Short title: Regulation of PSII function by light signaling

*** Corresponding author:** Hong-Lei Jin
Institute of Medical Plant Physiology and Ecology, School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, 510006 Guangzhou, People's Republic of China.
E-mail: jinhl@gzucm.edu.cn.
Tel: +86-20-39358634.

Light Signaling-Dependent Regulation of Photosystem II Biogenesis and Functional Maintenance

Xue Li[1], Hong-Bin Wang[1,2], and Hong-Lei Jin[2*]

[1]State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, 510275 Guangzhou, People's Republic of China.
[2]Institute of Medical Plant Physiology and Ecology, School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, 510006 Guangzhou, People's Republic of China.
* Correspondence: jinhl@gzucm.edu.cn.
ORCID IDs: 0000-0003-4957-0509 (H.-B.W); 0000-0001-9148-9937 (H.-L.J)

One sentence summary: The photoreceptors phytochrome and cryptochrome regulate photosystem II biogenesis, assembly, and repair mediated by regulation of gene expression by ELONGATED HYPOCOTYL5 (HY5).

Footnotes:

H. -L. J. and H.-B. W. designed the research project; X. L. and H.-L. J. performed the experiments; X. L. and H.-L. J. wrote the article. H.-L. J. and H.-B. W. revised the article.

This work was supported by the National Natural Science Foundation of China Grant (31770260 and 31970261), the Talent Support Project of Guangdong (2019TQ05N182), the National Science Fund for Distinguished Young Scholars Grant (31425003), and the National Science and Technology Major Project Foundation of China (grant no. 2016ZX08009003-005-005).

ABSTRACT

Light is a key environmental cue regulating photomorphogenesis and photosynthesis in plants. However, the molecular mechanisms underlying the interaction between light signaling pathways and photosystem function are unknown. Here, we show that various monochromatic wavelengths of light cooperate to regulate photosystem II (PSII) function in *Arabidopsis thaliana*. The photoreceptors cryptochromes and phytochromes modulate the expression of HIGH CHLOROPHYLL FLUORESCENCE173 (HCF173), which is required for PSII biogenesis by regulating PSII core protein D1 synthesis mediated by the transcription factor ELONGATED HYPOCOTYL5 (HY5). HY5 directly binds to the ACGT-containing element ACE motif and *G-box cis*-element present in the *HCF173* promoter and regulates its activity. PSII activity was decreased significantly in *hy5* mutants under various monochromatic wavelengths of light. Interestingly, we demonstrate that HY5 also directly regulates the expression of the genes associated with PSII assembly and repair, including *ALBINO3*, *HCF136*, *HYPERSENSITIVE TO HIGH LIGHT1*, *etc.*, which is required for the functional maintenance of PSII under photodamaging conditions. Moreover, deficiency of *HY5* broadly decreases the accumulation of other photosystem proteins besides PSII proteins. Thus, our study reveals an important role of light signaling in both biogenesis and functional regulation of the photosystem and provides insight into the link between light signaling and photosynthesis in land plants.

INTRODUCTION

Chloroplasts and non-photosynthetic plastid organelles are derived from cyanobacterial endosymbionts and many of the endosymbiont's genes have migrated to the plant cell nucleus by gene transfer (Timmis et al., 2004). Therefore, the chloroplast proteome of several thousand proteins is dominated by nuclear-encoded proteins (Barkan, 2011). The thylakoid membrane system, a major structural component of chloroplasts, functions in photosynthetic electron transport and ATP synthesis and consists of four major multimeric complexes: photosystem II (PSII), cytochrome b_6/f, PSI, and ATP synthase. PSII structure has been resolved to near-atomic resolution, and the protein subunits, cofactors, and coordinates for different ligands are largely known (Zouni et al., 2001; Loll et al., 2005). PSII biogenesis involves the collaborative assembly of over 30 different polypeptides and a multitude of nuclear-encoded regulatory proteins (Nickelsen and Rengstl, 2013). While plants depend on light for growth, they are sensitive to the damaging effects of radiation. PSII is the major component of the photosystem that is damaged by light (Liere and Börner, 2007). Therefore, PSII repair is required for its functional maintenance. PSII repair is a complex process, including protein phosphorylation, disassembly, and reassembly, which are regulated by numerous regulatory factors (Nickelsen and Rengstl, 2013; Lu, 2016).

