Arabidopsis FHY3 and HY5 Positively Mediate Induction of COP1 Transcription in Response to Photomorphogenic UV-B Light

Xi Huang, a,b,c,1 Xin Hao Ouyang, b,c,d,1 Panyu Yang, b,e On Sun Lau, c,2 Gang Li, c,3 Jigang Li, b,c, Haodong Chen, b, and Xing Wang Deng, b,c,f,4

a College of Life Sciences, Beijing Normal University, Beijing 100875, China
b Peking-Yale Joint Center for Plant Molecular Genetics and Agro-Biotechnology, National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing 100871, China
c Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520-8104
d Rice Research Institute, Sichuan Agricultural University, Chengdu, Sichuan 611130, China
e Department of Botany, College of Life Sciences, Hunan Normal University, Changsha 410081, China
f Shenzhen Institute of Crop Molecular Design, Shenzhen 518107, China

As sessile organisms, higher plants have evolved the capacity to sense and interpret diverse light signals to modulate their development. In Arabidopsis thaliana, low-intensity and long-wavelength UV-B light is perceived as an informational signal to mediate UV-B–induced photomorphogenesis. Here, we report that the multifunctional E3 ubiquitin ligase, CONSTITUTIVE PHOTOMORPHOGENESIS1 (COP1), a known key player in UV-B photomorphogenic responses, is also a UV-B–inducible gene. Two transcription factors, FAR-RED ELONGATED HYCOTYL3 (FHY3) and ELONGATED HYCOTYL5 (HY5), directly bind to distinct regulatory elements within the COP1 promoter, which are essential for the induction of the COP1 gene mediated by photomorphogenic UV-B signaling. Absence of FHY3 results in impaired UV-B–induced hypocotyl growth and reduced tolerance against damaging UV-B. Thus, FHY3 positively regulates UV-B–induced photomorphogenesis by directly activating COP1 transcription, while HY5 promotes COP1 expression via a positive feedback loop. Furthermore, FHY3 and HY5 physically interact with each other, and this interaction is diminished by UV-B. Together, our findings reveal that COP1 gene expression in response to photomorphogenic UV-B is controlled by a combinatorial regulation of FHY3 and HY5, and this UV-B–specific working mode of FHY3 and HY5 is distinct from that in far-red light and circadian conditions.

INTRODUCTION

Though a minor component of sunlight, UV-B light (280 to 315 nm) that reaches the earth’s surface exerts strong influences on plant growth and development. Plants have evolved the capacity to sense UV-B and perceive it not only as a damaging stimulus but also as an informational signal. In general, there are two broad categories of plant responses to UV-B, nonspecific and specific pathways, featuring UV-B–induced damage and photomorphogenetic responses, respectively. In response to high-fluence and short-wavelength UV-B light, plants experience stress-related physiological processes, including DNA damage, generation of reactive oxygen species, and inhibition of photosynthesis (Brosché et al., 2002; Frohnmeyer and Staiger, 2003). By contrast, low-fluence and long-wavelength UV-B acts as a positive signal that promotes plant photomorphogenic development, which is characterized by hypocotyl growth inhibition (Kim et al., 1998), flavonoid accumulation (Christie and Jenkins, 1996), and acclimation to UV-B stress (Kliebenstein et al., 2002). Such acclimation renders plants a certain level of tolerance against harmful UV-B (Frohnmeyer and Staiger, 2003; Ulm and Nagy, 2005; Hectors et al., 2007; Jenkins, 2009).

Molecularly, photomorphogenic UV-B is effective in triggering differential gene expression in Arabidopsis thaliana. Based on genetic and transcriptional analyses, several positive regulators involved in UV-B–specific responses have been isolated. They include the bZIP transcription factor ELONGATED HYCOTYL5 (HY5) (Ulm et al., 2004), the UV RESISTANCE LOCUS8 (UVR8) (Brown et al., 2005), and the multifunctional E3 ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENESIS1 (COP1) (Oravecz et al., 2006). Mutants for each of these three genes suffer from decreased activation of UV-B–inducible genes, leading to reduced inhibition of hypocotyl elongation, impaired anthocyanin accumulation, and defective acclimation under UV-B (Brown et al., 2005; Oravecz et al., 2006; Favory et al., 2009).

HY5, an extensively studied bZIP transcription factor, functions downstream of multiple photoreceptors and plays key
roles in promoting photomorphogenesis under diverse light conditions (Oyama et al., 1997; Ang et al., 1998; Osterlund et al., 2000; Ulm et al., 2004). HY5 targets a large number of light-responsive genes in vivo by directly binding to the ACGT-containing elements (ACEs) of their promoters (Oyama et al., 1997; Ang et al., 1998; Lee et al., 2007; Zhang et al., 2011). It also mediates crosstalk between light and hormone signaling, such as abscisic acid, gibberellins, and auxins (Oyama et al., 1997; Cluis et al., 2004; Lau and Deng, 2010), and integrates light and stress responses, such as low temperature (Catalá et al., 2011). In the UV-B-specific signaling pathway, HY5 serves as a hub that is required for the accumulation of transcripts of a subset of UV-B–responsive genes. HY5’s own UV-B–induced expression largely depends on UVR8 and COP1 (Ulm et al., 2004; Oravecz et al., 2006; Favory et al., 2009). However, the specific cis-element that is responsible for mediating low-fluence and long-wavelength UV-B–responsive gene expression remains undefined.

Recently, the molecular mechanism for UVR8-mediated UV-B perception was structurally described (Christie et al., 2012; Wu et al., 2012). Unlike the other photoreceptors, UVR8 possesses an internal chromophore shaped by the Trp residues Trp-233 and Trp-285. Without UV-B, UVR8 forms a symmetric homodimer that is stabilized by intra- and intermolecular interactions, principally through the Arg residues Arg-286 and Arg-338 and surrounding Trp residues. Exposure to UV-B leads to a conformational switch from dimeric to monomeric UVR8 within seconds. The monomeric UVR8 then triggers downstream signaling pathways (Rizzini et al., 2011; Wu et al., 2011; Christie et al., 2012; Wu et al., 2012). Upon UV-B irradiation, UVR8 rapidly accumulates in the nucleus and interacts with COP1 (Kaiserli and Jenkins, 2007). Interestingly, there appears to be residual nuclear UVR8, which constitutively associates with chromatin regions of several UV-B–activated genes, including HY5, regardless of the presence or absence of UV-B (Brown et al., 2005; Cloix and Jenkins, 2008).

In far-red and visible light–induced photomorphogenesis, which will be designated as traditional photomorphogenesis hereafter, COP1 is a central repressor that targets photomorphogenesis-promoting transcription factors, including HY5 for 26S proteasome-mediated degradation. The function of COP1 is modulated primarily via nucleocytoplasmic translocation and interaction with regulatory factors (Yi and Deng, 2005). By contrast, in UV-B–induced photomorphogenesis, COP1 is a positive regulator for HY5, triggering downstream UV-B–specific responses via an unknown mechanism (Favory et al., 2009).

