The Plant Cell, Vol. 26: 2858–2872, July 2014, www.plantcell.org 

# HUA ENHANCER1 Is Involved in Posttranscriptional Regulation of Positive and Negative Regulators in *Arabidopsis* Photomorphogenesis[C][W][OPEN]

**Huang-Lung Tsai,[a] Yi-Hang Li,[a] Wen-Ping Hsieh,[a] Meng-Chun Lin,[a] Ji Hoon Ahn,[b] and Shu-Hsing Wu[a,1]**

[a] Institute of Plant and Microbial Biology, Academia Sinica, Taipei 11529, Taiwan

[b] Creative Research Initiatives, Division of Life Sciences, Korea University, Seongbuk-Gu, Seoul 136-701, Korea

ORCID IDs: 0000-0003-3467-0507 (H.-L.T.); 0000-0002-7179-3138 (S.-H.W.)

**Light regulates growth and developmental processes in plants via global transcriptome adjustment, translational control, and multilayered posttranslational modification of proteins. The transcriptional activation and repression of light-responsive genes has been well documented; however, the impact of posttranscriptional regulation on conveying light signals has been less addressed. Here, we examined whether optimal photomorphogenesis in *Arabidopsis thaliana* requires the proper biogenesis of small regulatory RNAs that play pivotal roles in the posttranscriptional regulation of gene expression. *Arabidopsis* carrying a mutation in *HUA ENHANCER1* (*HEN1*), required for stabilization of small regulatory RNAs, showed defects in multiple aspects of photomorphogenic and skotomorphogenic development. HEN1 negatively regulated *Arabidopsis* photomorphogenesis. Light-activated *HEN1* expression depended on the photoreceptors phytochrome A (phyA), phyB, cryptochrome 1 (cry1), and cry2 and key transcriptional regulators ELONGATED HYPOCOTYL5 (HY5) and HY5-HOMOLOG. We also demonstrate the involvement of the small regulatory RNAs miR157d and miR319 in modulating the expression of a positive regulator, HY5, and negative regulators TEOSINTE BRANCHED1, CYCLOIDEA AND PCF family proteins, respectively, for optimal photomorphogenic development in *Arabidopsis*.**

## INTRODUCTION

Light is an important and dynamic environmental cue for many programmed growth and developmental processes in plants. The process of photomorphogenesis has been widely used for studies of light sensing and signaling pathways regulated at multiple levels. First, with light treatment, the photoreceptors or key light signaling molecules redistribute within different subcellular localizations to execute their functions (reviewed in Lorrain et al., 2006; Van Buskirk et al., 2012). Second, selective protein degradation modulates the protein abundance of both positive and negative regulators of light signaling pathways (reviewed in Yi and Deng, 2005; Henriques et al., 2009). Third, light-regulated protein phosphorylation involves the activation of both photoreceptors and downstream signaling molecules (Yeh et al., 1997; Ahmad et al., 1998; Yeh and Lagarias, 1998; Fankhauser and Chory, 1999; Colón-Carmona et al., 2000; Medzihradszky et al., 2013; Nito et al., 2013). Fourth, widespread translational regulation occurs in response to light or dark treatments (Juntawong and Bailey-Serres, 2012; Liu et al., 2012, 2013). Also, by interacting with PENTA1, phytochrome negatively regulates the translation of protochlorophyllide reductase mRNAs (Paik et al., 2012). Fifth, and likely the most well studied, is transcriptome adjustments in plants with light treatment (reviewed in Jiao et al., 2007). Recently, the splicing factor REDUCED RED-LIGHT RESPONSES IN CRY1CRY2 BACKGROUND1 was found to be a phyB-dependent signaling component (Shikata et al., 2012), suggesting alternative splicing could further increase the transcriptomic diversity.

Although light-modulated alteration in transcriptomes has been documented, most efforts have focused on the transcriptional activation or repression of light-regulated genes. Posttranscriptional regulation of gene expression in response to light signals has been less studied. A recent report showed that a key positive regulator in *Arabidopsis thaliana* photomorphogenesis, ELONGATED HYPOCOTYL5 (HY5), could activate the expression of *MIR* genes encoding microRNAs (miRNAs) by binding directly to their promoters (Zhang et al., 2011). Thus, small regulatory RNAs may participate in modulating the transcriptome shift during photomorphogenesis. Interestingly, phenotypic examination of *Arabidopsis* mutants carrying weak alleles of *ago1* revealed a light-hypersensitive phenotype (Sorin et al., 2005), so small regulatory RNAs may act as negative regulators of the light signaling pathways. However, additional studies are needed to clarify whether light regulates the biogenesis and/or actions of small regulatory RNAs in photomorphogenic *Arabidopsis*.

In many eukaryotic organisms, small regulatory RNAs play a pivotal role in the transcriptional, posttranscriptional, or translational regulation of gene expression (reviewed in Chen, 2010). After being processed from single-stranded RNA hairpin or double-stranded RNAs, miRNAs and small

[1] Address correspondence to shuwu@gate.sinica.edu.tw.
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Shu-Hsing Wu (shuwu@gate.sinica.edu.tw).

[C] Some figures in this article are displayed in color online but in black and white in the print edition.

[W] Online version contains Web-only data.

[OPEN] Articles can be viewed online without a subscription.

www.plantcell.org/cgi/doi/10.1105/tpc.114.126722

interfering RNAs are stabilized by the action of HUA ENHANCER1 (HEN1), a small RNA methyltransferase that adds a methyl group to the 3′-most nucleotide of small RNAs, thus protecting against 3′ uridylation and truncation (Ji and Chen, 2012).

Here, we report on a light-hypersensitive phenotype in an *Arabidopsis* mutant defective in *HEN1*. Our data reveal that light upregulated the expression of *HEN1*, a process requiring red (R), far-red (FR), or blue (B) light photoreceptors and key transcriptional regulators, HY5 and HY5-HOMOLOG (HYH). Among the miRNAs stabilized by HEN1, miR157d and miR319 could target transcripts of *HY5* and those of *TEOSINTE BRANCHED1*, *CYCLOIDEA AND PCF24* (*TCP24*), respectively, for cleavage during *Arabidopsis* photomorphogenesis. Light may upregulate *HEN1* expression for the proper biogenesis of small regulatory RNAs, and selected miRNAs could fine-tune the temporal expression patterns of key transcriptional regulators in *Arabidopsis* photomorphogenesis.

## RESULTS

### HYL1, HEN1, and HST Are Negative Regulators of *Arabidopsis* Photomorphogenesis

We evaluated whether small RNAs are involved in the light-regulated deetiolation process in *Arabidopsis* by examining the photomorphogenic phenotype of mutants defective in miRNA biogenesis genes, including *HYPONASTIC LEAVES1* (*HYL1*) (Lu and Fedoroff, 2000), *HEN1* (Park et al., 2002), and *HASTY* (*HST*) (Bollman et al., 2003). These genes are responsible for processing primary miRNA transcripts, stabilization, and export of mature miRNA duplexes (reviewed in Rogers and Chen, 2013; a simplified biogenesis scheme is shown in Supplemental Figure 1). The *hyl1-2*, *hst-3*, and *hst-6* mutants have shorter hypocotyls than the wild-type Columbia-0 (Col-0) under both dark and light conditions (Supplemental Figure 2), as described previously (Lu and Fedoroff, 2000; Bollman et al., 2003). Treatment with various fluences of continuous blue (Bc) light clearly exaggerated the light-induced inhibition of hypocotyl elongation in *hyl1* and *hst* mutants (Figure 1A). We also observed a similar light-dependent phenotype for *hen1-1* mutant as compared with its corresponding wild-type Landsberg *erecta* (L*er*) (Figure 1B; Supplemental Figure 2). The light hypersensitivity in these mutants implied that HYL1, HST, and HEN1 function as negative regulators in the deetiolation process in *Arabidopsis*.

### Negative Regulatory Roles of HEN1 in Multiple Aspects of Skotomorphogenic and Photomorphogenic Development

We surveyed light-regulated expression patterns of genes in miRNA biogenesis pathways, including *DICER LIKE1*, *SERRATE*, *HYL1*, *HEN1*, *HST*, and *ARGONAUTE1* in the public transcriptome data repository Genevestigator (Zimmermann et al., 2004). Only *HEN1* showed clear light responsiveness (Supplemental Figure 3). Therefore, we focused on HEN1 and its roles in deetiolating *Arabidopsis*.

We next examined the photomorphogenic phenotype of two independent *hen1* alleles, *hen1-5* (Vazquez et al., 2004) and *hen1-6* (Li et al., 2005), in a Col-0 background (Supplemental Figure 4A). Neither mutant expressed the full-length *HEN1* transcript (Supplemental

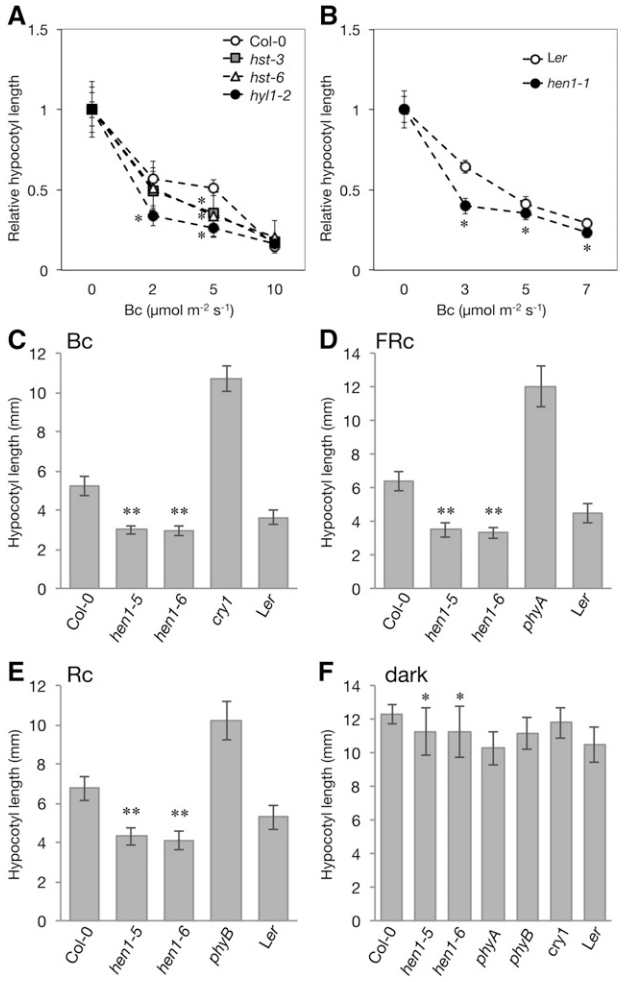


**Figure 1.** HEN1 Plays Multiple Roles in Skotomorphogenesis and Photomorphogenesis.

**(A)** and **(B)** Hypocotyl fluence rate responses of wild type and *hyl1-2*, *hst3*, and *hst6* mutants in the Col-0 **(A)** and *hen1-1* in the L*er* background **(B)** under Bc light at the indicated fluence rates. Hypocotyl lengths under each indicated fluence rate were normalized to that under the dark. Data are mean ± SD. *Significantly different from the corresponding wild type ($P < 0.01$, Student's *t* test; $n = 16$ to 32).

**(C)** to **(F)** Hypocotyl lengths of 4-d-old seedlings of wild types and mutants *hen1-5* and *hen1-6* in the Col-0, *phyA*, *phyB*, and *cry1* in the L*er* background grown under Bc (5 μmol $m^{-2}$ $s^{-1}$) **(C)**, FRc (0.125 $Wm^{-2}$) **(D)**, Rc (5 μmol $m^{-2}$ $s^{-1}$) **(E)**, and dark **(F)**. Data are mean ± SD. ** and *, significantly different from the corresponding wild type (Col-0) ($P < 10^{-14}$ and $P < 0.05$, Student's *t* test; $n = 14$ to 20).