The PSII reaction center protein, D1 (encoded by the chloroplast gene *psbA*), is damaged by light and must be rapidly turned over and replaced with newly synthesized D1 for PSII reassembly and repair. Therefore, the efficient synthesis of D1 is important for PSII biogenesis, assembly, and repair. In land plants, D1 protein synthesis increased up to 100-fold when induced by light, but without an equivalent increase in *psbA* mRNA levels, indicating that translation is the pivotal regulation step (Fromm et al., 1985; Klein et al., 1988; Malnoe et al., 1988; Krupinska and Apel, 1989). Previous studies have revealed that

HIGH CHLOROPHYLL FLUORESCENCE173 (HCF173) and HCF244, two regulators of *psbA* mRNA translation, promote D1 biosynthesis (Schult et al., 2007). LOW PHOTOSYNTHETIC EFFICIENCY 1 (LPE1), a nuclear-encoded chloroplast-targeted pentatricopeptide repeat (PPR) protein, plays a vital role in D1 translation by promoting the association of HCF173 and *psbA* mRNA (Jin et al., 2018). The expression of *HCF173*, but not *LPE1*, was drastically induced by light (Jin et al., 2018), but the mechanism of regulation remains unclear.

In addition to being the primary energy source for plant growth, light is a key environmental factor affecting plant development. More than 32% of genes respond to changing light conditions in *Arabidopsis thaliana* (Ma et al., 2001). At least four types of photoreceptors perceive different wavelengths of light, including phytochromes (PHY), the red (R) and far-red (FR) light receptors; cryptochromes (CRY) and phototropins, the blue (B) and ultraviolet-A (UV-A) light receptors, respectively; and the ultraviolet-B (UV-B) light receptor UV RESISTANCE LOCUS 8 (UVR8) (Cashmore et al., 1999; Briggs and Christie, 2002; Quail, 2002; Rizzini et al., 2011). Photoreceptors transmit light signals to downstream transcription factors, such as the basic helix-loop-helix (bHLH) proteins phytochrome-interacting factors (PIFs) (Leivar and Quail, 2011) and the bZIP protein ELONGATED HYPOCOTYL5 (HY5) (Osterlund et al., 2000), and thereby affect plant growth and development. HY5 regulates diverse signaling pathways by directly binding to the *cis*-regulatory elements of promoters in a sequence-specific manner (Gangappa and Botto, 2016). In darkness (D) condition, the E3 ubiquitin ligase CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1) is enriched in the nucleus in darkness and specifically targets positive photomorphogenic factors such as HY5 for ubiquitination and degradation (Lau and Deng, 2012; Huang et al., 2014). Although the mechanisms by which light signals regulate chloroplast gene

117 expression are well understood, the relationship between light signaling
118 pathways and PSII function remains largely unknown.
119 In this study, we demonstrate that the light signaling pathway is required
120 for regulation of PSII biogenesis and functional maintenance. We found that
121 various monochromatic wavelengths of light cooperate to regulate PSII
122 function in Arabidopsis. Photoreceptor-mediated light signaling regulates PSII
123 function, including PSII biogenesis, assembly, and repair, through the
124 transcription factor HY5. HY5 directly binds to the promoter of genes related to
125 PSII assembly and repair, which is required for the functional maintenance of
126 PSII.
127
128

RESULTS

Various Monochromatic Wavelengths of Light Cooperatively Contribute to PSII activity, D1 Protein Accumulation, and *HCF173* Expression