Here, we show that COP1 gene expression is induced by photomorphogenic UV-B. FAR-RED ELONGATED HYPOCOTYL3 (FHY3) and HY5 activate COP1 expression by respectively targeting their distinct motifs in the COP1 promoter region, contributing to UV-B–induced photomorphogenesis and tolerance against damaging UV-B. The genetic and molecular evidence presented in this study demonstrates that in the UV-B–specific responses, FHY3 is a positive regulator upstream of COP1, while HY5 forms a positive feedback loop on COP1. Our findings uncover the elaborate control of photomorphogenic UV-B on COP1 by dual transcriptional regulation to ensure efficient early signaling.

RESULTS

COP1 Expression Is Induced by Photomorphogenic UV-B

In response to low-fluence and long-wavelength UV-B, a set of genes was found to be activated in a temporal manner (Oravecz et al., 2006). To determine how COP1 is regulated by photomorphogenic UV-B, we examined the expression pattern of COP1 in 4-d-old seedlings grown under no UV-B light (−UV-B) and then transferred to UV-B light (+UV-B). The accumulation of COP1 transcripts rises within 1 h and reaches a peak of eightfold induction after 3 h of exposure to +UV-B and then decreases (Figure 1A). However, when using 4-d-old seedlings grown in darkness and then transferred to far-red, red, or blue light conditions, we found the expression of COP1 was only slightly affected. These results suggest that, compared with monochromatic far-red and visible light signals, photomorphogenic UV-B preferably induces COP1 expression. Besides, COP1 protein abundance continues to increase over 12 h of photomorphogenic UV-B irradiation (Figure 1B). These results show that photomorphogenic UV-B induces COP1 expression at both mRNA and protein levels.

FHY3 Binds to the COP1 Promoter via an FHY3 Binding Site Motif in Vitro and in Vivo

The induction of COP1 by photomorphogenic UV-B prompted us to explore transcription factors involved in the regulation of

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**Figure 1.** COP1 Is Induced by UV-B at the mRNA and Protein Levels.  
(A) Changes in COP1 transcript levels in 4-d-old wild-type (Col) seedlings transferred from darkness (Dk) to far-red (FR), red (R), or blue (B) light conditions or from −UV-B to +UV-B and harvested at indicated time points. The transcript level at 0 h was set as 1. Error bars represent standard deviation (SD) of three biological replicates.  
(B) Changes in COP1 protein levels in 4-d-old wild-type (Col) seedlings transferred from −UV-B to +UV-B and harvested at indicated time points. Anti-RPN6 was used as a loading control.
COP1 expression. Examination of the COP1 promoter identified a putative FHY3 binding site (FBS; Lin et al., 2007) – 190 bp upstream of the COP1 start codon (Figure 2A). A 1143-bp fragment of the COP1 promoter, which was reported to be sufficient for COP1 promoter activity (Kang et al., 2009) was cloned and used for yeast one-hybrid assays. We found that the transcriptional activation domain fused FHY3 (AFHY3), but not the AD control, induced LacZ reporter gene expression driven by the COP1 promoter (Figures 2A and 2B). Site-directed mutagenesis of this putative FBS abolished AFHY3 binding to the COP1 promoter. Subsequently, an electrophoretic mobility shift assay (EMSA) was performed to confirm the direct binding of FHY3 to the FBS on the COP1 promoter. The first 200 amino acids of FHY3, C-terminally fused to glutathione S-transferase, (GST-FHY3N) was previously shown to harbor DNA binding activity (Lin et al., 2007; Li et al., 2010). In our assay, addition of GST-FHY3N, but not GST, resulted in slower migration of the FBS probe, indicating that FHY3 is able to bind to the COP1 promoter. When assayed with a probe with the FBS site mutated, GST-FHY3N was not able to bind to the COP1 promoter fragment (Figure 2D).

To investigate whether FHY3 protein associates with the COP1 promoter in vivo, a chromatin immunoprecipitation (ChIP) assay was performed following the regular PCR and quantitative PCR (qPCR) analyses. In 4-d-old –UV-B– and +UV-B–grown seedlings, the “a” fragment, which contains the FBS motif of the COP1 promoter, was found to be enriched by antibodies against native or transgenic FHY3. However, the “b” fragment, which is further upstream of the COP1 promoter, was used as a negative control and was not enriched (Figure 2F; see Supplemental Figure 1A online). The reduced enrichment in +UV-B–grown seedlings can probably be ascribed to the lower protein level of FHY3 under +UV-B (Figures 2G and 2H; see Supplemental Figures 1B and 1C online). Taken together, these results indicate that FHY3 protein directly binds to the COP1 promoter through the FBS motif under both –UV-B and +UV-B.

**HY5 Binds to the COP1 Promoter via an ACE Element in Vitro and in Vivo**

In addition to the FBS motif, three putative ACEs, typical HY5 binding sites, are present in the COP1 promoter region. Yeast one-hybrid assays were again used to identify the ACE(s) that is important for HY5 binding. AD-HY5 was able to bind the wild-type COP1 promoter, whereas mutation in ACE3 resulted in little affinity of AD-HY5 to the COP1 promoter, demonstrating that ACE3 is the dominant, if not the only, site for HY5 binding (Figure 2C). The ACE3 is located – 80 bp upstream of the COP1 start codon and 100 bp downstream of the FBS motif (Figure 2A). HY5-HOMOLOG (HYH) overlaps in function with HY5 in the stimulation of some gene expression by UV-B (Brown and Jenkins, 2008), but no obvious signal for HYH binding to the COP1 promoter was detected (see Supplemental Figure 2 online). In an EMSA test, the wild-type but not the mutated ACE3 probe was bound by the recombinant GST-HY5, as evidenced by the presence of bands with retarded mobility (Figure 2E).

Furthermore, ChIP-PCR analysis validated the in vivo association of HY5 protein with the COP1 promoter in –UV-B– and +UV-B–grown wild-type seedlings. Anti-HY5 antibodies were found to enrich the ACE3-containing “a” fragment, but not the control “b” fragment (Figure 2F). ChIP-qPCR assays confirmed that the COP1 promoter can be immunoprecipitated by antibodies against HY5 under both –UV-B and +UV-B (Figures 2G and 2H). These data illustrate that through the ACE3 site, HY5 is also able to bind to the COP1 promoter regardless of photomorphogenic UV-B treatment.