Figure 4B). Similar to *hen1-1*, both *hen1-5* and *hen1-6* mutants were hypersensitive to Bc (Figure 1C; Supplemental Figure 5A). Under continuous far-red (FRc) and red (Rc) light, *hen1-5* and *hen1-6* both had a short hypocotyl as compared with the wild type (Figures 1D and 1E; Supplemental Figures 5B and 5C). The appropriate hypocotyl elongation of *hen1-6* could be restored by introducing a genomic fragment of *HEN1* into *hen1-6* plants (Supplemental Figure 6), so loss of functional *HEN1* is indeed responsible for the light hypersensitivity in *hen1*. Consistent with a shorter hypocotyl in *hen1-1*

compared with its corresponding wild-type L*er* in the dark, the hypocotyl lengths of *hen1-5* and *hen1-6* were also slightly shorter than that of their corresponding wild-type Col-0 (Figure 1F; Supplemental Figure 5D).

We also observed additional light-hypersensitive responses in *hen1-1*. Time-lapse imaging revealed that the cotyledons of the *hen1-1* mutant opened faster than the L*er* wild type (Figure 2A). Light also triggers the accumulation of anthocyanin in wild-type seedlings (Ahmad et al., 1995; Maier et al., 2013; Shin et al., 2013). Light-grown *hen1-1* showed high anthocyanin levels (Figure 2B), which is consistent with *hen1-1* being light hypersensitive.

Mutations in negative regulators of photomorphogenesis, such as CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) and PHYTOCHROME INTERACTING FACTORs (PIFs), also resulted in aberrant skotomorphogenic development, including short hypocotyl (Leivar and Quail, 2011), opened hook (Mazzella et al., 2014) and reduced antigravity responses (Kim et al., 2011). In the dark, hypocotyl lengths were shorter for the *hen1-1* mutant than L*er* (Figure 1B). Also, in the dark, the apical hooks of *hen1-1* seedlings were partially opened and reached a plateau at an angle of 136°, much smaller than the 181° for wild-type seedlings (Figure 2C). Additionally, *hen1-1* showed reduced response to a change in gravity orientation (Figure 2D).

Therefore, consistent with it playing negative roles in light responses as do COP1 and PIFs, HEN1 has broad effects on both skotomorphogenesis and photomorphogenesis.

### phyA, phyB, cry1, HY5, and HYH Regulate the Light-Responsive Expression of *HEN1*

We investigated whether a broad spectrum or specific wavelengths of light could induce the expression of *HEN1*. R, FR, and

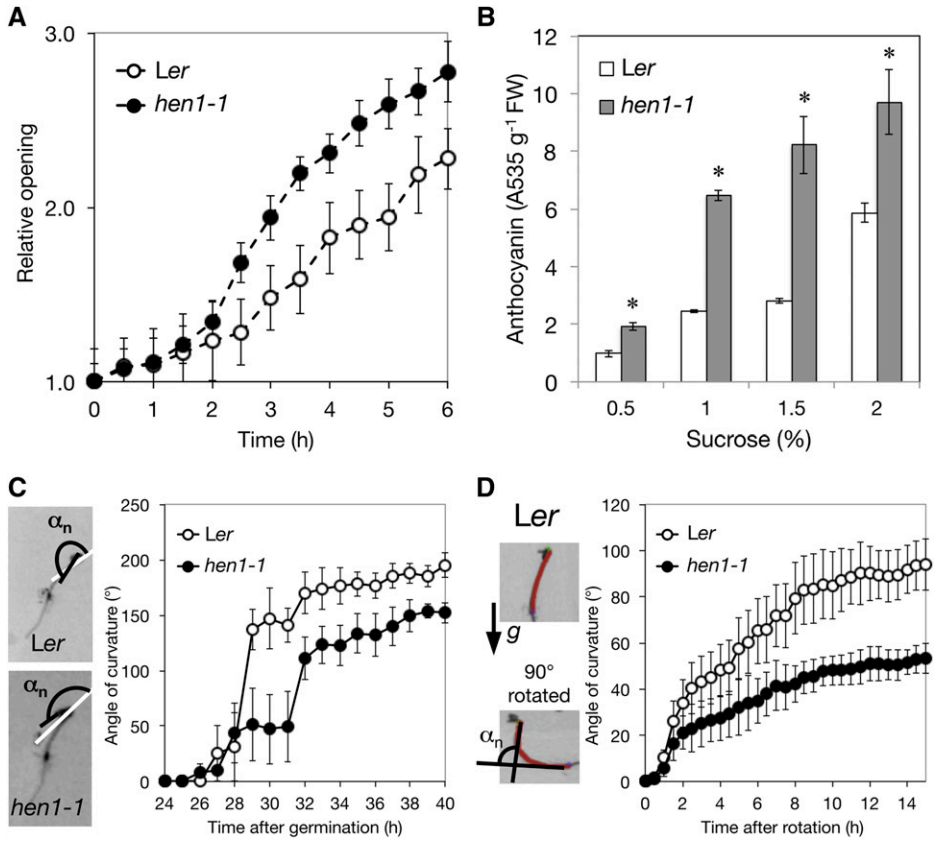


**Figure 2.** The *hen1-1* Mutant Shows Broad Skotomorphogenic and Photomorphogenic Defects.

**(A)** Cotyledon opening of 4-d-old etiolated seedlings under continuous white light (Wc; 100 μmol $m^{-2}$ $s^{-1}$) analyzed by measuring cotyledon tip-to-tip distances for the wild type and *hen1-1* mutant. Data are mean ± SE from six biological replicates.

**(B)** Anthocyanin level in 4-d-old L*er* and *hen1-1* seedlings grown under 16-h W light (100 μmol $m^{-2}$ $s^{-1}$)/8-h dark cycles in the presence of sucrose with indicated concentrations. Data are mean ± SD from two biological replicates (each with two technical replicates). *Significantly different from the wild type ($P < 0.05$, Student's *t* test; $n = 4$).

**(C)** Kinetics of hook formation in *hen1-1* and wild-type seedlings under the dark. The images show apical hook angles ($\alpha_n$) at 1-h intervals after 24-h germination at 22°C. Data are mean ± SE from three biological replicates.

**(D)** The hypocotyl turning angles at 0.5-h intervals after 90° change in seedling direction for the wild type and *hen1-1* were measured. Images show a representative L*er* seedling at time 0 and 15 h. The arrow indicates the gravity direction. The hypocotyl was traced in red to determine the angle of hypocotyl curvature. Data are mean ± SE from four biological replicates.

[See online article for color version of this figure.]

B could all induce *HEN1* expression (Figures 3A to 3C). We also examined whether the induction of *HEN1* by R, FR, and B light depended on functional photoreceptors in the *Arabidopsis* photoreceptor mutant *phyA* under FR light, *phyB* under R light, and *cry1* under B light. The light responsiveness of *HEN1* was largely compromised in *phyA* and *phyB* mutants under FRc and Rc light, respectively (Figures 3A and 3B), so the FR and R light responsiveness of *HEN1* depended on functional phyA and phyB, respectively. However, under B light, the expression of *HEN1* could still be induced but to a lesser extent in the *cry1* mutant as compared with the wild type (Figure 3C). This result suggested that cry1 was not the sole photoreceptor contributing to the B-light-inducible expression of *HEN1*. We therefore examined whether additional

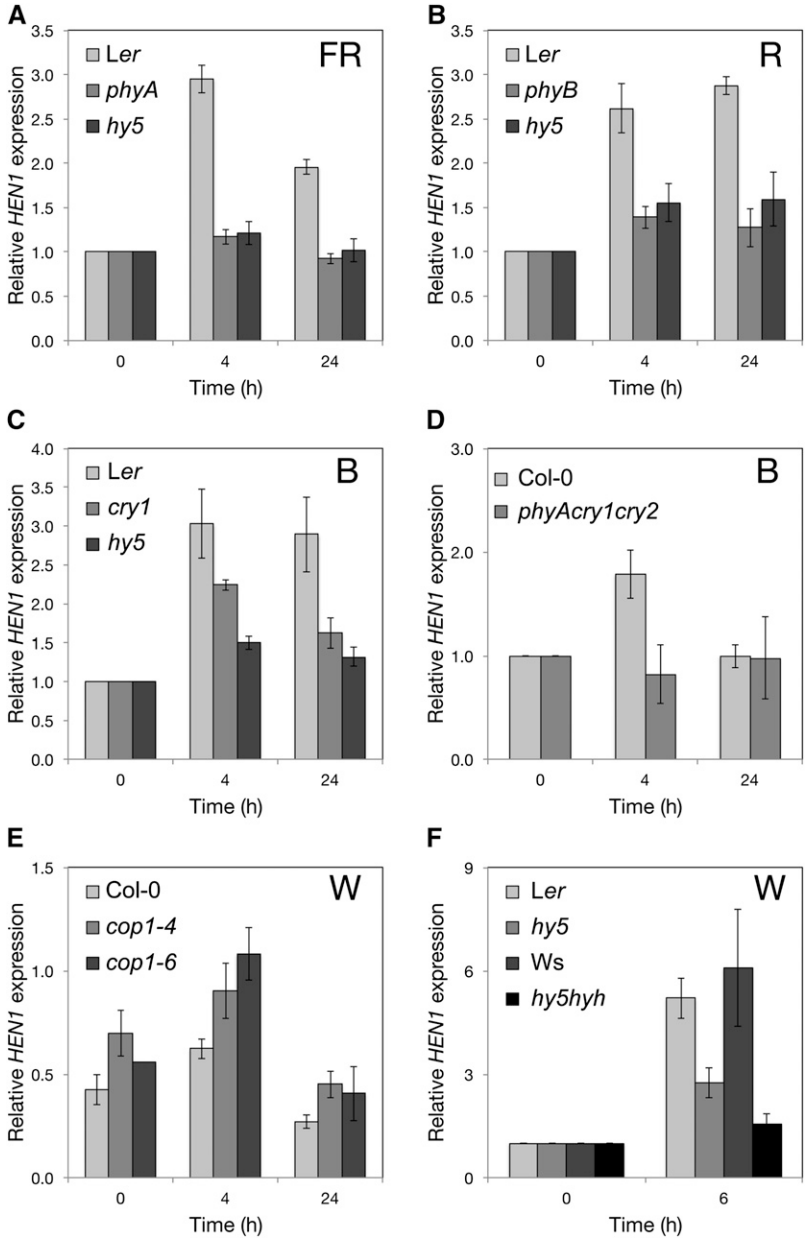


**Figure 3.** The Expression of *HEN1* Is Upregulated by Light and Requires Photoreceptors and HY5.

Real-time qRT-PCR analysis of the mRNA level of *HEN1* in etiolated wild type (L*er*, Wassilewskija, and Col-0), *hy5*, and the light signaling mutants *phyA* **(A)**, *phyB* **(B)**, *cry1* **(C)**, *phyA cry1 cry2* **(D)**, *cop1-4*, *cop1-6* **(E)**, and *hy5 hyh* **(F)** on exposure to FR (1.13 $Wm^{-2}$), R (18 $\mu mol\ m^{-2}\ s^{-1}$), B (15 $\mu mol\ m^{-2}\ s^{-1}$), and W (100 $\mu mol\ m^{-2}\ s^{-1}$), respectively, at the indicated times. The expression of *UBQ10* was an internal control. Except for the relative *HEN1* level in **(E)** being 1000-multiplied mean values of $2^{-\Delta CT}$ ($\Delta C_T = C_{T,\ HEN1} - C_{T,\ UBQ10}$) for clarity, the level of *HEN1* at each time is normalized to that of etiolated seedlings (0 h). Data are mean ± SD from three biological replicates (each with three technical replicates).

