript II reverse transcriptase (Invitrogen). cDNA was analyzed by relative qPCR. Specific primers (Supplemental Table S1) were designed, and the PCR products were sequenced to validate that only the desired target region was amplified. Actin (At5g09810) was used as an endogenous control to normalize the relative expression level. cDNA from 6-d-old dark-grown Col-0 wild-type seedlings was used for normalization and defined as 1. The data were analyzed with ABI 7500 System SDS software (version 1.2.3). At least three independently performed experiments on independent tissue samples were analyzed.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Analysis of H3K9ac and gene expression patterns of different ecotype seedlings grown in the dark or in white light.

Supplemental Figure S2. Characterization of *Arabidopsis* T-DNA insertion line in the *HD1* gene.

Supplemental Table S1. RT-qPCR primers for six representative light-regulated genes and actin.

Supplemental Table S2. ChIP-qPCR primers for six representative light-regulated genes.

Supplemental Table S3. Primers for characterization of *hd1* T-DNA insertion line.

Supplemental Table S4. ChIP-qPCR primers for Pol II binding assay in *hd1* mutant.

Supplemental Table S5. Primers for At2g34420 and At2g34430 loci.

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Table S1. RT-qPCR primers for six representative light-regulated genes and actin

TIGR Number	Sequence	Amplicon size (bp)
At2g34430-F	GGAGTTGAAGGTGAAGGAGC	227
At2g34430-R	TTCCAAGATGCAACAAACCG	
At2g37130-F	TAAGATTATCAAAGACGCACTCG	172
At2g37130-R	AGTCTCAACATCTCCCAAGTAGC	
At2g42790-F	GTCGGCGGATACGAGTGTC	260
At2g42790-R	GGTAGCCACGGTAACGAAGG	
At3g54890-F	CAGCTTACCTTGACGGTTCTGC	202
At3g54890-R	GGTAGTGCTGCCCATTCCTG	
At5g38430-F	GCTCTCCTCTGCCGCTGTG	143
At5g38430-R	CTCTCCCCCATTGCTTGTG	
At5g62300-F	GTAAATCATTGACTCCTTCCTTC	229
At5g62300-R	AGCTCCACGAACCAAATCAGTAC	
At5g09810-F	GGTGTCATGGTTGGTATGGGTC	199
At5g09810-R	CCTCTGTGAGTAGAACTGGGTGC	

Table S2. ChIP-qPCR primers for six representative light-regulated genes

+: Downstream of ATG; -: Upstream of ATG

Group I: for H3K4me3

TIGR Number	Sequence	Position	Amplicon size (bp)
At2g34430-F	TCTCTCCTCTCCTGCTTTGACC	+20	102
At2g34430-R	GGCTTGGAGGCTTTGCG	+105	
At2g37130-F	TAAGATTATCAAAGACGCACTCG	+602	260
At2g37130-R	AGTCTCAACATCTCCCAAGTAGC	+839	
At2g42790-F	GTCGGCGGATACGAGTGTC	+98	349
At2g42790-R	GGTAGCCACGGTAACGAAGG	+427	
At3g54890-F	TGACGGTTCTGCTCCTGGG	+173	284
At3g54890-R	ACAACCTCCAATGTTGAAATCAG	+434	
At5g38430-F	GCTCTCCTCTGCCGCTGTG	+14	143
At5g38430-R	CTCTTCCCCCATTGCTTGTG	+137	
At5g62300-F	TGGAGCTAAGGACAAGAGACTGAG	+401	168
At5g62300-R	GGAGTGGTTCCAACAACAAGC	+548	

Group II: for H3K9ac, H3K9me2 and H3K27me3

TIGR Number	Sequence	Position	Amplicon size (bp)
At2g34430-F	TCTCTCCTCTCCTGCTTTGACC	+20	102
At2g34430-R	GGCTTGGAGGCTTTGCG	+105	
At2g37130-F	GTTTAGAACGATTAAGAAACCCC	-216	288
At2g37130-R	ATGGAAGATGGAGAATAACTGTA	+49	
At2g42790-F	TCGAAAAATGAATGTTGGGTTGG	-462	274
At2g42790-R	CCCAACTTGGACTAACGGTGATG	-211	
At3g54890-F	TGCAGTGGATAAATGGCAAAATAG	-283	248
At3g54890-R	AGGAGAAAGATGTGGACGAGAGG	-58	

At5g38430-F	CCAAAAGGAAGGAGCCAAAGC	-250	288
At5g38430-R	GTAACCACAGCGGCAGAGGAG	+17	
At5g62300-F	CGTCGAGCTTCGTTTCGTC	-144	156
At5g62300-R	ACCAGCCTTCCCTGGTTTC	+23	