**FY3 and HY5 Are Capable of Mediating COP1 Activation in Response to UV-B**

Since FHY3 and HY5 exhibit direct binding to the COP1 promoter via FBS and ACE3, respectively, these two transcription factors might contribute to the regulation of COP1 expression. To test this hypothesis, we first took advantage of the transgenic line FYH3p:FHY3-GR/hy5-3–4 expressing an FHY3-glucocorticoid receptor (GR) fusion protein driven by the FHY3 native promoter (Lin et al., 2007). The fusion protein will translocate from the cytoplasm into the nucleus after treatment with dexamethasone (DEX) (Lloyd et al., 1994). The seedlings were grown under –UV-B for 4 d and then treated with DEX before transferred to –UV-B and +UV-B, respectively. The expression of COP1 is induced within 2 h under +UV-B but not –UV-B (Figure 3A). Nevertheless, FYH3, an identified target of FHY3 in far-red light signaling (Lin et al., 2007), is induced under –UV-B instead of +UV-B (see Supplemental Figure 3A online), consistent with no change in the FHY1 mRNA level in wild-type seedlings grown under –UV-B and then transferred to +UV-B (see Supplemental Figure 3B online).

Then, a dual luciferase assay was conducted in Nicotiana benthamiana to check the effects of FYH3 and HY5 on COP1 transcription. Recombinant reporter constructs were generated to allow the wild-type or mutated COP1 promoter to drive expression of the firefly luciferase (LUC) reporter gene (Figure 3B). Under photomorphogenic UV-B, the overexpression of FYH3 leads to a 3.7-fold increase in the activity of the LUC reporter under the COP1 promoter (Figure 3C). By contrast, it had no effect when either UV-B irradiation was absent (Figure 3C) or the FBS motif in the COP1 promoter was disrupted (Figure 3D). ACE3 mutation still led to the induction of LUC expression by FYH3 (Figure 3D). In a parallel analysis, HY5 also activated the COP1 promoter-driven LUC expression in a UV-B–dependent manner, though with reduced effectiveness compared with FYH3 (Figure 3C). With the mutated ACE3 construct, HY5 was no longer able to promote LUC expression (Figure 3D). The FBS mutation exerted no influence on the induction of LUC expression by HY5 (Figure 3D). Altogether, these results demonstrate that upon UV-B irradiation, FHY3 and HY5 can promote transcription of COP1 in plant cells.

Furthermore, to get more insight how FHY3 and HY5 activate COP1, the dual luciferase assay was perform in Arabidopsis. Under photomorphogenic UV-B, in wild-type plants, the transiently overexpressed FHY3 and HY5 led to 2.6- and 2.0-fold increases, respectively, in the activity of the LUC reporter under the COP1 promoter. However, little reporter activity was detected when uvr8-6 plants were infiltrated (Figure 3E), indicating that the loss of UVR8 function impinges on FHY3 and HY5 in COP1 activation. Together with the fact that UVR8 is a UV-B receptor (Rizzini et al., 2011; Wu et al., 2011; Christie et al., 2012; Wu et al., 2012), we
Figure 2. FHY3 and HY5 Bind to the COP1 Promoter in Vitro and in Vivo.

(A) Diagram of fragments of the COP1 promoter. The adenine of the translational start codon (ATG) is designated as position +1. Orange and blue blocks indicate putative FBS and ACEs, respectively. Arrows indicate the fragments amplified in ChIP-PCR assays. The wild-type and mutated sites of the COP1 promoter subfragments are shown in uppercase and lowercase letters, respectively.

(B) COP1p::LacZ

WT    mFBS
AD    AD-FHY3

COP1p::LacZ

WT    mACE1    mACE2    mACE3    mACE1+2    mACE1+3    mACE2+3    mACE1+2+3
AD    AD-HY5

(D) COP1p-FBS

COP1p-mFBS

Protein

GST    GST-FHY3N

+E

GST-FHY3N

FP

(F) - UV-B

+ UV-B

Input no Ab α-FHY3 α-HY5

a

b

(G) fhy3-1 Col

UV-B

hy5-215 Col

UV-B

FHY3

HY5

RPN6

UV-B

+UV-B

% of input

% of input

H

COP1pro-FBS

COP1pro-ACE3

No Ab α-FHY3 α-HY5

No Ab α-FHY3 α-HY5

No Ab α-HY5

No Ab α-HY5

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(A) Diagram of fragments of the COP1 promoter. The adenine of the translational start codon (ATG) is designated as position +1. Orange and blue blocks indicate putative FBS and ACEs, respectively. Arrows indicate the fragments amplified in ChIP-PCR assays. The wild-type and mutated sites of the COP1 promoter subfragments are shown in uppercase and lowercase letters, respectively.
conclude that the activation of COP1 by FHY3 and HY5 is dependent on UVR8-initiated UV-B signaling.

**FHY3 Is Critical for UV-B–Induced Photomorphogenesis at the Seeding Stage**

As shown above, FHY3 is able to bind to the COP1 promoter and activate its transcription under UV-B. COP1 is a positive regulator of UV-B–induced photomorphogenesis. Therefore, it is possible that FHY3 may be critical in this pathway.

As inhibition of hypocotyl elongation is a characteristic response to photomorphogenic UV-B (Favory et al., 2009; Gardner et al., 2009), the relative hypocotyl length, a percentage defined as the hypocotyl length under +UV-B relative to the length under −UV-B, was measured using 4-d-old seedlings grown in typical photomorphogenic UV-B experimental conditions (see Supplemental Figure 4 online). Consistent with earlier reports (Oravecz et al., 2006; Favory et al., 2009; Gardner et al., 2009), a UV-B photoreceptor mutant urv8-6 and a key promoter mutant cop1-4 showed much longer relative hypocotyl length than the wild type, but such a defect was not found in the other photoreceptor mutants phyAB, phyABDE, and cry1 cry2 or the DNA repair mutant urv1-1. These observations confirmed that the condition used is specific to UV-B–induced photomorphogenesis instead of UV-B stress.

Interestingly, we found that fhy3-4 mutants displayed a dramatically reduced ability to inhibit hypocotyl elongation with UV-B irradiation compared with wild-type Nossen-0 (No-0) (Figure 4A). The relative hypocotyl length in No-0 seedlings was around 35% but was 53% in fhy3-4 mutants. This defect was also observed in four additional fhy3 mutant alleles, fhy3-1, fhy3-3, fhy3-5, and fhy3-10 (see Supplemental Figures 5A and 5B online). Photomorphogenic UV-B accelerates biosynthesis of sunscreen flavonoids (Oravecz et al., 2006). Quantitatively, we found that there was less anthocyanin content in fhy3-4 than in No-0 (Figures 4B).