B-light photoreceptors, including phyA (Poppe et al., 1998) and cry2, could function in parallel to cry1 in inducing *HEN1* expression under B light. The B light induction of *HEN1* was abolished in the *phyA cry1 cry2* triple mutant (Figure 3D), which indicates that phyA and cry2 could be accessory B light photoreceptors for *HEN1* induction.

*HY5* encodes a key transcription factor and is upregulated by various qualities of light signals, including R, FR, and B light (Chang et al., 2008). Therefore, we examined whether the light induction of *HEN1* was regulated by HY5. The phyB- and phyA-induced expression of *HEN1* primarily depended on HY5 (Figures 3A and 3B). The induction of *HEN1* by B light also depended on the expression of HY5 (Figure 3C). Thus, HY5 acts downstream of multiple photoreceptors for the B light induction of *HEN1*. We examined the HY5-dependent induction of *HEN1* in *cop1* mutants that can accumulate a high level of HY5 protein in the dark (Osterlund et al., 2000). As expected, we detected increased *HEN1* transcripts in *cop1-4* and *cop1-6* mutants (McNellis et al., 1994) in the dark and subsequent deetiolation (Figure 3E).

We noted the residual upregulation of *HEN1* in the *hy5* mutant under various light conditions (Figures 3A to 3C), which indicates that additional factors may function in addition to or in parallel with HY5 to induce *HEN1*. Because the function of HYH is partially redundant to that of HY5 in promoting photomorphogenesis (Holm et al., 2002), HYH may also contribute to the light-upregulated expression of *HEN1*. Indeed, the light-induced expression of *HEN1* was further compromised in the *hy5 hyh* double mutant under white light (W) (Figure 3F). Therefore, HY5 and HYH are the primary activators of *HEN1* expression under light.

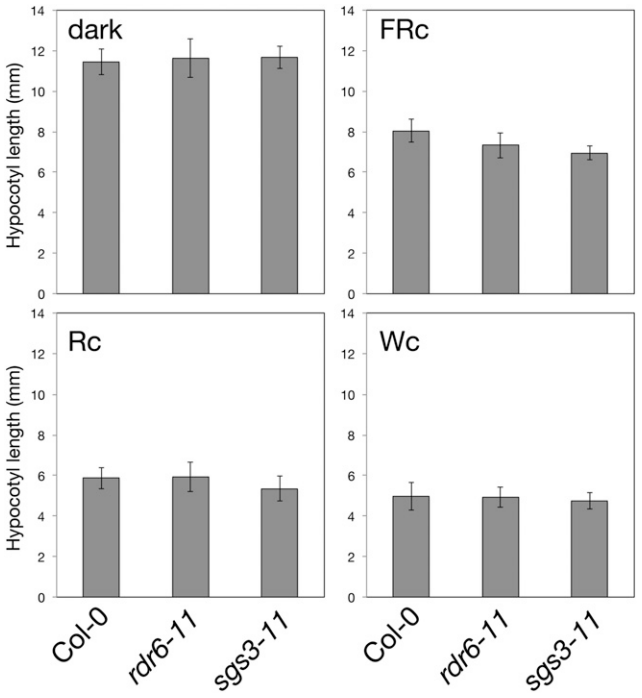


**Figure 4.** *Arabidopsis* Mutants Defective in ta-siRNA Biogenesis Show Normal Photomorphogenic Development.

Hypocotyl lengths of seedlings of the wild type and mutants defective in *RDR6* and *SGS3* measured after growth in the dark, FRc (0.125 $Wm^{-2}$), Rc (5 $\mu$mol $m^{-2}$ $s^{-1}$), and Wc (15 $\mu$mol $m^{-2}$ $s^{-1}$) for 4 d. Data are mean ± SD.

## Small Regulatory RNAs and *Arabidopsis* Photomorphogenesis

Light upregulates *HEN1* to attenuate photomorphogenesis possibly by stabilizing small regulatory RNAs to silence the expression of key regulators in this important physiological process. HEN1 methylates small regulatory RNAs generated from various biogenesis pathways. To narrow down the classes of small regulatory RNAs that may be involved, we first examined the photomorphogenic development of *Arabidopsis* mutants defective in key genes for the biogenesis of *trans*-acting small interfering RNAs (ta-siRNAs). *Arabidopsis* mutants defective in *RNA-DEPENDENT RNA POLYMERASE6* (*RDR6*) and *SUPPRESSOR OF GENE SILENCING3* (*SGS3*) (Peragine et al., 2004; Vazquez et al., 2004; Xie et al., 2005; Yoshikawa et al., 2005) showed a normal photomorphogenic phenotype under the light qualities examined (Figure 4). Despite a slightly shorter hypocotyl in *sgs3* under FR light (Figure 4), fluence response analysis revealed a comparable hypocotyl length for *sgs3* and the wild type across a broad range of FR intensities (Supplemental Figure 7). Thus, reduced expression of ta-siRNAs alone is insufficient to confer the light hypersensitivity seen in *hen1*. Accordingly, we focused on characterizing miRNAs for their roles downstream of HEN1 in modulating the posttranscriptional expression of light-responsive genes.

## Increased HEN1 Levels Lead to Increased miRNA Abundance

HEN1 is a methyltransferase functioning to stabilize small RNAs (Li et al., 2005). Our results showed that light could upregulate the expression of *HEN1*. We next examined whether the increase in *HEN1* mRNAs leads to increased accumulation of HEN1 protein and consequently more small regulatory RNAs. We generated transgenic *Arabidopsis* carrying a HA-tagged HEN1 (HA-HEN1) under the control of a β-estradiol inducible promoter (*XVE:HA-HEN1*) (Zuo et al., 2006). HA-HEN1 was previously shown to be a biologically active protein (Yang et al., 2007). We first induced *HA-HEN1* expression in etiolated seedlings of transgenic plants under dark conditions to eliminate the effect of light-upregulated *HEN1*. *HEN1* transcript expression was increased with β-estradiol treatment (Figure 5A). Although too little endogenous HEN1 protein was present to be detected with anti-HEN1 antibody in etiolated seedlings, a specific protein with the expected size of HA-HEN1 (109 kD) recognized both by anti-HEN1 and anti-HA antibodies was induced to accumulate with β-estradiol treatment (Figure 5B). Etiolated seedlings treated with 0.1 and 1 μM β-estradiol showed proportionally increased *HA-HEN1* transcript and protein levels (Figures 5A and 5B). The increased HA-HEN1 protein level was accompanied by increased expressions of the miR157 and miR159/319 in dark-grown

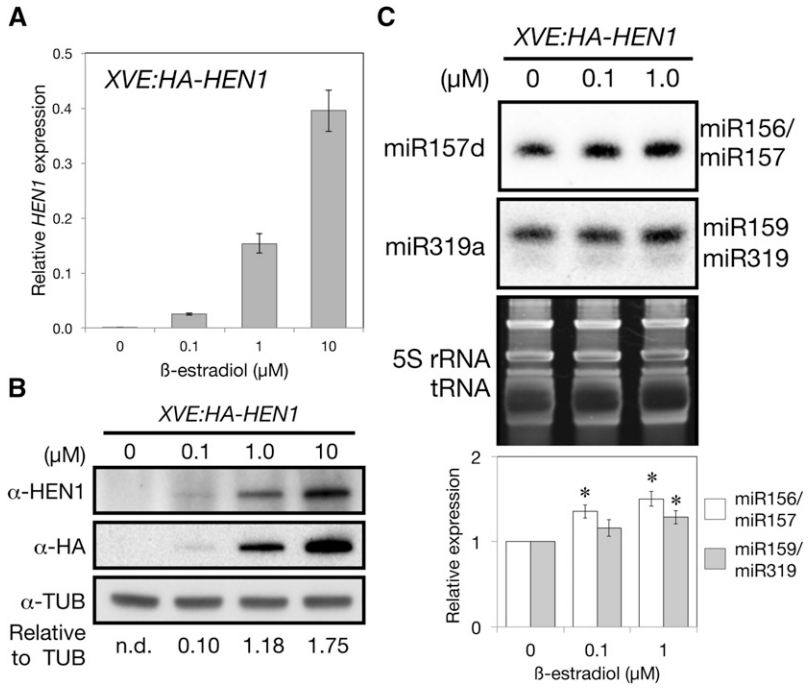


**Figure 5.** *HEN1* Plays a Rate-Limiting Role for miRNA Accumulation in Etiolated Seedlings.

The 3-d-old etiolated T2 seedlings of *XVE:HA-HEN1* were treated with 0 to 10 μM β-estradiol for 24 h in the dark.

**(A)** qRT-PCR analysis of the mRNA level of *HEN1* relative to that of *UBQ10*. Data are mean ± SD from two biological replicates (each with three technical replicates).

**(B)** Immunoblot analysis of protein level of HA-HEN1 with the antibodies pig anti-HEN1 (α-HEN1), mouse anti-HA (α-HA), and mouse anti-tubulin (α-TUB). Numbers below the α-TUB blot indicate the HA-HEN1 protein levels in the α-HA blot relative to TUB signals. n.d., nondetectable.

**(C)** Small RNA analyses of miR156/157 and miR159/319. DNA oligos used for each blot are indicated to the left of each blot. The bar graph shows 5S rRNA- and tRNA-normalized miRNA fold increase in HA-HEN1-induced seedlings. Data are mean ± SD quantitated using ImageJ on three biological replicates, shown in Supplemental Figure 8. *Significantly different from the noninduced sample (0 μM) ($P < 0.05$, Student's *t* test; $n = 3$).

seedlings (Figure 5C; Supplemental Figure 8). This suggested that, in etiolated seedlings, the level of endogenous miRNA precursors exceeded the functional capacity of endogenous HEN1 protein.

The results in Figures 3 and 5 imply that when wild-type etiolated seedlings were exposed to light, the increased *HEN1* transcripts likely resulted in increased protein level of HEN1 to promote miRNA biogenesis/accumulation. Thus, the light-induced increase of HEN1 plays a regulatory role in increasing miRNAs in the deetiolation process.

### *HY5* Transcript Is a Target of miR157d

The results in Figures 1 and 2 indicate that HEN1 is a negative regulator of photomorphogenesis. Light upregulates the expression of *HEN1* (Figure 3), and increased levels of HEN1 will result in increased levels of miRNAs (Figure 5). Therefore, in the deetiolating process, the increased miRNAs may target transcripts of positive regulators of photomorphogenesis for cleavage. Such cleavage may result in the accumulation of site-specific sequence signatures seen in the degradome database of 5′-end sequences of transcripts after miRNA-mediated cleavage (PARE, https://mpss.udel.edu/dbs/index.php?SITE=at_pare) (German et al., 2008). A search of such signatures revealed an enriched signature within the 5′-untranslated region (UTR) of the *HY5* transcript. This putative cleavage site was located at a position opposite to nucleotides 11-12 of miR157d, rather than the canonical cleavage position 10-11, possibly because of the presence of a single nucleotide bulge on miR157d when pairing with the *HY5* transcript (Figure 6A).