Prompted by our previous observation that PSII activity and accumulation of the PSII core protein D1 are induced by white (W) light (Jin et al., 2018), we examined whether PSII activity and D1 accumulation are regulated by specific wavelengths of light. Arabidopsis wild-type (WT) Columbia-0 (Col-0) seedlings were grown in darkness (D) for five days, transferred to W light (100 μmol photons m^{-2} s^{-1}), blue (B) light (58 μmol photons m^{-2} s^{-1}), red (R) light (44 μmol photons m^{-2} s^{-1}), or far-red (FR) light (10 μmol photons m^{-2} s^{-1}) for 0 to 48 h (Fig. 1A, E, I and M), and then harvested and subjected to immunoblot and chlorophyll fluorescence analysis. As previously observed for W light (Jin et al., 2018; Fig. 1B and C), both PSII activity and D1 protein levels increased substantially following exposure to all of the specific wavelengths of light (Fig. 1F, J, N, G, K and O), indicating that B, R, and FR light induce PSII activity and D1 protein accumulation.

We previously demonstrated that LPE1 and HCF173 cooperatively regulate D1 synthesis and PSII biogenesis. Light induces the association of LPE1 and *psbA* mRNA, which promotes PSII biogenesis (Jin et al., 2018). We showed that light induces the expression of *HCF173* but not *LPE1* in etiolated Arabidopsis Col-0 seedlings (Jin et al., 2018). However, the mechanism by which light regulates HCF173 in PSII biogenesis is unclear. To provide insight into the mechanism by which light induces *HCF173* expression, we investigated whether *HCF173* expression is also regulated by specific wavelengths of light. Reverse transcription quantitative PCR (RT-qPCR) analysis showed that *HCF173* transcript levels were significantly elevated over a two-day period under the various light conditions compared with the D condition, with at least a 7-fold induction under W, B, R, and FR light (Fig. 1D,

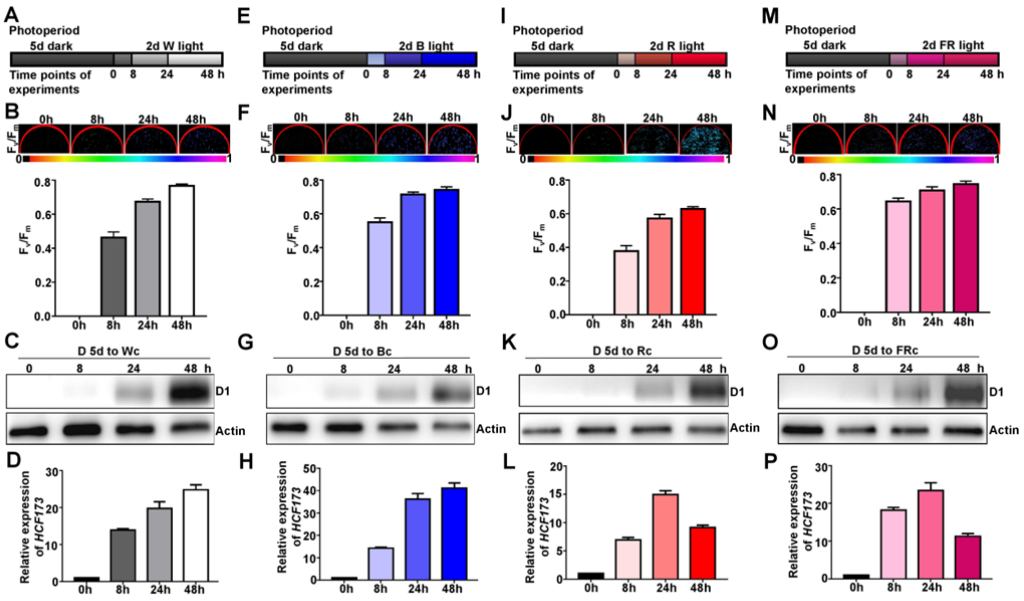

Figure 1. Single wavelengths of light regulate PSII activity, as well as D1 and HCF173 protein accumulation during greening.