Next, we examined the expression of several marker genes responsive to photomorphogenic UV-B in 4-d-old seedlings grown under −UV-B and +UV-B. We found that the photomorphogenic UV-B–mediated transcript accumulation of EARLY LIGHT-INDUCIBLE PROTEIN2 (ELIP2) (Figure 4C), At4g15480 (Figure 4D), and DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN2A (DREB2A) (Figure 4E) was diminished in fhy3-4, but HY5 (Figure 4F) accumulated slightly more transcripts. Next, we continued to analyze the temporal expression of these genes using 4-d-old seedlings grown under −UV-B and then transferred to +UV-B for various time periods. The temporal induction of all these genes by photomorphogenic UV-B was much weaker in fhy3-4 than in No-0 (Figures 4G to 4J). These results demonstrate that FHY3 positively contributes to the transcriptional response to photomorphogenic UV-B, particularly at an early stage.

Furthermore, introduction of a 35S promoter–driven 3FLAG-FHY3-3HA construct in fhy3-4 (see Supplemental Figure 5C online) rescues the defects in its hypocotyl growth (Figure 4A) and anthocyanin accumulation (Figure 4B), supporting that FHY3 is involved in UV-B–induced photomorphogenesis at the seeding stage.

**FAR-RED IMPAIRED RESPONSE1 (FAR1)** is homologous to FHY3 and shares functional redundancy with FHY3 in phytochrome A (phyA) signaling, circadian clock, chloroplast development, and chlorophyll biosynthesis (Wang and Deng, 2002; Hiltingbrunner et al., 2005; Allen et al., 2006; Lin et al., 2007; Genoud et al., 2008; Li et al., 2010; Li et al., 2011; Ouyang et al., 2011; Tang et al., 2012). Thus, we are interested if FAR1 plays a role in UV-B–induced photomorphogenesis as well. Surprisingly, far1 mutants showed no apparent difference from wild-type seedlings in UV-B–promoted hypocotyl growth (see Supplemental Figure 6 online). In addition, the fhy3 far1 double mutants displayed the same extent of hypocotyl growth in the presence of UV-B as that of fhy3 single mutant (see Supplemental Figure 6 online). These results suggest that FAR1 is not essential in photomorphogenic UV-B response.

**A T-DNA Insertion hy5 Null Mutant Exhibits Impaired UV-B–Induced Photomorphogenesis**

Previous studies have reported reduced UV-B–mediated gene activation in hy5-1 and hy5-215 mutants and impaired UV-B tolerance in hy5-1, suggesting that HY5 is a positive regulator in mediating gene expression and protective survival under UV-B (Ulir et al., 2004; Oravecz et al., 2006; Gruber et al., 2010; Stracke et al., 2010). Here, we found that hy5-ks50, a T-DNA insertion hy5 null mutant, is also impaired in UV-B–induced photomorphogenesis. The relative hypocotyl length of the mutant seedlings was around 74% (see Supplemental Figure 7A online), and there was much less anthocyanin content in hy5-ks50 than that in Wassi-lewskija (Ws) (see Supplemental Figure 7B online). These defects were restored through the overexpression of 35S:HY5 (see Supplemental Figure 7 online). This indicates that the hy5-ks50
null mutant, similar to the other *hy5* alleles reported, is defective in UV-B–induced photomorphogenesis and thus was used for further investigation in our analysis.

**Both fhy3 and hy5 Mutants Are Hypersensitive to Damaging UV-B**

Earlier studies reported that UV-B–induced photomorphogenesis is a fundamental event for plants to acquire tolerance against damaging UV-B (Favory et al., 2009). Based on the observations of abnormal UV-B–induced photomorphogenesis in our *fhy3* and *hy5* mutants, we attempted to investigate if they suffer from UV-B–induced damage. Wild-type seedlings were apparently resistant to the UV-B stress. By contrast, the leaves of *fhy3*-4 mutants shrivelled, and *hy5*-ks50 mutants displayed even more severe defects with shrunken and pale leaves (Figure 5). The hypersensitivity to UV-B stress in *fhy3*-4 and *hy5*-ks50 mutants might be ascribed to their failure to properly respond to photomorphogenic UV-B, further demonstrating the involvement of FHY3 and HY5 in UV-B–induced photomorphogenesis and acclimation.

**Absence of FHY3 or HY5 Results in Abnormal Accumulation of COP1 Transcript and Protein under Photomorphogenic UV-B**

To further explore the roles of FHY3 and HY5 in UV-B–specific signaling, especially in regulating *COP1* expression in vivo, temporal expression of UV-B responsive genes was analyzed using 4-d-old seedlings grown under -UV-B and then transferred to +UV-B for various time periods.
Figure 4. FHY3 Is Involved in UV-B–Induced Photomorphogenesis at the Seedling Stage.

(A) Phenotypes of 4-d-old wild-type (No-0), fhy3-4, and 3FLAG-FHY3-3HA/fhy3-4 seedlings grown under −UV-B and +UV-B. Quantitative analysis of hypocotyl length is presented as percentage of −UV-B. Error bars represent sd of three biological replicates. Bar = 1 mm.

(B) Quantitative analysis of anthocyanin accumulation of the seedlings in (A). Error bars represent sd of three biological replicates.

(C) to (F) The transcript levels of UV-B–induced marker genes ELIP2 (C), At4g15480 (D), DREB2A (E), and HY5 (F) in 4-d-old wild-type (No-0) and fhy3-4 seedlings grown under −UV-B to +UV-B. The transcript level in No-0 under −UV-B was set as 1. Error bars represent sd of three biological replicates.

(G) to (J) The transcript levels of UV-B–induced marker genes ELIP2 (G), At4g15480 (H), DREB2A (I), and HY5 (J) in 4-d-old wild-type (No-0) and fhy3-4 seedlings transferred from −UV-B to +UV-B and harvested at indicated time points. The transcript level at 0 h in No-0 was set as 1. Error bars represent sd of three biological replicates.

[See online article for color version of this figure.]
CHALCONE SYNTHASE (CHS), which encodes a key enzyme at the first committed step in anthocyanin biosynthesis, is another UV-B-induced marker gene (Oravecz et al., 2006). In No-0, CHS was rapidly activated with a peak of 64-fold induction after 3 h of UV-B treatment and starts to fall afterwards. In fhy3-4, the CHS transcript rose with a diminished and retarded peak of 39-fold increase after 6 h of UV-B treatment (Figure 6A). COP1 transcript peaked with 6.3- and 3.9-fold increases in No-0 and fhy3-4, respectively, after 6 h of UV-B treatment (Figure 6B). At the protein level, compared with No-0, less COP1 protein accumulated in fhy3-4 over 12 h of photomorphogenic UV-B irradiation (Figure 6C). In parallel assays, the UV-B-induced transcription of CHS was found to be almost completely lost in hy5-ks50 (Figure 6D). COP1 transcript peaked with 6.7- and 3.6-fold increase in Ws and hy5-ks50, respectively (Figure 6E). The increase in COP1 protein mediated by photomorphogenic UV-B was largely abolished in hy5-ks50 (Figure 6F). These results indicate that both FHY3 and HY5 are required for the full activation of COP1 and COP1 protein accumulation mediated by photomorphogenic UV-B.