We confirmed the presence of this cleaved product of *HY5* mRNA in photomorphogenic *Arabidopsis* by 5′ rapid amplification of cDNA ends (RACE) (Figure 6A). Because *HY5* was not previously predicted as a target of miR157d, we investigated the authenticity of the targeted regulation of *HY5* by miR157d by examining the sequences of both *MIR157d* and *HY5* from 513 sequenced *Arabidopsis* tracks from the *Arabidopsis* 1001 Genomes database (http://signal.salk.edu/atg1001/3.0/gebrowser.php). The sequence encoding mature miR157d was fully conserved among the 513 *Arabidopsis* tracks. In addition, analysis of single nucleotide polymorphisms (SNPs) revealed 100% conservation at the miR157d target region on the *HY5* transcript among the 513 genome tracks (see Methods), whereas frequent SNPs were observed across the 5′-UTR and the rest of the *HY5* cDNA (Figure 6A). Therefore, sequences for complementary regions between miR157d and

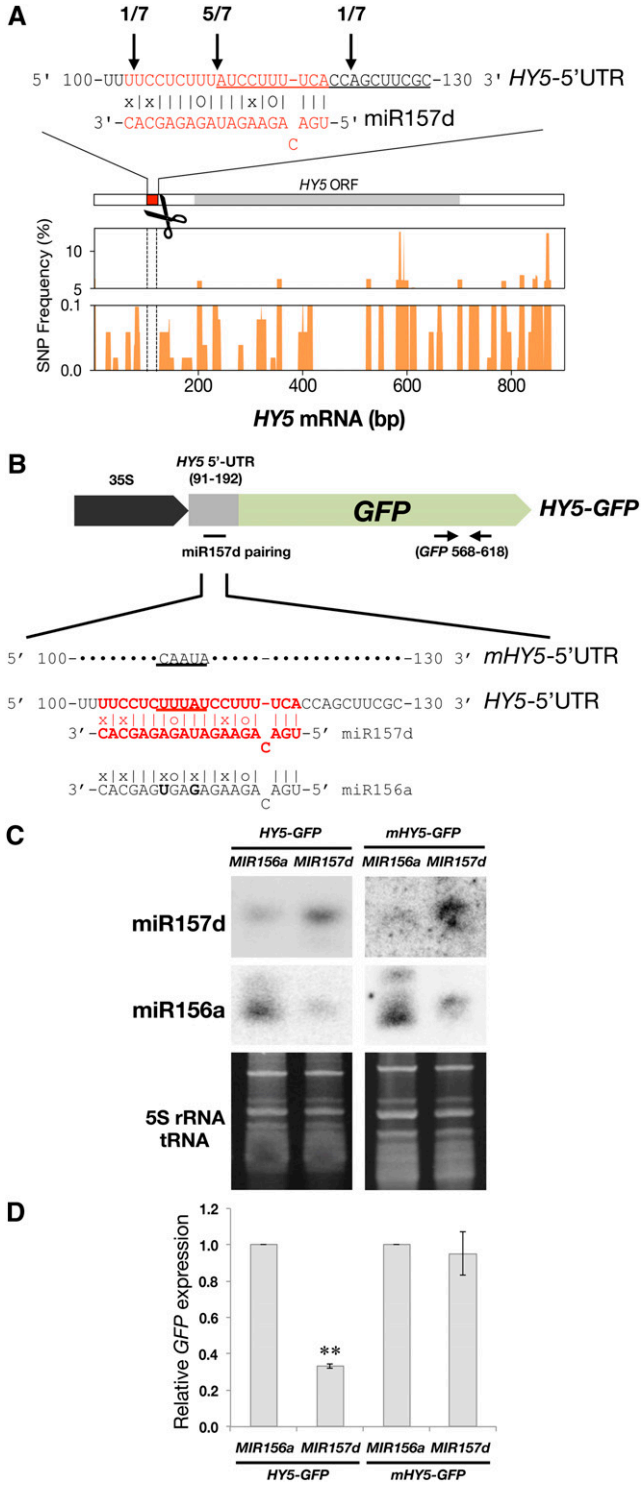


**Figure 6.** *HY5* Transcript Is Targeted by miR157d.

**(A)** The sequence in red represents the pairing region between miR157d and the 5′-UTR of *HY5* mRNA. Numbers and vertical arrows indicate *HY5* cleaved ends validated by 5′ RACE. Straight lines and "o" symbols represent perfect matches and G-U matches, respectively. The enriched signature for *HY5* in the PARE database is underlined. SNP frequency for *HY5* in 513 *Arabidopsis* tracks summed using a 10-bp sliding window across the *HY5* cDNA.

**(B)** The *HY5* 5′-UTR (91 to 192 nucleotides; gray box) containing the miR157d pairing region (102 to 120 nucleotides) was fused with *GFP* cDNA to generate the *35S:HY5-GFP* construct for *N. benthamiana* co-infiltration assays with *35S:MIR156a* or *35S:MIR157d*. The pairing region (sequences in red) of *HY5-GFP* was mutated (nucleotides underlined) to generate *35S:mHY5-GFP*. Horizontal arrows indicate primer binding sites for *GFP* qRT-PCR analysis.

**(C)** RNA gel blot analyses of miR156a and miR157d transcripts in co-infiltration assays. DNA oligos used for each blot are indicated to the left of each blot. 5S rRNA and tRNA levels are loading controls. Each sample contained 2 μg total RNA.

**(D)** qRT-PCR analysis of *HY5-GFP* and *mHY5-GFP* transcripts. ***HY5-GFP* level reduced only when coinfiltrated with *35S:MIR157d* as compared with *35S:MIR156a* ($P < 0.01$, Student's *t* test; $n = 6$). Data are mean ± SE from two biological replicates (each with three technical replicates).

*HY5* were preferentially retained, possibly because of functional constraint.

We next demonstrated that *HY5* is an authentic target of miR157d using *Agrobacterium tumefaciens*-mediated transient expression assays. Tobacco (*Nicotiana tabacum*) leaves were coinfiltrated with agrobacteria harboring binary vectors constitutively expressing constructs of *MIR157d* and *HY5* 5′-UTR (91 to 192 bp) fused with *GFP* cDNA (*HY5-GFP*) (Figures 6B and 6C). The miR156a, which shares high sequence identity with miR157d, with only two additional mismatches within the targeting region on the *HY5* 5′-UTR, and *mHY5-GFP* carrying mutations in miR157d target site were used as controls (Figures 6B and 6C). The *HY5-GFP* transcript level was specifically suppressed by miR157d but not miR156a (Figure 6D). Moreover, *mHY5-GFP* was resistant to miR157d (Figure 6D), which suggests that there is specific targeting of *HY5* 5′-UTR by miR157d.

### HEN1 and HY5 Form a Negative Feedback Regulatory Loop in Photomorphogenic *Arabidopsis*

To examine whether HEN1 executes its negative roles in photomorphogenesis at least in part by regulating the level of miR157d, we first examined the accumulation of miR157d in the *hen1-1* mutant. Consistent with previously investigated miRNAs in *hen1* mutants (Li et al., 2005), miR157d showed only a trace uridylated signal, which confirms that the accumulation of miR157d depended on functional HEN1 (Supplemental Figure 9). Furthermore, miRNA-specific profiling of the small RNAs in deetiolating seedlings indicated that light enhanced the accumulation of miR157d in photomorphogenic *Arabidopsis* (Figure 7A).

With *HY5* as a direct target of miR157d (Figure 6), its transcript level is expected to be increased in the *hen1* mutant, which has a reduced level of miR157d. Indeed, the light-induced expression of *HY5* was further elevated in the *hen1-1* mutant as compared with the wild type (L*er*) under photomorphogenesis (Figure 7B). The increased *HY5* mRNA level also resulted in higher HY5 protein level in *hen1-1* than L*er* seedlings throughout the photomorphogenic process (Figure 7C;

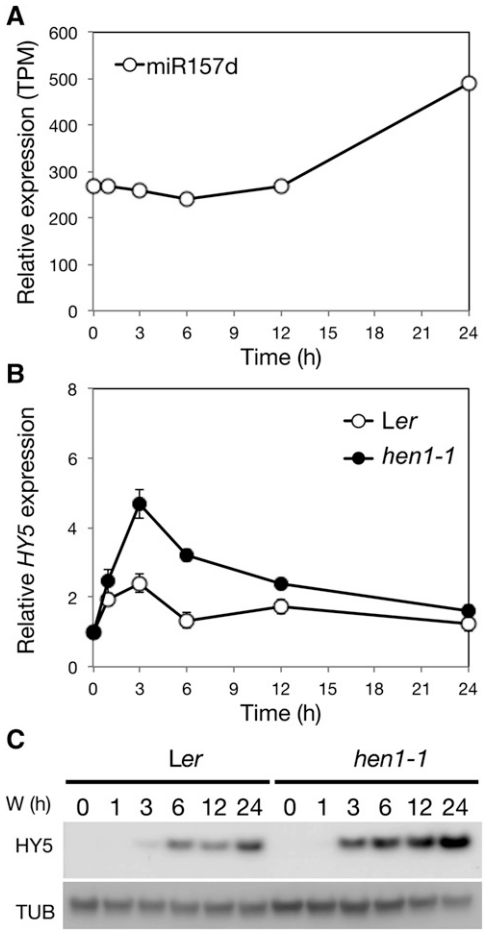


**Figure 7.** Light Responsiveness and Regulatory Roles of miR157d on *HY5* during Photomorphogenesis.

**(A)** The light responsiveness of miR157d. TPM, transcripts per million.
**(B)** qRT-PCR analysis of the mRNA level of *HY5* in wild-type (L*er*) and *hen1-1* mutant seedlings with *UBQ10* as an internal control. The expression was normalized to that of the corresponding genotype at 0 h. Data are mean ± SD from three biological replicates (each with three technical replicates).
**(C)** Representative immunoblot analysis of HY5 protein level in the wild type (L*er*) and *hen1-1* during photomorphogenesis with tubulin (TUB) as a loading control.

Supplemental Figure 10). The increased level of the positive regulator HY5 could enhance the light sensitivity of the *hen1* mutant, which is consistent with the light-hypersensitive phenotype of *hen1* (Figures 1 and 2).

By contrast, in the *MIR157d*-overexpression line (*MIR157dox*; Supplemental Figure 11), *HY5* transcript and protein levels were reduced (Figures 8A and 8B; Supplemental Figure 12). As expected, *MIR157dox* plants showed light hyposensitivity as compared with the wild type (Col-0) (Figure 8C). To further examine whether the light hyposensitivity of *MIR157dox* was due to reduced accumulation of HY5, we introduced a miR157d-resistant *HY5* overexpression construct (*mHY5* as in Figure 6B) into *MIR157dox* (denoted *mHY5/MIR157dox*). As expected, HY5 was overaccumulated in deetiolating *mHY5/MIR157dox* seedlings (Figure 8D). In contrast to the parental line (*MIR157dox*), *mHY5/MIR157dox* lines were light hypersensitive, and the degree of light hypersensitivity was proportional to HY5 protein levels in three independent *mHY5/MIR157dox* lines (Figure 8D). We cannot entirely rule out that miR157d may target additional positive regulators of photomorphogenesis. However, our results clearly indicated that the light hyposensitivity of *MIR157dox* seedlings could be rescued if HY5 protein levels were manipulated to increase (Figure 8D).

Taken together, HEN1 has a negative regulatory role on *HY5* expression via the action of miR157d. Together with our findings of HY5-dependent upregulation of *HEN1* (Figure 3), this demonstrates that *HY5* and *HEN1* form a negative feedback regulatory loop in photomorphogenesis.

### miR319 Regulates the Expression of Negative Regulators of *Arabidopsis* Photomorphogenesis

Because HEN1 is required for general miRNA biogenesis, the light hypersensitivity observed in the *hen1* mutants could be a combined result of the actions of multiple miRNA-target pairs affecting photomorphogenesis. We wondered whether additional miRNAs or miRNA target genes played roles in photomorphogenesis regulation. Five TCPII subfamily transcription factor genes, *TCP2*, *3*, *4*, *10*, and *24*, are known targets of miR319 (Jones-Rhoades et al., 2006). Ectopic expression of *TCP2/3/4* with mutated miR319 target sites results in light-hyposensitive phenotypes, so TCP2/3/4 are hypothesized to be negative regulators of hypocotyl elongation (Palatnik et al., 2003; Koyama et al., 2007; Zhao et al., 2013). We confirmed the presence of a miR319-mediated cleaved signature for transcripts of a selected TCPII gene, *TCP24*, in photomorphogenic *Arabidopsis* (Figure 9A). The expression of both *MIR319* and TCPII family members (*TCP2/3/4/10/24*) was transiently upregulated by light (Figures 9B and 9C).