Table S3. Primers for characterization of *hdl* T-DNA insertion line

	Sequence
1	ATGGATACTGGCGGCAATTCG
2	GCGTGGACCGCTTGCTGCAACT
3	TCATGCTCCGGCATCTTGTCTTC

Table S4. CHIP-qPCR primers for Pol II binding assay in *hdl* mutant

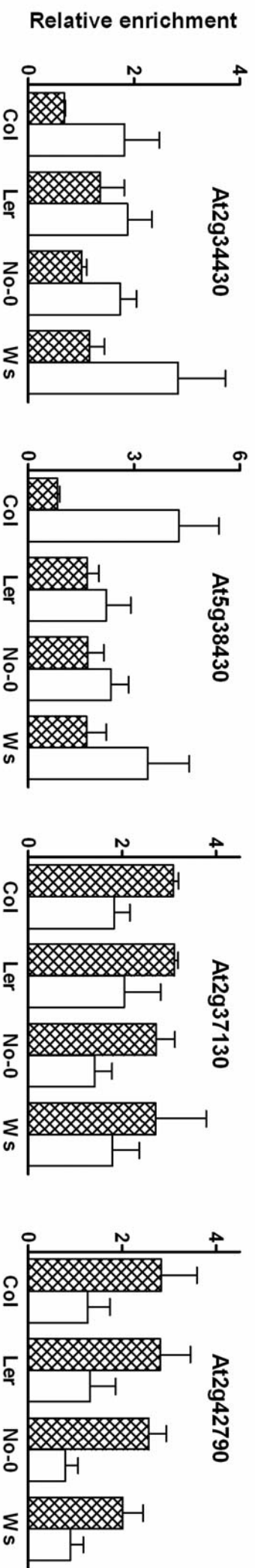
TIGR Number	Sequence	Amplicon size (bp)
At2g34430-F	TCTCTCCTCTCCTGCTTTGACC	102
At2g34430-R	GGCTTGGAGGCTTTGCG	
At2g37130-F	TAAGATTATCAAAGACGCACTCG	172
At2g37130-R	AGTCTCAACATCTCCCAAGTAGC	
At2g42790-F	GTCGGCGGATACGAGTGTC	349
At2g42790-R	GGTAGCCACGGTAACGAAGG	
At5g38430-F	GCTCTCCTCTGCCGCTGTG	143
At5g38430-R	CTCTTCCCCCATTGCTTGTG	

Table S5. Primers for At2g34420 and At2g34430 loci

Primer Name	Sequence	Amplicon size (bp)
M-9-F	GAGTCTTCAGATAAAACGCCAAC	264
M-9-R	CGTTCAAGCCATCGTCACC	
M-8-F	GGTCGGCCAAATGGTCG	273
M-8-R	CCATTGGGGCTACTCAAGTTATCC	
M-7-F	GTCCAATCCTCCGTCGCTGAAG	176
M-7-R	CCGACCCAGAGACCTTCGCC	
M-6-F	CGGTGTCCCATCCGTAGTC	248
M-6-R	GATTATAATGGCTTCCTCAACC	
M-5-F	TGGACCCTTTGGCTTGGC	290
M-5-R	TCCCAAATCTCATAAGCCAATC	
M-4-F	GTGATTGGCTTATGAGATTTGG	243
M-4-R	TGAGAGCATGAAGTGGATTGG	
M-3-F	GTTTTCTTTAATTCCAATCCACTTC	254
M-3-R	CAACAAAATCAATCAAAGGTATCAG	
M-2-F	TTAACTGATACCTTTGATTGATTTG	293
M-2-R	CTTAGAACGTTGCATACTACCCAC	
M-1-F	GCAACGTTCTAAGTATGTTTGGAC	298
M-1-R	GCCTTGATTTCTATTGGTTGTTG	
M-0-F	TCAACAACCAATAGAAATCAAGGC	224
M-0-R	CCGGTCAAAGCAGGAGAGG	
M+1-F	TCTCTCCTCTCCTGCTTTGACC	102
M+1-R	GGCTTGGAGGCTTTGCG	
M+2-F	AAAGCCTCCAAGCCCACC	274
M+2-R	CTTCTCCGAACTTCACTCCG	
M+3-F	CGGTTTGGTTCAAGGCT	260

M+3-R	CTCCTTCACCTTCAACTCC	
M+4-F	GGAGTTGAAGGTGAAGGAGC	227
M+4-R	TTCCAAGATGCAACAAACCG	
M+5-F	GTGTGTATCCGGTTTGTTGCA	165
M+5-R	TGAACACCGAATTAGGTCTAAGTATG	