COP1 and HY5 Genetically Interact with FHY3 in Mediating UV-B Photomorphogenic Responses

In order to further define the genetic role of FHY3 in the photomorphogenic UV-B signaling pathway, we examined the possible genetic interaction between COP1, FHY3, and HY5. The seedlings of a previously described COP1 overexpression line, COP1OE (see Supplemental Figure 8 online), displayed a stronger inhibition of hypocotyl elongation upon UV-B treatment (Figure 7A). When introduced into fhy3-4, the COP1 overexpression completely suppressed the reduced inhibition of hypocotyl elongation found in fhy3-4, to a level that is similar to COP1OE (Figure 7A). Quantitative analysis of the CHS transcripts confirmed that COP1OE/fhy3-4 phenocopies COP1OE (Figure 7B). Therefore, we conclude that COP1 acts downstream of FHY3.

GUS-COP1/cop1-5 was previously found to rescue the constitutive photomorphogenesis of cop1-5 in darkness by overexpressing COP1 (von Anim et al., 1997; see Supplemental Figure 8 online). Under photomorphogenic UV-B, GUS-COP1/cop1-5 exhibited comparable hypocotyl growth to No-0. When crossed with COP1/cop1-5, hy5-ks50 showed no recovery of hypocotyl shortening (Figure 7C). Besides, in response to photomorphogenic UV-B, GUS-COP1/cop1-5 displayed stronger induction of CHS than No-0, but GUS-COP1/hy5-ks50 failed to induce CHS like hy5-ks50 (Figure 7D). In the absence of HY5, COP1 overexpression was not sufficient to regain the regular response to photomorphogenic UV-B, suggesting epistasis of hy5-ks50 mutation over COP1 overexpression. According to the phenotypes of COP1OE/fhy3-4 and GUS-COP1/hy5-ks50, we deduce that the main function of HY5 is downstream of FHY3 and COP1.

Next, we crossed fhy3-1 with hy5-215, which was reported to have a reduced inhibition of hypocotyl elongation under +UV-B (Jiang et al., 2012). We observed that fhy3-1 hy5-215 double mutant seedlings closely resembled the hy5-215 single mutants in hypocotyl growth upon UV-B treatment (Figure 7E). Consistently, fhy3-1 hy5-215 mimicked hy5-215 in its photomorphogenic UV-B-mediated CHS induction (Figure 7F). These results also agree with the notion that HY5 acts as a main downstream transcription factor of COP1 in the central pathway in UV-B-mediated photomorphogenesis. It is likely that participating in COP1 gene activation is one of the regulatory roles of HY5.

Photomorphogenic UV-B Affects the Functional Interaction between FHY3 and HY5

It was previously noted that the antagonistic roles of FHY3 and HY5 in phyA signaling and their cooperative functions in the circadian clock both rely on their physical interaction (Li et al., 2010; Li et al., 2011). It is therefore of interest to examine whether FHY3 and HY5 functionally interact in UV-B responses. We first conducted firefly luciferase complementation imaging (LCI) assays (Chen et al., 2008) by transiently overexpressing CLuc and NLuc fusion proteins in N. benthamiana leaf cells. Obvious LUC activity was observed when CLuc-FHY3N and HY5-NLuc were coexpressed under –UV-B, whereas much less was observed under +UV-B (Figure 8A). This result suggests that UV-B attenuates the association between FHY3 and HY5 in plant cells.

In addition, the presence of both the FBS motif and the ACE element in the COP1 promoter also hints at a possible functional interaction between FHY3 and HY5. Thus, we examined whether the loss of HY5 or FHY3 affected the other with regard to their protein abundance and their affinity to the COP1 promoter. In hy5-215, the protein level of FHY3 is comparable to that of the wild type (Figure 8B). ChIP of FHY3 also precipitated the FBS motif-containing promoter fragment of COP1 at a similar efficiency with the wild type (Figure 8C). However, in hy5-3-1, more HY5 proteins accumulate (Figure 8B), and antibodies against HY5 enriched twofold more DNA fragments containing the ACE3 in the hy3-1 mutant than that in the wild type (Figure 8D). These observations reveal that FHY3 or HY5 are each able to associate with the COP1 promoter in the absence of the other.

DISCUSSION

The E3 ubiquitin ligase COP1 is a well-known repressor in traditional photomorphogenesis, primarily via mediating the degradation...
of photomorphogenesis-promoting transcription factors (Yi and Deng, 2005). By contrast, COP1 plays a positive role in UV-B–induced photomorphogenesis, but how photomorphogenic UV-B regulates COP1 is unknown. Our analyses reveal that COP1 is a UV-B–inducible gene, whose full activation requires transcription factors FHY3 and HY5. Furthermore, FHY3 and HY5 are engaged in UV-B–induced photomorphogenesis and acclimation and show a genetic interaction with COP1 (Figure 9).

Thus, this work uncovers a functional mode of FHY3 and HY5 beyond phyA signaling and circadian regulation.

Photomorphogenic UV-B Regulates COP1 Expression via FHY3 and HY5 Binding LREs

During the last decades, the reprogramming of the plant transcriptome by light has been extensively examined. Transcriptional
reprogramming requires light-responsive cis-elements (LREs), which are commonly present in the promoter regions of light-regulated genes. Combinations of LREs confer distinct transcriptional responses to various light signals and further depict a sophisticated light regulated transcriptional network (Puente et al., 1996; Jiao et al., 2007). Such transcriptional regulation is also coupled with UV-B specific signaling. Photomorphogenic UV-B promotes differential gene expression (Ulm et al., 2004; Brown et al., 2005; Oravecz et al., 2006; Favory et al., 2009), histone modification, and chromatin remodeling (Casati et al., 2008). In Arabidopsis, although the UVBox, a promoter motif, is required for the induction of ARABIDOPSIS THALIANA NAC DOMAIN PROTEIN13 by shorter-wavelength and high-intensity UV-B, this element is not regulated by longer-wavelength UV-B (Safrany et al., 2008). In our study, COP1 was induced upon treatment with photomorphogenic UV-B (Figure 1). The FBS motif and the ACE element in the COP1 promoter serve as LREs to positively mediate COP1 expression in this transcriptional response to photomorphogenic UV-B (Figures 2 and 3).