The induction of *MIR319* by light has the potential to reduce the expression of *TCPs*. To assess the role of miR319 on photomorphogenesis, we analyzed *MIR319a* overexpression lines, *jaw-D* mutants (Palatnik et al., 2003), known to show reduced expression of TCPII members (Nag et al., 2009). Because TCPs are negative regulators in hypocotyl elongation, their reduced levels may confer *Arabidopsis* seedlings with light hypersensitivity. Indeed, multiple alleles of *jaw-D* mutants showed a light hypersensitive phenotype (Figure 9D).

### Temporal Expression Adjustment of *HEN1*, *HY5*, and *TCP24*

To elucidate the regulatory relationship between HEN1, HY5, and TCP24 in deetiolating *Arabidopsis* seedlings, we examined their expression by time-course analysis. The positive regulator *HY5* quickly responded to light signals, followed by the two negative regulators, *HEN1* and *TCP24* (Figure 10A). This finding suggested that in deetiolating seedlings, positive regulators such as HY5 could efficiently transmit the light signals in a timely manner. With time, negative regulators such as *HEN1* and *TCP24* are expressed to attenuate the actions of the positive regulator(s), so the light responses could be properly fine-tuned and/or optimized.

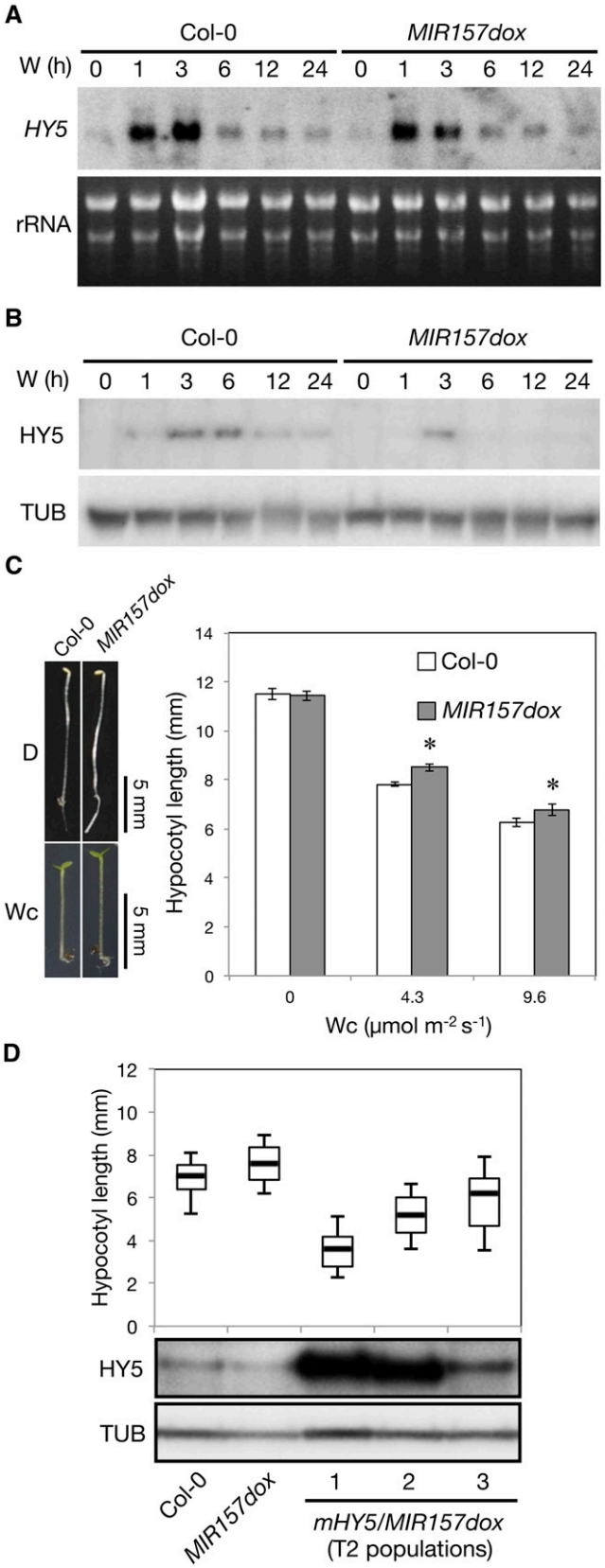


**Figure 8.** The Effect of *MIR157d* Overexpression on Photomorphogenesis.

**(A)** RNA gel blot analysis of *HY5* with 5 μg total RNA from 4-d-old etiolated Col-0 and *MIR157dox* seedlings exposed to W (50 μmol $m^{-2}$ $s^{-1}$) for the times indicated. The level of rRNA was a loading control. Numbers below the blot indicate *HY5* level relative to the rRNA in each sample. One representative blot from three independent biological replicates is shown.

**(B)** Representative immunoblot analysis of HY5 in wild type (Col-0) and *MIR157d* overexpression line (*MIR157dox*) during photomorphogenesis with TUB as a loading control.

**(C)** Hypocotyl lengths of *MIR157dox* seedlings under Wc of indicated fluence rates. Data are mean ± SE. *Significantly different from wild-type *Arabidopsis* (Col-0) ($P < 0.01$, Student's *t* test; $n = 13$ to 15).

**(D)** Box plots ($n = 65$ to 98) show hypocotyl lengths of the wild type (Col-0), *MIR157dox*, and three independent *mHY5/MIR157dox* lines grown for 4 d under Wc (5 μmol $m^{-2}$ $s^{-1}$). The top, middle, and bottom of the box represent the 25, 50, and 75 percentiles, respectively, and the top and bottom black lines are the 10th and 90th percentiles, respectively. HY5 levels in etiolated seedlings exposed to Wc (50 μmol $m^{-2}$ $s^{-1}$) for 6 h were determined by immunoblot analysis with TUB as a loading control.

[See online article for color version of this figure.]

We also examined the expression behaviors of *HEN1*, *HY5*, and *TCP24* in response to various light fluence rates. Light at lower fluence rate could trigger the expression of all three genes (Figure 10B). High light fluence rate further boosted the expression of *HY5* but not *TCP24* (Figure 10B). The higher expression ratio of positive to negative regulators may contribute to the exaggerated light responses under high light intensity.

## DISCUSSION

### HEN1 Is a Negative Regulator That Functions to Attenuate Light Signals during Photomorphogenesis in *Arabidopsis*

As a key enzyme in miRNA/small interfering RNA biogenesis, HEN1 likely functions to stabilize small RNA species that target the mRNAs of key regulators for degradation in light signaling pathways. From our observations, we postulate a light signaling pathway involving HEN1 in regulating *Arabidopsis* photomorphogenesis (Figure 11). Briefly, in wild-type *Arabidopsis*, environmental B, R, and FR light signals could be perceived by the photoreceptors cry1/cry2/phyA, phyB, and phyA, respectively. The light signals primarily converge at HY5 and HYH to induce the expression of *HEN1*, which is needed to stabilize and increase the levels of miRNAs, including but not limited to miR157d and miR319. With the progression of deetiolation, miR157d and miR319 could trigger miRNA-mediated cleavage of *HY5* and *TCP* mRNAs. This process helps to reduce or shape the temporal expression kinetics of these genes to properly attenuate the actions of both the positive (HY5) and the negative (TCPs) regulators of photomorphogenesis (Figure 11).

Our observations also reveal the complex nature of photomorphogenic development driven by a regulatory cascade or network of intertwined positive and negative regulators. HEN1 may stabilize miRNAs to prevent the overproduction of these regulators, for an antagonistic balance between positive and negative regulators. Nevertheless, the light-hypersensitive

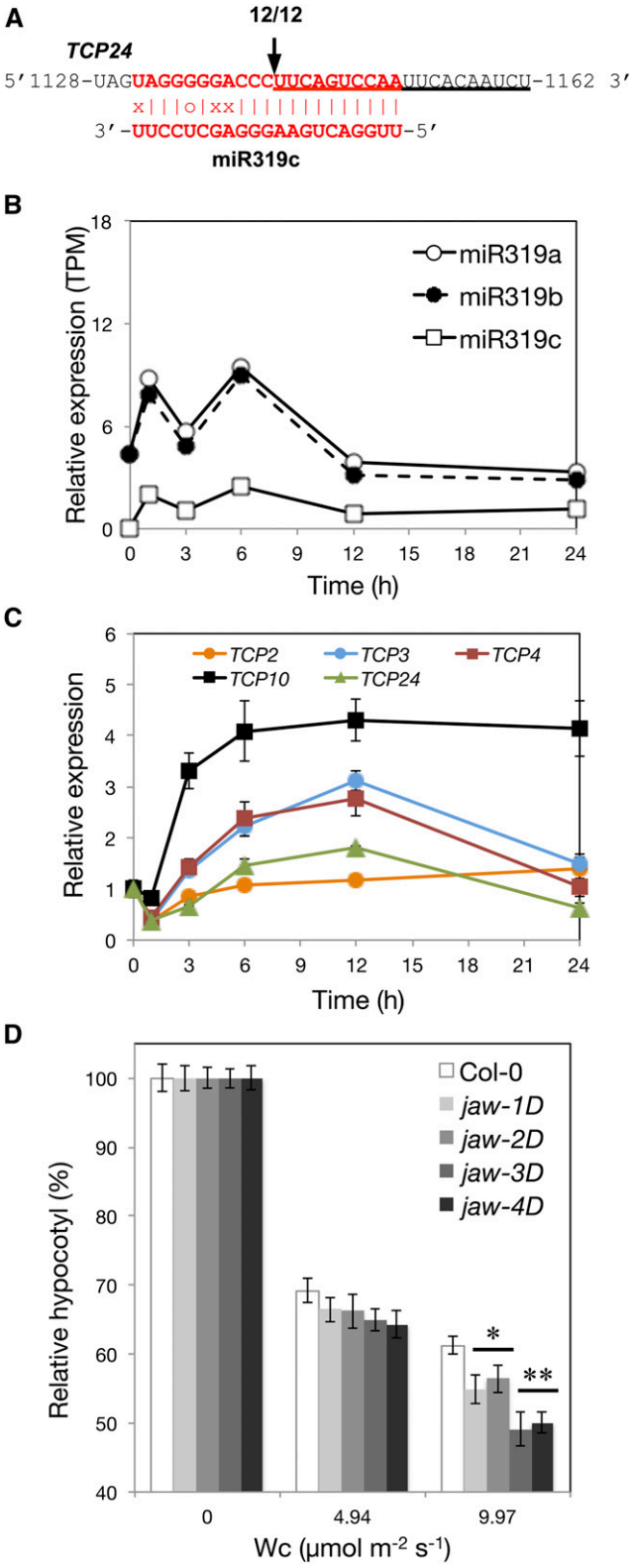


**Figure 9.** Light Responsiveness and Regulatory Role of miR319 in *TCP* Levels in Photomorphogenic *Arabidopsis.*

phenotype of *hen1* suggests that the more predominant role of HEN1 is to desensitize positive regulators in the light signaling pathway.