A CHIP-qPCR



B RT-qPCR

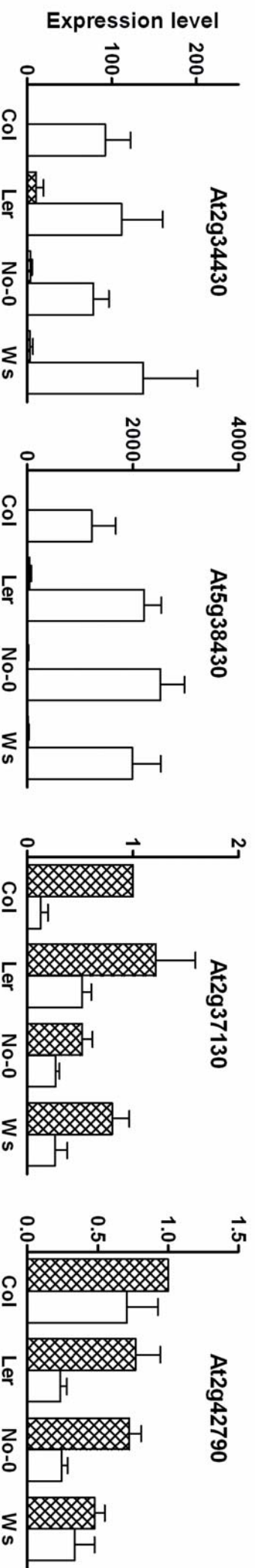


Figure S1. Analysis of H3K9ac and gene expression patterns of different ecotype seedlings grown in the dark (checked columns) or white light (white columns) for six days. A, H3K9ac patterns of different ecotype seedlings. The representations of relative enrichment and error bars are the same as Fig. 1B. B, Gene expression patterns at representative gene loci in different ecotypes. The data were calculated as for Fig. 1A.

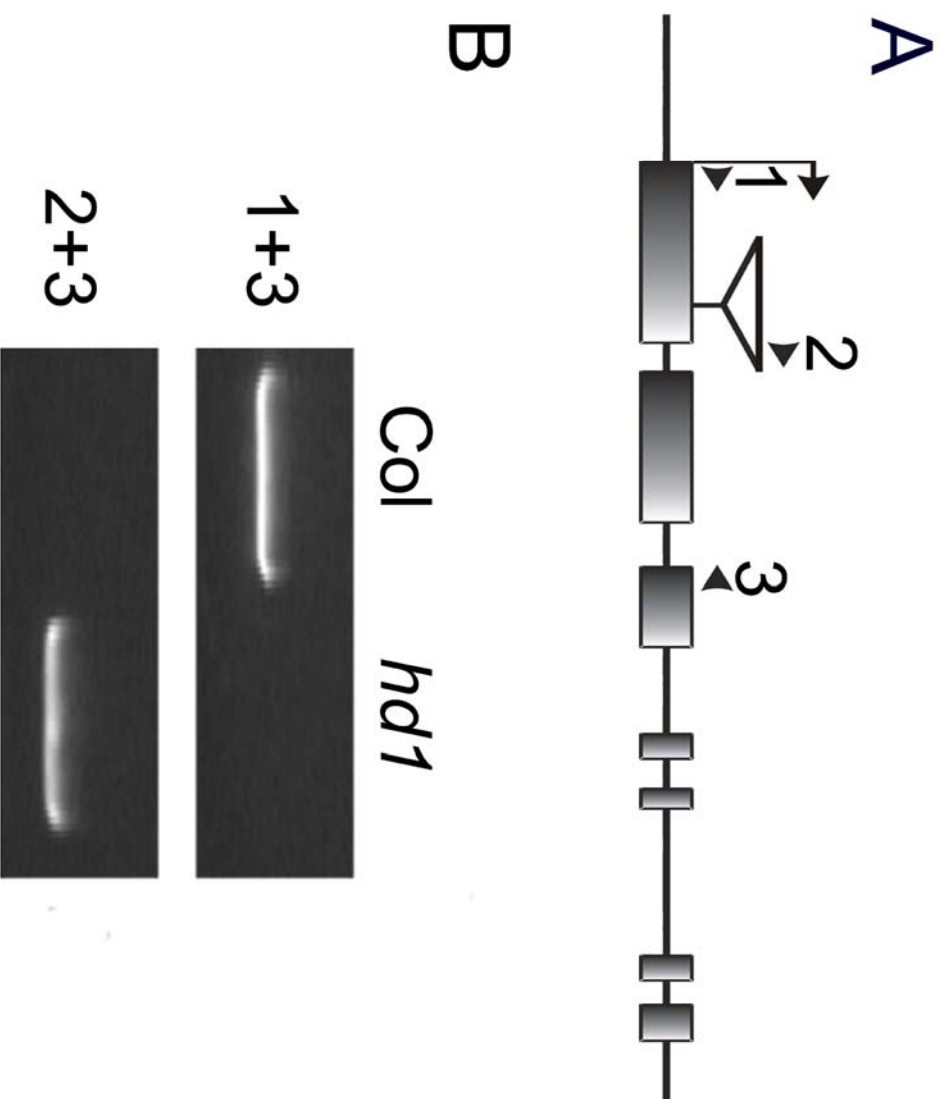


Figure S2. Characterization of Arabidopsis T-DNA insertion line in the *HD1* gene (At4g38130). A, Cartoon of T-DNA insertion in the *HD1* gene. The exons are indicated by rectangles. Numbered arrowheads represent primers used in panel B (see Table S3 for primer sequences). B, Genotyping of *hd1* mutant seedlings using PCR-based reverse genetic screen with the primers shown in panel A.