As a multifunctional E3 ligase, COP1 is subjected to changes in activity in response to diverse stimuli. For example, the cryptochromes rapidly inactivate COP1 through their direct association (Wang et al., 2001; Yang et al., 2001), four functional redundant SUPPRESSOR OF PHYA (SPA) proteins act as regulatory sub-units of COP1-SPA heterogeneous complexes (Saijo et al., 2003; Zhu et al., 2008), and at a relatively slower rate, light inhibits its E3 ligase activity partially by excluding it from the nucleus (von Arnim and Deng, 1994). Here, we establish how COP1 is controlled at the transcriptional level in Arabidopsis. Specifically, the accurate composition of the LREs for FHY3 and HY5 in the COP1 promoter (Figure 2) defines its ability to positively respond to the stimulus of photomorphogenic UV-B (Figure 3).
FHY3 Is Associated with UV-B–Induced Photomorphogenesis

UV-B–induced photomorphogenesis was first observed in Arabidopsis in the late 1990s and expanded light control of plant development beyond the traditional photomorphogenesis by far-red and visible light wavelengths (Kim et al., 1998). To date, this response has been shown in several plant species, such as maize (Zea mays) (Casati and Walbot, 2003; Casati et al., 2008), cucumber (Cucumis sativus) (Shinkle et al., 2010), and moss (Physcomitrella patens) (Wolf et al., 2011). In Arabidopsis, besides HY5, UVR8, and COP1, there are a growing number of factors found to be involved in the responses to photomorphogenic UV-B, including two potential Damaged DNA Binding Protein1 (DDB1) binding WD40 proteins, REPRESSOR OF UV-B PHOTOMORPHOGENESIS1 (RUP1) and RUP2. They are UVR8-interacting proteins and act downstream of UVR8 and COP1 in a negative feedback loop (Gruber et al., 2010).

Recently, the identification of genome-wide binding sites of FHY3 profoundly extended our understanding of the diversity of FHY3 function, especially in environmental adaptation (Ouyang et al., 2011; Stirnberg et al., 2012; Tang et al., 2012). In response to photomorphogenic UV-B, FHY3 functions primarily via activating COP1 expression. Also, it was found that photomorphogenic UV-B entrains the plant clock. Without UV-B pulses, amplified circadian rhythms are eliminated in clock genes like CIRCADIAN CLOCK ASSOCIATED1, GIGANTEA, and EARLY FLOWERING4 (ELF4) (Fehér et al., 2011). Among these genes, ELF4 loses its rhythmic expression in fhy3 mutants in visible light and it needs FHY3 for transcriptional activation (Li et al., 2011). Thus, FHY3 might be a link between UV-B–induced photomorphogenesis and the circadian clock. Interestingly, FAR1 does not seem to function in early photomorphogenic UV-B responses (see Supplemental Figure 6 online). Based on previous promoter-swapping experiments, FHY3 may undertake a divergent role from FAR1 through protein subfunctionalization (Lin et al., 2008).

The Expression and Activity of FHY3 Is Regulated by Photomorphogenic UV-B

Time-course microarray-based expression profiling of light-responsive genes has documented the light regulation of transcription factors (Tepperman et al., 2001, 2004; Jiao et al., 2003). For example, HY5 protein accumulates under various light conditions (Oyama et al., 1997; Hardtke et al., 2000; Osterlund et al., 2000). FHY3 is repressed by far-red light (Lin et al., 2007) but...
is activated by photomorphogenic UV-B (see Supplemental Figures 3B and 9A online). This differential regulation shows that FHY3 can be specifically controlled by distinct wavelengths of light. Meanwhile, FHY1, a target of FHY3 under far-red light, shows no transcriptional response to UV-B (see Supplemental Figure 3 online). Such distinct transcriptional behaviors of FHY3 and FHY1 indicate a fine-tuning of light signals that differentiates the far-red light pathway from the UV-B pathway. Additionally, though FHY3 mRNA level is rapidly upregulated by photomorphogenic UV-B, its protein level is eventually downregulated (Figure 2G; see Supplemental Figures 1B, 9B, and 9C online). This observation indicates that FHY3 might be regulated by photomorphogenic UV-B in a posttranscriptional manner, which awaits further investigation.

FHY3 contains an N-terminal C2H2 zinc finger domain, a central putative consorpsase domain, and a C-terminal SWIM zinc finger domain (named after SW2/SNF and MuDR transposases) (Makarova et al., 2002). The C2H2 zinc finger domain mediates its DNA binding activity, and the transposase catalytic domain and SWIM motif are important for its transcriptional activation activity (Lin et al., 2008). FHY3 shows high affinity to the COP1 promoter (Figure 2), whose FBS-containing region is on the list of global FBSs (Ouyang et al., 2011). A series of ChIP assays collectively suggests that FHY3 binds to the COP1 promoter in far-red, dark, −UV-B, and +UV-B conditions (Ouyang et al., 2011; Figure 2; see Supplemental Figure 1 online). However, our earlier microarray data did not detect altered COP1 mRNA levels in response to FHY3 translocation into the nucleus in far-red light or dark conditions (Ouyang et al., 2011). The transcriptional activation activity of FHY3 toward the COP1 promoter is strictly induced by the UV-B signaling (Figures 3C and 3E).

**COP1 and HY5 Form a Positive Feedback Loop in UV-B-Induced Photomorphogenesis**

As one of the core transcription factors in light control of plant development, HY5 is induced by light and its protein accumulation is promoted by multiple photoreceptors under distinct wavelengths of light (Oyama et al., 1997; Hardtke et al., 2000; Osterlund et al., 2000). The SPA-COP1 complexes are substrate receptors for CULLIN4-DDB1 E3 ligases, which ubiquitinate HY5 for selective degradation by the 26S proteasome (Osterlund et al., 2000; Saijo et al., 2003; Chen et al., 2006, 2010; Zhu et al., 2008). In UV-B–induced photomorphogenesis, HY5 acts downstream of UVR8 and COP1 (Ulm et al., 2004; Oravecz et al., 2006; Favory et al., 2009). Meanwhile, HY5 directly associates with the COP1 promoter via the ACE element independent of UV-B irradiation (Figure 2) and induces COP1 expression specifically dependent on UV-B signaling (Figures 3C and 3E). Thus, COP1 and HY5 form a positive feedback loop in UV-B photomorphogenic responses.

Upon UV-B treatment, three-fourths of the early UV-B–activated genes lose induction in cop1-4, but more than a half remain unchanged in hy5-1 (Oravecz et al., 2006), suggesting there is a group of COP1-dependent genes that are regulated independent of HY5. Besides, there may no longer be a COP1-mediated degradation of HY5 under UV-B (Favory et al., 2009). In this case, the mutual transcriptional regulation between COP1 and HY5 might stand as one of their interaction modes. Still, the positive feedback is important, together with FHY3-induced COP1 expression, to produce adequate early signal transducers to maintain UVR8-COP1–mediated signaling. Downstream of UVR8-COP1, RUP1 and RUP2 rapidly accumulate their transcripts and proteins and result in a negative feedback loop to repress UV-B–induced gene expression. Meanwhile, RUP1 and RUP2 are likely to sequester UVR8 from COP1. In these two ways, RUP1 and RUP2 prevent an exaggerated UV-B photomorphogenesis response and further balance UV-B acclimation and plant development (Gruber et al., 2010; Figure 9). A recent study on another negative regulator, SALT TOLERANCE, that might antagonize HY5 transcriptional activity (Jiang et al., 2012) also supports coordination of positive and negative feedback processes as crucial for appropriate UV-B photomorphogenic responses in planta.