### Accumulation of *HEN1* Transcripts in Early Photomorphogenesis

Our data indicate that the full light responsiveness of *HEN1* depends on the photoreceptors phyA, phyB, cry1, and cry2 (Figures 3A to 3D). HY5 and HYH are the primary positive regulators that convey the light signal to induce *HEN1* expression during early photomorphogenesis (Figure 3F). However, the promoter region of *HEN1* contains no putative HY5 binding *cis*-element such as a G-box (CACGTG) or ACE box (ACGT-containing element) (Chattopadhyay et al., 1998; Shin et al., 2007). A recent HY5 ChIP-chip experiment also did not reveal *HEN1* (Lee et al., 2007). Thus, *HEN1* is likely not the immediate target of HY5. The normal light-induced expression of *HEN1* in the *b-box-containing protein 22/light-regulated zinc finger protein1* (*bbx22/lzf1*) mutant excluded BBX22/LZF1, a HY5-dependent positive regulator of photomorphogenesis (Chang et al., 2008), as responsible for *HEN1* activation. Revealing the immediate transcription factor(s) governing the light responsiveness of *HEN1* awaits the identification of more positive regulators in the HY5 lineage of the light-signaling pathway.

### miRNA-Mediated Posttranscriptional Regulation in Photomorphogenic *Arabidopsis*

Etiolated *hen1-1*, *hyl1*, and *hst* seedlings have short hypocotyls (this study; Lu and Fedoroff, 2000; Bollman et al., 2003). However, these mutants still exhibit light-hypersensitive phenotypes by showing exaggerated light-mediated inhibition of hypocotyl elongation (Figure 1; Supplemental Figure 2). Because photomorphogenesis is normal for mutants defective in a ta-siRNA biogenesis pathway (Figure 4), miRNAs may play a more prominent role in the posttranscriptional regulation of photomorphogenesis.

We observed that the expression of miR157d and miR319 was increased by light in deetiolating seedlings (Figures 7 and 9). Such upregulation could be accomplished by increasing the transcription of primary transcripts of the *MIR* genes, by enhancing the capability of the processing complex to generate

**(A)** 5′-RACE analysis of the cleavage site of *TCP24* mRNA within the *TCP24*-miR319c duplex.

**(B)** The light responsiveness of miR319 members.

**(C)** qRT-PCR analysis of the mRNA level of *TCP* genes. The expression of each gene under W light at times indicated was normalized to that of *UBQ10* and was relative to that of etiolated seedlings (0 h). Data are mean ± SE from two biological replicates (each with two technical replicates).

**(D)** The hypocotyl lengths of different allelic *jaw-D* mutant seedlings under Wc of the indicated fluence rates normalized to the length under the dark. Data are mean ± SE. * and **, significantly different from wild-type *Arabidopsis* (Col-0) ($P < 0.05$ and $P < 0.01$, Student's *t* test; $n = 24$ to 27).

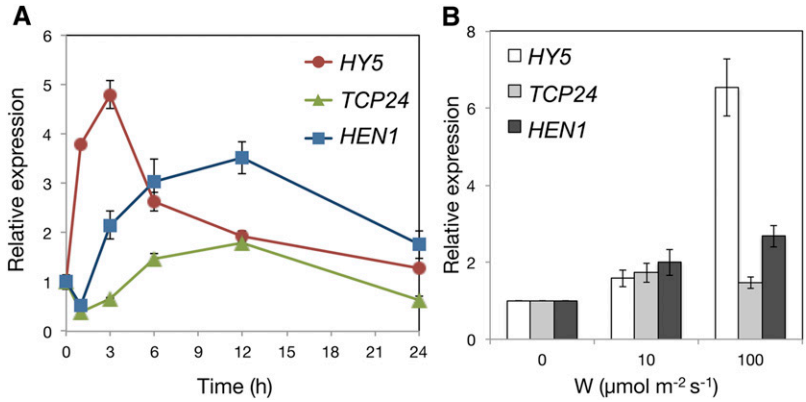


**Figure 10.** Responsiveness of *HY5*, *TCP24*, and *HEN1* to Light over Time and Fluence Rates during Deetiolation.

**(A)** qRT-PCR analysis of the mRNA levels of *HY5*, *TCP24*, and *HEN1* in deetiolating seedlings of the wild type (Col-0) at the indicated times of exposure to W (100 μmol $m^{-2}$ $s^{-1}$). Relative expression of each gene was normalized to that of *UBQ10* and was relative to that of etiolated seedlings.
**(B)** qRT-PCR analysis of *HY5*, *TCP24*, and *HEN1* expression in deetiolating Col-0 seedlings exposed to the indicated fluence rates for 6 h.

more precursors of miRNAs, stabilization, and/or transport of miRNAs. Among genes essential for the miRNA biogenesis pathway, only the *HEN1* transcript is upregulated by light (Supplemental Figure 3). By artificially increasing the level of HA-HEN1 protein in dark-grown seedlings, miR157d/319 levels were increased (Figure 5C). This finding is indicative of a rate-limiting role for HEN1 in the biogenesis of these two miRNAs in young seedlings. Thus, the light responsiveness of *HEN1* expression may serve as a signal to boost the biogenesis of miRNAs during early deetiolation. The increased miR157d and miR319 expression then imposes posttranscriptional regulation of gene expression in photomorphogenic *Arabidopsis*.

A future goal is to reveal additional miRNA-mRNA pairs that together achieve an optimized equilibrium of positive and negative regulators in deetiolating *Arabidopsis*. This investigation will require a comprehensive profiling of both mRNA and small regulatory RNAs and pairwise functional characterizations during photomorphogenic development.

## METHODS

### Plant Materials and Growth Conditions

Seeds of *hyl1-2* (Song et al., 2007), *hst-3* (Bollman et al., 2003), *hst-6* (Bollman et al., 2003), *rdr6-11* (Peragine et al., 2004), *sgs3-11* (Peragine et al., 2004), *hen1-5* (SALK_049197) (Vazquez et al., 2004), *hen1-6* (SALK_090960) (Li et al., 2005), *hy5-1* (Koornneef et al., 1980), *cry1* (Koornneef et al., 1980), *phyA-201* (Nagatani et al., 1993), *phyB-5* (Reed et al., 1993), *jaw-1D* (CS6948), *jaw-2D* (CS6949), *jaw-3D* (CS6950), and *jaw-4D* (CS6951) mutants were obtained from the ABRC. The mutants *hen1-1* (Park et al., 2002), *phyA cry1 cry2* (Duek and Fankhauser, 2003), *cop1-4*/*cop1-6* (McNellis et al., 1994), and *hy5 hyh* (Holm et al., 2002) were kindly provided by Xuemei Chen, Christian Fankhauser, Hsu-Liang Hsieh, and Christian Hardtke, respectively.

Seeds of *Arabidopsis thaliana* Col-0, L*er*, and mutant plants were germinated on half-strength Murashige and Skoog medium with 0.8% agar and stratified at 4°C in the dark for 3 d, exposed to white light for 4 h to induce germination, and placed in the dark at 22°C for 20 h. For measuring hypocotyl length after light treatments, seedlings were moved to 22°C growth chambers for 4 d under various light conditions. Control seedlings were kept in the dark for 4 d. For *HEN1* expression analysis, cold-stratified seeds were allowed to grow in the dark for 4 d at 22°C, then treated with light for the times indicated. LED light sources (Daina Electronics) were used for B (470 ± 30 nm), R (660 ± 25 nm), and FR light (730 ± 25 nm). Fluence rates used for measuring hypocotyl length under W, B, R, or FR light are in Figures 1, 4, 8, and 9 and Supplemental Figures 2, 5, 6, and 7. Hypocotyl length was analyzed using ImageJ (Schneider et al., 2012). The fluence rate was measured with use of an LI-250 radiometer (LI-COR).

### Apical Hook and Antigravitropism Measurement

Seeds were germinated on a vertical plate in the dark for 24 h before seedlings were photographed every hour. The angle of curvature of the hook was measured as described (Vandenbussche et al., 2010). For the antigravitropism analysis, seedlings were grown on a vertical plate in the dark for 4 d before the plate was rotated for 90° and photographed every 30 min. The hypocotyl curvature from antigravitropism was measured using HypoPhen (Kami et al., 2012).

### Anthocyanin Quantification

L*er* and *hen1-1* seedlings were grown for 4 d on half-strength Murashige and Skoog media containing 0.8% agar and sucrose at the indicated concentrations (Figure 2B) under 16 h light/8 h dark to enhance anthocyanin accumulation. Whole seedlings were collected and weighed for anthocyanin extraction and quantification as described (Lange et al., 1971).

### RNA Isolation and Detection

Total RNA was isolated from seedlings by the pine-tree method as described (Chang et al., 1993) or with the *mir*Vana miRNA isolation kit (Ambion). First-strand cDNA was synthesized and used for quantitative RT-PCR (qRT-PCR) as described (Wu et al., 2008) with the gene-specific primers in Supplemental Table 1. Small RNA gel blot analysis was performed as described (Chiou et al., 2006) with the miRNA-specific oligos in Supplemental Table 1. Signal intensities in Figures 5B and 5C and Supplemental Figure 12 were analyzed using ImageJ (Schneider et al., 2012).

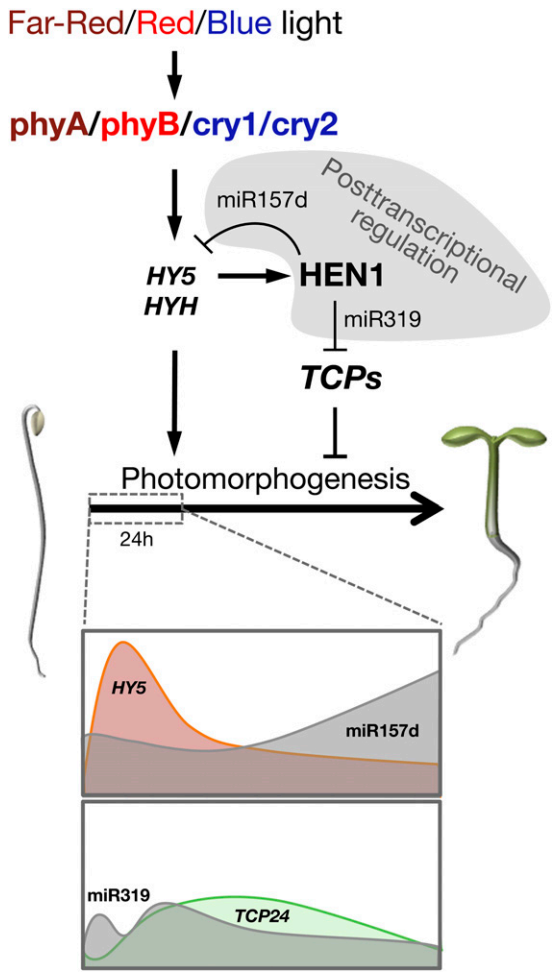


**Figure 11.** Diagram Depicting the Regulation and Function of HEN1 in Light Signal Transduction in Early Photomorphogenesis in *Arabidopsis*

The light-induced expression of *HEN1* depends on the photoreceptors and positive regulators HY5/HYH. The increased HEN1 in photomorphogenic seedlings leads to the accumulation of miR157d and miR319 to fine-tune the expression patterns of both positive and negative regulators of photomorphogenesis. During early photomorphogenic development, miR157d targets *HY5* transcript for degradation, resulting in a negative feedback regulatory loop between HEN1 and HY5. miR319 negatively regulates the expression of *TCP24*, a negative regulator of photomorphogenesis.

For RNA gel blot analyses of *HEN1* and *HY5* transcripts, total RNA was isolated from seedlings by the PureLink Plant RNA Reagent method (Invitrogen). Five micrograms of total RNA was separated on 1% formaldehyde agarose gel and transferred to positively charged nylon membrane (GE Healthcare). *HEN1* and *HY5* cDNAs were amplified with specific primers (Supplemental Table 1) and used as templates for probe labeling.