**The Combinatorial Functional Interaction of the FHY3-HY5 Pair Is Distinct in UV-B–Specific Response from That in Far-Red Light Signaling and Circadian Regulation**

Two contrasting working models between FHY3 and HY5 were hypothesized in recent years. In far-red light responses, FHY3 and FAR1 directly activate FHY1 and FHL, which produce chaperone proteins to facilitate the phyA-FHY1/FHL nuclear import...
complex for downstream signaling, while they are down-regulated by phyA in a negative feedback loop (Lin and Wang, 2004; Hiltpool et al., 2005; Genoud et al., 2008). HY5 acts antagonistically to FHY3 and suppresses FHY1/FHL expression by interfering with FHY3/FAR1 binding to the FHY1/FHL promoters (Li et al., 2010). However, FHY3 and HY5 work in concert in circadian expression of ELF4. FHY3 is tied to circadian regulation in the daytime phase, relied on its role in gating red light signaling to the circadian clock and in maintaining the rhythmic expression of ELF4. FHY3/FAR1 and HY5 directly activate ELF4 expression during the day (Allen et al., 2006; Li et al., 2011).

In UV-B photomorphogenic responses, FHY3 and HY5 share some similarity in their temporal mRNA accumulation upon UV-B treatment (see Supplemental Figure 9 online), their mutant phenotypes and their effects on COP1 and CHS expression. Our data clearly defined a set of two cis-elements, the FBS motif and the ACE element, that act in the FHY3- and HY5-mediated transcriptional modulation of COP1 in response to UV-B. In the COP1 promoter, there is probably little steric hindrance for FHY3 and HY5 binding since the cis-elements bound by these two proteins are ~100 bp away (Figure 2A). This distance is even greater than that in the ELF4 promoter (30 bp). Additionally, photomorphogenic UV-B diminishes the physical contact of FHY3 and HY5 (Figure 8A), possibly due to the reduced FHY3 protein abundance (Figure 2G; see Supplemental Figures 1B, 9B, and 9C online). Either FHY3 or HY5 alone can maintain a low level of UV-B-mediated COP1 upregulation (Figures 6B and 6E), to show affinity to the COP1 promoter (Figures 8C and 8D), and to accomplish limited photomorphogenic responses to UV-B when the other one is absent (Figure 4; see Supplemental Figure 7 online). These data suggest that FHY3 and HY5 work in a non-competitive manner. FHY3 acts as a positive regulator of UV-B-induced photomorphogenesis through directly activating COP1 transcription, while HY5 forms a positive feedback on COP1. Such dual transcriptional regulation is required for the full activation of UV-B-induced COP1 gene expression. To some extent, HY5 might compensate for phy3 mutation by elevating its own mRNA and protein levels (Figures 4F and 6B). Compared with far-red light and circadian conditions, photomorphogenic UV-B endows the same set of signaling intermediates, FHY3 and HY5, a distinct working mode.

Together, our data support a refined UV-B signaling model (Figure 9). In UV-B photomorphogenic responses, a core UV-B signaling pathway is established by UVR8-COP1-HY5. Upon UV-B treatment, UVR8 undergoes a rapid conformational switch from homodimer to monomer, which enables its interaction with COP1. The UVR8-COP1 complex triggers downstream signaling events, including gene expression regulated by HY5, which is repressed by a negative feedback formed by RUPS. In UV-B regulation of gene expression, COP1 is one of the UV-B–inducible genes. FHY3 and HY5, whose physical interaction is altered by UV-B, directly bind to the FBS motif and the ACE element in the COP1 promoter respectively and activate the transcription of COP1. This combinatorial transcriptional modulation helps produce abundant COP1 and thus ensures a more active UVR8-COP1-HY5 UV-B core signaling pathway.

METHODS

Plants and Growth Conditions

The wild-type Arabidopsis thaliana used in this study was of the Columbia (Col), Ws, No-0, and Landsberg erecta ecotypes. Some of the mutants and transgenic lines used in this study were described previously: phy3-1 and far-1 (Whitehead et al., 1993; Kang et al., 2009), phy3-3, phy3-4, phy3-5, and phy3-10 (Wang and Deng, 2002), phy3-4 far-1-2 and FHY3p:FHY3-GR/ phy3-4 (Lin et al., 2007), 3FLAG-FHY3-3HA/phy3-4 (Li et al., 2011), hy5-215, and hy5-ks50 (Oyama et al., 1997), 3SS-HYS/hy5-ks50 (Hardtke et al., 2000), COP1OE, and cop1-4 (McNellis et al., 1994), GUS-COP1/cop1-5 (von Arnim et al., 1997), phyAB (Reed et al., 1994), phyABDE (Franklin et al., 2003), cry1 cry2 (Mao et al., 2005), uvr8-6 (Favory et al., 2009), and uvrh-1-1 (Liu et al., 2001).

The Arabidopsis seeds were surface-sterilized and sown on solid 1% Murashige and Skoog medium supplemented with 1% Suc for biochemical assays or with 0.3% Suc for phenotypic analysis and cold treated at 4°C for 4 d. Then, for photomorphogenic UV-B treatment, seedlings were grown under continuous white light (3 μmol m⁻² s⁻¹, measured by LI-250 Light Meter; LI-COR Biosciences) supplemented with Philips TL20W/01RS narrowband UV-B tubes (1.5 μmol m⁻² s⁻¹, measured by a TN-340 UV-B light meter) under a 350-nm cutoff (half-maximal transmission at 350 nm) filter ZUL0330 (UV-B; Asahi Spectra) or a 300-nm cutoff (half-maximal transmission at 300 nm) filter ZUL0300 (+UV-B; Asahi Spectra). For damaging UV-B treatment, seedlings were grown under continuous white light (30 μmol m⁻² s⁻¹, supplemented with narrowband UV-B (1.5 μmol m⁻² s⁻¹) for 12 d and then were irradiated by Philips TL40W/12RS broadband UV-B tubes (20 μmol m⁻² s⁻¹) with ZUL0350 (~UV-B) or ZUL0300 (+UV-B) filters for 12 h. The UV-B light conditions are essentially identical to those reported recently (Oravecz et al., 2006; Favory et al., 2009). For monochromatic light treatment, cold-treated seeds were grown in darkness for 4 d and then transferred to far-red light (0.5 μmol m⁻² s⁻¹), red light (10 μmol m⁻² s⁻¹), or blue light (4 μmol m⁻² s⁻¹).