### Validation of miRNA-Directed Cleavage on Targets

Modified 5′ RACE with the GeneRacer Kit (Invitrogen) was used to validate cleavage sites on transcripts of *HY5* and *TCP24*. The primers for 5′ RACE are in Supplemental Table 1.

### Analysis of Frequency of SNPs

We used the *Arabidopsis* 1001 Genomes database (http://signal.salk.edu/atg1001/3.0/gebrowser.php) for analysis of *HY5* SNPs from multiple sequence alignments of 513 *Arabidopsis* tracks (available on April 29, 2013). The SNP frequency was calculated for each position in *HY5* exon regions by comparing *HY5* sequences from different tracks to that of Col-0.

### Construction of *MIR* Genes, *HY5-GFP*/*mHY5-GFP* Reporters, *mHY5* cDNA, and *XVE:HA-HEN1* System

*MIR156a* and *MIR157d* were amplified from *Arabidopsis* genomic DNA with corresponding forward and reverse primers (Supplemental Table 1) and inserted into the pBIN61 vector digested by *Xba*I and *Sma*I and under the control of the 35S promoter. The fragment of *MIR157d* driven by the 35S promoter was subcloned into the *Hin*dIII site of the pCAMBIA 1390 binary vector to generate *MIR157dox* line in *Arabidopsis* (Col-0). For the *HY5-GFP* reporter, *HY5* 5′-UTR (91 to 192 bp) containing the miR157d pairing region was amplified with *HY5-5′UTR* forward and reverse primers (Supplemental Table 1) and fused to the N terminus of GFP, and *HY5-GFP* fragment was excised by *Xba*I and *Sma*I and inserted in the pBIN61 vector to generate the *HY5-GFP* reporter. The miR157d-pairing region on the *HY5-GFP* reporter was further mutated by site-directed mutagenesis with *HY5-5′UTRm* forward and reverse primers (Supplemental Table 1) to generate the *mHY5-GFP* reporter. This mutated 5′-UTR was introduced by RT-PCR with primers *HY5*-5′UTRm-*Xba*I-F and *HY5stop-Eco*RI-R (Supplemental Table 1) to generate *mHY5* cDNA. The *Xba*I-*Eco*RI *mHY5* cDNA fragment was inserted into a Basta resistance (bar)-modified pCAMBIA 1390 under the control of the 35S promoter. The *HEN1* genomic DNA fragment containing the entire coding region was amplified with primers in Supplemental Table 1. The *HEN1* genomic DNA was inserted into a modified pER10-bar digested by *Kpn*I and *Spe*I to generate a 3×HA epitope translationally fused *HEN1* fragment in the *XVE:HA-HEN1* construct for XVE-induction in *Arabidopsis* (Col-0) (Zuo et al., 2006).

### *Nicotiana benthamiana* Transient Expression

Transient expression assay was performed as described (Voinnet et al., 2003). Leaves of 4-week-old *N. benthamiana* were coinfiltrated with cultures of *Agrobacterium tumefaciens* (C58C1) carrying *MIR157d* (or *MIR156a*) and *HY5-GFP* (or *mHY5-GFP*) reporter. Agrobacteria were brought to $OD_{600}$ = 1.0 for input by mixing *MIR* and reporter cultures in a 9:1 ratio. Infiltrated tissues were collected after 3 d, and total RNA was isolated for small RNA gel blot and qRT-PCR analyses.

### XVE:HA-HEN1 Induction

The T2 seed populations from independent transgenic lines of *XVE:HA-HEN1* (Col-0) were germinated on the growth medium described above containing phosphinothricin (10 μg/μL) for the selection. The seeds were allowed to grow in the dark at 22°C for 3 d and transferred into 20 mL 3 mM MES buffer (pH 5.7) containing 0, 0.1, 1, or 10 μM β-estradiol dilutions carried by 20 μL DMSO for 24-h dark induction before collection for *HA-HEN1* and miRNA analyses.

### Sequencing of Small RNAs

Four-day-old etiolated *Arabidopsis* seedlings were treated with white light (100 μmol $m^{-2}$ $s^{-1}$) for 0, 1, 3, 6, 12, and 24 h. Total RNA was isolated with the *mir*Vana miRNA isolation kit. The size-fractionated small RNAs were used to generate libraries for sequencing by the Illumina platform. Sequencing reads mapped to miR157d and miR319a/b/c were normalized to the total reads and extracted for profiling the light responsiveness in the deetiolation process.

### Immunoblot Analyses

Total protein was extracted from 4-d-old etiolated seedlings as described (Chang et al., 2011) at different times of W light treatment. HY5 protein was detected by polyclonal anti-HY5 antibody (Liu et al., 2012). HA-tagged HEN1 protein was detected by incubation with the antibodies mouse monoclonal anti-HA (H3663; Sigma-Aldrich) or pig polyclonal anti-HEN1 (Yang et al., 2007). Endogenous α-tubulin was detected by incubation with a mouse monoclonal anti-α-tubulin antibody (T5168; Sigma-Aldrich).

### Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative data library with the following locus identifiers: *HYL1* (At1g09700), *HEN1* (At4g20910), *HST* (At3g05040), *PHYA* (At1g09570), *PHYB* (At2g18790), *CRY1* (At4g08920), *CRY2* (At1g04400), *HY5* (At5g11260), *HYH* (At3g17609), *COP1* (At2g32950), *RDR6* (At3g49500), *SGS3* (At5g23570), *TCP2* (At4g18390), *TCP3* (At1g53230), *TCP4* (At3g15030), *TCP10* (At2g31070), *TCP24* (At1g30210), *MIR156A* (At2g25095), *MIR157D* (At1g48742), *MIR319A* (At4g23713), *MIR319B* (At5g41663), and *MIR319C* (At2g40805).

### Supplemental Data

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

**Supplemental Figure 1**. Schematic Overview of Canonical miRNA Biosynthesis (Modified from Rogers and Chen, 2013).

**Supplemental Figure 2**. Skotomorphogenesis and Photomorphogenesis Are Defective in miRNA Biogenesis Mutants.

**Supplemental Figure 3**. The Transcript Level of *HEN1* Is Light Upregulated.

**Supplemental Figure 4**. Molecular Characterization of *hen1-5* and *hen1-6* Mutants.

**Supplemental Figure 5**. The *hen1* Mutants Are Hypersensitive to Blue (B), Far-Red (FR), and Red (R) Light.

**Supplemental Figure 6**. Functional Complementation of *hen1* Light-Hypersensitive Phenotype.

**Supplemental Figure 7**. Effect of FR Fluence Rate on Hypocotyl Extension in Wild-Type and *sgs3-11* Mutant Seedlings.

**Supplemental Figure 8**. The Levels of miR157 and miR159/319 Are Increased Accompanied with Increased Level of HA-HEN1 Protein.

**Supplemental Figure 9**. The Expressions of miR157d and miR319 Are Compromised in *hen1-1* Mutant.

**Supplemental Figure 10**. HY5 Is Increased in the *hen1-1* Mutant during Photomorphogenesis.

**Supplemental Figure 11**. Overaccumulation of miR157d Detected in the *MIR157dox* Line.

**Supplemental Figure 12**. *HY5* Transcript Levels Are Decreased in the *MIR157dox* Transgenic Line.

**Supplemental Table 1**. Primers and Oligos Used in This Study.

## ACKNOWLEDGMENTS

We thank Yi-Chen Wu for constructing the *HEN1* genomic fragment for the complementation experiment, Jing-Fen Wu for constructing the *mHY5* overexpression and *XVE:HA-HEN1* inducible constructs, Tzyy-Jen Chiou and Kuo-Chen Yeh for helpful discussion, Ho-Ming Chen for initial search of the PARE database, Mei-Jane Fang for technical assistance, Xuemei Chen for providing seeds of the *hen1-1* mutant and the anti-HEN1 antibody, and Christian Frankhauser, Hsu-Liang Hsieh, and Christian Hardtke for providing seeds of the *phyA cry1 cry2* triple mutant, *cop1* mutant alleles, and *hy5 hyh* double mutant. This research was supported by a National Research Foundation of Korea grant funded by the Korean government (Ministry of Science, ICT, and Future Planning; 2008-0061988) to J.H.A., an Academia Sinica postdoctoral fellowship to H.-L.T., and the Foresight Project L20-2 and Investigator Award to S.-H.W. from Academia Sinica.

## AUTHOR CONTRIBUTIONS

H.-L.T. and S.-H.W. designed the research, analyzed the data, and wrote the article. H.-L.T., Y.-H.L., W.-P.H., and M.-C.L. performed the research. J.H.A. contributed experimental materials.

Received April 20, 2014; revised June 17, 2014; accepted July 1, 2014; published July 22, 2014.

## REFERENCES

**Ahmad, M., Lin, C., and Cashmore, A.R.** (1995). Mutations throughout an Arabidopsis blue-light photoreceptor impair blue-light-responsive anthocyanin accumulation and inhibition of hypocotyl elongation. Plant J. **8:** 653–658.

**Ahmad, M., Jarillo, J.A., Smirnova, O., and Cashmore, A.R.** (1998). The CRY1 blue light photoreceptor of Arabidopsis interacts with phytochrome A in vitro. Mol. Cell **1:** 939–948.

**Bollman, K.M., Aukerman, M.J., Park, M.Y., Hunter, C., Berardini, T.Z., and Poethig, R.S.** (2003). HASTY, the Arabidopsis ortholog of exportin 5/MSN5, regulates phase change and morphogenesis. Development **130:** 1493–1504.

**Chang, C.-S.J., Maloof, J.N., and Wu, S.-H.** (2011). COP1-mediated degradation of BBX22/LZF1 optimizes seedling development in Arabidopsis. Plant Physiol. **156:** 228–239.

**Chang, C.-S.J., Li, Y.H., Chen, L.T., Chen, W.C., Hsieh, W.P., Shin, J., Jane, W.N., Chou, S.J., Choi, G., Hu, J.M., Somerville, S., and Wu, S.-H.** (2008). LZF1, a HY5-regulated transcriptional factor, functions in Arabidopsis de-etiolation. Plant J. **54:** 205–219.

**Chang, S., Puryear, J., and Cainey, J.** (1993). A simple and efficient method for isolating RNA from pine trees. Plant Mol. Biol. Rep. **11:** 113–116.

**Chattopadhyay, S., Ang, L.H., Puente, P., Deng, X.W., and Wei, N.** (1998). Arabidopsis bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression. Plant Cell **10:** 673–683.

**Chen, X.** (2010). Small RNAs - secrets and surprises of the genome. Plant J. **61:** 941–958.

**Chiou, T.J., Aung, K., Lin, S.I., Wu, C.C., Chiang, S.F., and Su, C.L.** (2006). Regulation of phosphate homeostasis by microRNA in Arabidopsis. Plant Cell **18:** 412–421.

**Colón-Carmona, A., Chen, D.L., Yeh, K.C., and Abel, S.** (2000). Aux/IAA proteins are phosphorylated by phytochrome in vitro. Plant Physiol. **124:** 1728–1738.

**Duek, P.D., and Fankhauser, C.** (2003). HFR1, a putative bHLH transcription factor, mediates both phytochrome A and cryptochrome signalling. Plant J. **34:** 827–836.

**Fankhauser, C., and Chory, J.** (1999). Light receptor kinases in plants! Curr. Biol. **9:** R123–R126.

**German, M.A., et al**. (2008). Global identification of microRNA-target RNA pairs by parallel analysis of RNA ends. Nat. Biotechnol. **26:** 941–946.

**Henriques, R., Jang, I.C., and Chua, N.H.** (2009). Regulated proteolysis in light-related signaling pathways. Curr. Opin. Plant Biol. **12:** 49–56.

**Holm, M., Ma, L.G., Qu, L.J., and Deng, X.W.** (2002). Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in Arabidopsis. Genes Dev. **16:** 1247–1259.

**Ji, L., and Chen, X.** (2012). Regulation of small RNA stability: methylation and beyond. Cell Res. **22:** 624–636.

**Jiao, Y., Lau, O.S., and Deng, X.W.** (2007). Light-regulated transcriptional networks in higher plants. Nat. Rev. Genet. **8:** 217–230.

**Jones-Rhoades, M.W., Bartel, D.P., and Bartel, B.** (2006). MicroRNAS and their regulatory roles in plants. Annu. Rev. Plant Biol. **57:** 19–53.

**Juntawong, P., and Bailey-Serres, J.** (2012). Dynamic light regulation of translation status in *Arabidopsis thaliana*. Front. Plant Sci. **3:** 66.

**Kami, C., Hersch, M., Trevisan, M., Genoud, T., Hiltbrunner, A., Bergmann, S., and Fankhauser, C.** (2012). Nuclear phytochrome A signaling promotes phototropism in Arabidopsis. Plant Cell **24:** 566–576.

**Kim, K., Shin, J., Lee, S.H., Kweon, H.S., Maloof, J.N., and Choi, G.** (2011). Phytochromes inhibit hypocotyl negative gravitropism by regulating the development of endodermal amyloplasts through phytochrome-interacting factors. Proc. Natl. Acad. Sci. USA **108:** 1729–1734.

**Koornneef, M., Rolff, E., and Spruit, C.J.P.** (1980). Genetic-control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L) Heynh. Z. Pflanzenphysiol. **100:** 147–160.

**Koyama, T., Furutani, M., Tasaka, M., and Ohme-Takagi, M.** (2007). TCP transcription factors control the morphology of shoot lateral organs via negative regulation of the expression of boundary-specific genes in Arabidopsis. Plant Cell **19:** 473–484.

**Lange, H., Shropshire, W., and Mohr, H.** (1971). An analysis of phytochrome-mediated anthocyanin synthesis. Plant Physiol. **47:** 649–655.

**Lee, J., He, K., Stolc, V., Lee, H., Figueroa, P., Gao, Y., Tongprasit, W., Zhao, H., Lee, I., and Deng, X.W.** (2007). Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. Plant Cell **19:** 731–749.

**Leivar, P., and Quail, P.H.** (2011). PIFs: pivotal components in a cellular signaling hub. Trends Plant Sci. **16:** 19–28.

**Li, J., Yang, Z., Yu, B., Liu, J., and Chen, X.** (2005). Methylation protects miRNAs and siRNAs from a 3′-end uridylation activity in Arabidopsis. Curr. Biol. **15:** 1501–1507.

**Liu, M.-J., Wu, S.-H., Chen, H.-M., and Wu, S.-H.** (2012). Widespread translational control contributes to the regulation of Arabidopsis photomorphogenesis. Mol. Syst. Biol. **8:** 566.

**Liu, M.-J., Wu, S.-H., Wu, J.-F., Lin, W.-D., Wu, Y.-C., Tsai, T.-Y., Tsai, H.-L., and Wu, S.-H.** (2013). Translational landscape of photomorphogenic Arabidopsis. Plant Cell **25:** 3699–3710.

**Lorrain, S., Genoud, T., and Fankhauser, C.** (2006). Let there be light in the nucleus! Curr. Opin. Plant Biol. **9:** 509–514.

**Lu, C., and Fedoroff, N.** (2000). A mutation in the Arabidopsis HYL1 gene encoding a dsRNA binding protein affects responses to abscisic acid, auxin, and cytokinin. Plant Cell **12:** 2351–2366.

**Maier, A., Schrader, A., Kokkelink, L., Falke, C., Welter, B., Iniesto, E., Rubio, V., Uhrig, J.F., Hülskamp, M., and Hoecker, U.** (2013). Light and the E3 ubiquitin ligase COP1/SPA control the protein stability of the MYB transcription factors PAP1 and PAP2 involved in anthocyanin accumulation in Arabidopsis. Plant J. **74:** 638–651.

**Mazzella, M.A., Casal, J.J., Muschietti, J.P., and Fox, A.R.** (2014). Hormonal networks involved in apical hook development in darkness and their response to light. Front. Plant Sci. **5:** 52.

**McNellis, T.W., von Arnim, A.G., Araki, T., Komeda, Y., Miséra, S., and Deng, X.W.** (1994). Genetic and molecular analysis of an allelic series of cop1 mutants suggests functional roles for the multiple protein domains. Plant Cell **6:** 487–500.

**Medzihradszky, M., et al**. (2013). Phosphorylation of phytochrome B inhibits light-induced signaling via accelerated dark reversion in Arabidopsis. Plant Cell **25:** 535–544.

**Nag, A., King, S., and Jack, T.** (2009). miR319a targeting of TCP4 is critical for petal growth and development in Arabidopsis. Proc. Natl. Acad. Sci. USA **106:** 22534–22539.

**Nagatani, A., Reed, J.W., and Chory, J.** (1993). Isolation and initial characterization of Arabidopsis mutants that are deficient in phytochrome A. Plant Physiol. **102:** 269–277.

**Nito, K., Wong, C.C., Yates, J.R., III., and Chory, J.** (2013). Tyrosine phosphorylation regulates the activity of phytochrome photoreceptors. Cell Reports **3:** 1970–1979.

**Osterlund, M.T., Hardtke, C.S., Wei, N., and Deng, X.W.** (2000). Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature **405:** 462–466.

**Paik, I., Yang, S., and Choi, G.** (2012). Phytochrome regulates translation of mRNA in the cytosol. Proc. Natl. Acad. Sci. USA **109:** 1335–1340.

**Palatnik, J.F., Allen, E., Wu, X., Schommer, C., Schwab, R., Carrington, J.C., and Weigel, D.** (2003). Control of leaf morphogenesis by microRNAs. Nature **425:** 257–263.

**Park, W., Li, J., Song, R., Messing, J., and Chen, X.** (2002). CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana*. Curr. Biol. **12:** 1484–1495.

**Peragine, A., Yoshikawa, M., Wu, G., Albrecht, H.L., and Poethig, R.S.** (2004). SGS3 and SGS2/SDE1/RDR6 are required for juvenile development and the production of trans-acting siRNAs in Arabidopsis. Genes Dev. **18:** 2368–2379.

**Poppe, C., Sweere, U., Drumm-Herrel, H., and Schäfer, E.** (1998). The blue light receptor cryptochrome 1 can act independently of phytochrome A and B in *Arabidopsis thaliana*. Plant J. **16:** 465–471.

**Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M., and Chory, J.** (1993). Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell **5:** 147–157.

**Rogers, K., and Chen, X.** (2013). Biogenesis, turnover, and mode of action of plant microRNAs. Plant Cell **25:** 2383–2399.

**Schneider, C.A., Rasband, W.S., and Eliceiri, K.W.** (2012). NIH Image to ImageJ: 25 years of image analysis. Nat. Methods **9:** 671–675.

**Shikata, H., Nakashima, M., Matsuoka, K., and Matsushita, T.** (2012). Deletion of the RS domain of RRC1 impairs phytochrome B signaling in Arabidopsis. Plant Signal. Behav. **7:** 933–936.

**Shin, D.H., Choi, M., Kim, K., Bang, G., Cho, M., Choi, S.B., Choi, G., and Park, Y.I.** (2013). HY5 regulates anthocyanin biosynthesis by inducing the transcriptional activation of the MYB75/PAP1 transcription factor in Arabidopsis. FEBS Lett. **587:** 1543–1547.

**Shin, J., Park, E., and Choi, G.** (2007). PIF3 regulates anthocyanin biosynthesis in an HY5-dependent manner with both factors directly binding anthocyanin biosynthetic gene promoters in Arabidopsis. Plant J. **49:** 981–994.

**Song, L., Han, M.H., Lesicka, J., and Fedoroff, N.** (2007). Arabidopsis primary microRNA processing proteins HYL1 and DCL1 define a nuclear body distinct from the Cajal body. Proc. Natl. Acad. Sci. USA **104:** 5437–5442.

**Sorin, C., Bussell, J.D., Camus, I., Ljung, K., Kowalczyk, M., Geiss, G., McKhann, H., Garcion, C., Vaucheret, H., Sandberg, G., and Bellini, C.** (2005). Auxin and light control of adventitious rooting in Arabidopsis require ARGONAUTE1. Plant Cell **17:** 1343–1359.

**Van Buskirk, E.K., Decker, P.V., and Chen, M.** (2012). Photobodies in light signaling. Plant Physiol. **158:** 52–60.

**Vandenbussche, F., Petrásek, J., Zádníková, P., Hoyerová, K., Pesek, B., Raz, V., Swarup, R., Bennett, M., Zazímalová, E., Benková, E., and Van Der Straeten, D.** (2010). The auxin influx carriers AUX1 and LAX3 are involved in auxin-ethylene interactions during apical hook development in Arabidopsis thaliana seedlings. Development **137:** 597–606.

**Vazquez, F., Gasciolli, V., Crété, P., and Vaucheret, H.** (2004). The nuclear dsRNA binding protein HYL1 is required for microRNA accumulation and plant development, but not posttranscriptional transgene silencing. Curr. Biol. **14:** 346–351.

**Voinnet, O., Rivas, S., Mestre, P., and Baulcombe, D.** (2003). An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. Plant J. **33:** 949–956.

**Wu, J.-F., Wang, Y., and Wu, S.-H.** (2008). Two new clock proteins, LWD1 and LWD2, regulate Arabidopsis photoperiodic flowering. Plant Physiol. **148:** 948–959.

**Xie, Z., Allen, E., Wilken, A., and Carrington, J.C.** (2005). DICER-LIKE 4 functions in trans-acting small interfering RNA biogenesis and vegetative phase change in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **102:** 12984–12989.

**Yang, Z., Vilkaitis, G., Yu, B., Klimasauskas, S., and Chen, X.** (2007). Approaches for studying microRNA and small interfering RNA methylation in vitro and in vivo. Methods Enzymol. **427:** 139–154.

**Yeh, K.-C., and Lagarias, J.C.** (1998). Eukaryotic phytochromes: light-regulated serine/threonine protein kinases with histidine kinase ancestry. Proc. Natl. Acad. Sci. USA **95:** 13976–13981.

**Yeh, K.-C., Wu, S.-H., Murphy, J.T., and Lagarias, J.C.** (1997). A cyanobacterial phytochrome two-component light sensory system. Science **277:** 1505–1508.

**Yi, C., and Deng, X.W.** (2005). COP1 - from plant photomorphogenesis to mammalian tumorigenesis. Trends Cell Biol. **15:** 618–625.

**Yoshikawa, M., Peragine, A., Park, M.Y., and Poethig, R.S.** (2005). A pathway for the biogenesis of trans-acting siRNAs in Arabidopsis. Genes Dev. **19:** 2164–2175.

**Zhang, H., He, H., Wang, X., Wang, X., Yang, X., Li, L., and Deng, X.W.** (2011). Genome-wide mapping of the HY5-mediated gene networks in Arabidopsis that involve both transcriptional and post-transcriptional regulation. Plant J. **65:** 346–358.

**Zhao, J., Favero, D.S., Peng, H., and Neff, M.M.** (2013). Arabidopsis thaliana AHL family modulates hypocotyl growth redundantly by interacting with each other via the PPC/DUF296 domain. Proc. Natl. Acad. Sci. USA **110:** E4688–E4697.

**Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L., and Gruissem, W.** (2004). GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiol. **136:** 2621–2632.

**Zuo, J., Hare, P.D., and Chua, N.H.** (2006). Applications of chemical-inducible expression systems in functional genomics and biotechnology. Methods Mol. Biol. **323:** 329–342.