Site-Directed Mutagenesis and Plasmid Construction

For yeast one-hybrid assays, to generate the COP1U:LacZ reporter construct, a reported COP1 promoter region (Kang et al., 2009) was amplified and cloned into the EcoRI-Xhol site of pLaclZiiμ (Lin et al., 2007). LacZ reporter genes driven by mutant COP1 promoters were generated using the COP1U:LacZ reporter plasmid as the template and the QuikChange site-directed mutagenesis kit (Stratagene). The AD-FHY3, AD-HYS, and AD-HYH constructs were described previously (Wang and Deng, 2002; Li et al., 2010). For EMSA assays, the GST-HYS and GST-FHY3N constructs were described previously (Ang et al., 1998; Lin et al., 2007), for LCI assays, NLuc, CLuc, HY5- NLuc, and CLuc-FHY3N were described previously (Murai et al., 2001). For anti-HY5 immunoblot analyses, Arabidopsis seeds were ground to a fine powder and total proteins were eluted in 2× SDS loading buffer. For all other immunoblots, Arabidopsis seeds were homogenized in a protein powder and total proteins were eluted in 2× SDS loading buffer.
Quantitative Real-Time PCR

Total RNA was extracted from Arabidopsis seedlings using the RNeasy plant mini kit (Qiagen). Reverse transcription was performed using the SuperScript II first-strand cDNA synthesis system (Invitrogen) according to the manufacturer’s instructions. Real-time qPCR analysis was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) with a Bio-Rad CFX96 real-time PCR detection system. Each experiment was repeated with three independent samples, and RT-PCR reactions were performed in three technical replicates for each sample. The primers used for quantitative RT-PCR are listed in Supplemental Table 1 online.

Yeast One-Hybrid Assays

Plasmids of AD fusions were cotransformed with the LacZ reporter genes driven by wild-type and mutant COP1 promoters into the yeast strain EGY48. Transformants were grown on proper dropout plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside for blue color development. Yeast transformation was conducted as described in the Yeast Protocols Handbook (Clontech).

ChIP

Arabidopsis seedlings grown under −UV-B and +UV-B for 4 d were used for ChIP assays following the procedure described previously (Lee et al., 2007). Briefly, 5 g of seedlings were first cross-linked with 1% formaldehyde under vacuum, and then the samples were ground to powder in liquid nitrogen. The chromatin complexes were isolated and sonicated and then incubated with anti-H3 (Millipore), anti-flag (Sigma-Aldrich), anti-FHY3, and anti-HY5 antibodies. The precipitated DNA was recovered and analyzed by PCR methods using the primers in Supplemental Table 1 online. Real-time PCR was performed as described above.

EMSA

EMSAs were performed using the biotin-labeled probes and the Lightshift Chemiluminescent EMSA kit (Pierce) according to the manufacturer’s instructions. Briefly, 0.5 μg of GST or GST fusion proteins were incubated together with biotin-labeled probes in 20-μL reaction mixtures containing 10 mM Tris-HCl, 150 mM KCl, 1 mM DTT, 50 ng/μL poly (dI-dC), 2.5% glycerol, 0.05% Nonidet P-40, 100 μM ZnCl2, and 0.5 μg/μL BSA for 20 min at room temperature and separated on 6% native polyacrylamide gels in Tris-Gly buffer. The labeled probes were detected according to the instructions provided with the EMSA kit. Sequences of the complementary oligonucleotides used to generate the biotin-labeled probes are shown in Supplemental Table 1 online.

Transient Transcription Dual-Luciferase Assay

Transient dual-luciferase assays in Nicotiana benthamiana and Arabidopsis were performed as described previously (Helliens et al., 2005). After infiltration, plants were left under −UV-B or +UV-B for 3 d, and then leaf samples were collected. Firefly luciferase and Renilla luciferase were assayed using the dual luciferase assay reagents (Promega) and were performed as previously described (Liu et al., 2008). Briefly, leaf discs (1 to 2 cm in diameter) were excised, ground in liquid nitrogen, and homogenized in 100 μL of the passive lysis buffer. Eight microliters of this crude extract was mixed with 40 μL of luciferase assay buffer, and the firefly LUC activity was measured using a GLOMAX 20/20 luminometer (Promega). Forty microliters of Stop and Glow Buffer was then added to the reaction, and the Renilla luciferase (REN) activity was measured. Four biological replicates were measured for each sample.

Anthocyanin and Hypocotyl Measurement

Anthocyanins were extracted and quantified as previously described (Noh and Spalding, 1998). Briefly, Arabidopsis seedlings were harvested and placed into extraction solution (18% 1-propanol and 1% HCl) and boiled for 3 min. Then, the mixture was left in darkness for at least 3 h at room temperature. After a brief centrifugation to pellet the tissue debris, the supernatant was removed and diluted with the extraction solution. The anthocyanin content was presented as A535 = 2(A650) g−1 fresh weight.

For each line under each condition (−UV-B or +UV-B), hypocotyl length was analyzed using three biological replicates. In each replicate, at least 30 Arabidopsis seedlings were measured. The relative hypocotyl length was presented as the percentage of the hypocotyl length under +UV-B with respect to that under −UV-B (percentage of −UV-B).

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: COP1 (At2g32950), ELIP2 (At1g14690), At4g15480, DREB2A (At5g00540), CHS (At5g13930), FYH3 (At3g22170), HY5 (At5g11260), HYH (At3g17609), and FHY1 (At1g37678).

Supplemental Data

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

Supplemental Figure 1. 3FLAG-FHY3-3HA Associates with the COP1 Promoter under −UV-B and +UV-B.

Supplemental Figure 2. HYH Does Not Bind to the COP1 Promoter.

Supplemental Figure 3. FHY1 Expression Is Not Induced by Photomorphogenic UV-B.

Supplemental Figure 4. Relative Hypocotyl Length of Wild-Type and Various Mutant Seedlings.

Supplemental Figure 5. Additional fhy3 Alleles Are Defective in UV-B–Induced Hypocotyl Growth.

Supplemental Figure 6. FAR1 Is Barely Involved in UV-B–Induced Photomorphogenesis.

Supplemental Figure 7. UV-B–Induced Photomorphogenesis Is Impaired in hy5-ks50.

Supplemental Figure 8. COP1 Is Overexpressed in COP1OEd/COP1−/−.

Supplemental Figure 9. COP1, HY5, and FHY3 Show Rapid Expression Responses to Photomorphogenic UV-B.

Supplemental Table 1. Summary of Primers Used in This Study.
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AUTHOR CONTRIBUTIONS

X.H., X.O., and X.W.D. designed the research. X.H., X.O., P.Y., O.S.L., G.L., J.L., and H.C. performed the research. X.H. and X.O. analyzed the data. X.H. and X.W.D. wrote the article.

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