(A, E, I, M) Schematic representation of the experimental set-up used with white (W), blue (B), red (R), and far-red (FR) light during greening. Five-day-old Arabidopsis Col-0 seedlings were maintained under darkness and were then transferred to different light conditions and harvested at 0, 8, 24, and 48 h for analysis.

(B, F, J, N) False-color images representing the maximal photochemical efficiency of PSII (F_v/F_m) during W, B, R, and FR light-induced greening of etiolated wild-type Arabidopsis seedlings. After growth in darkness for 5 days, etiolated seedlings were illuminated for 0, 8, 24, or 48 h, and false-color images representing F_v/F_m were captured.

(C, G, K, O) Western blot analysis of D1 proteins isolated from 5 d etiolated Arabidopsis Col-0 seedlings during W, B, R, or FR light-induced greening for the indicated periods. Anti-Actin was used as a sample loading control.

(D, H, L, P) RT-qPCR analysis showing the relative expression of *HCF173* after five-day-old etiolated Arabidopsis Col-0 seedlings were transferred to W, B, R, or FR light conditions for the indicated periods. Data are represented as mean ± SEM. All experiments were repeated at least three times with similar results.

H, L and P). Transcript levels peaked at 24 h under FR and R light conditions in contrast with peaks under W and B light at 48 h. After five days of continuous treatment under D, W, B, R, and FR light conditions, *HCF173* transcript levels were significantly up-regulated in each of the light conditions compared with the D condition, with at least a 20-fold induction (Supplemental Fig. S1). These results indicate that specific wavelengths of light, including B, R, and FR, induce *HCF173* expression.

Both Cryptochromes and Phytochrome Influence *HCF173* Expression

To examine whether photoreceptors regulate *HCF173* expression, we quantified *HCF173* transcript levels in Col-0 and the phytochrome and cryptochrome single, double, and triple mutants. The seedlings were grown in the D condition for five days, and then transferred to W light for 0 to 48 h. After 8 h of exposure to W light, *HCF173* expression was significantly lower in the *cry1-304*, *phyA-211*, and *phyB-9* single mutants, the *cry1 cry2* and *phyA phyB* double mutants, and the *phyA phyB cry1* and *cry1 cry2 phyA* triple mutants than in Col-0. However, *HCF173* expression was higher in the *cry2* single mutant than in Col-0 (Fig. 2A). After 24 h of exposure to W light, *HCF173* expression was significantly lower in the *phyA-211* and *phyB-9* single mutants, the *cry1 cry2* and *phyA phyB* double mutants, and the *phyA phyB cry1* and *cry1 cry2 phyA* triple mutants than in Col-0. However, *HCF173* expression was similar in the *cry1* and *cry2* single mutants and in Col-0 (Fig. 2A). After 48 h of exposure to W light, *HCF173* expression was significantly lower in the *cry1-304* and *phyA-211* single mutants, the *cry1 cry2* and *phyA phyB* double mutants, and the *phyA phyB cry1* and *cry1 cry2 phyA* triple mutants compared to Col-0. However, *HCF173* expression was much higher in the *cry2* single mutant than in Col-0 (Fig. 2A). These data demonstrate that *HCF173* expression is redundantly regulated by phytochrome and cryptochrome during light induction, and that CRY2 has a minor role in this process.

To confirm this, we quantified *HCF173* transcript levels in WT Col-0, cryptochrome mutants, and phytochrome mutants grown under continuous W light for five days. After induction by W light, *HCF173* expression in *phyA*, *phyB*, *cry1*, or *cry2* single mutants and a *phyA phyB* double mutant showed no obvious differences compared with Col-0. By contrast, *HCF173* expression in the *cry1 cry2* double mutant was slightly lower than in the wild type. *HCF173*

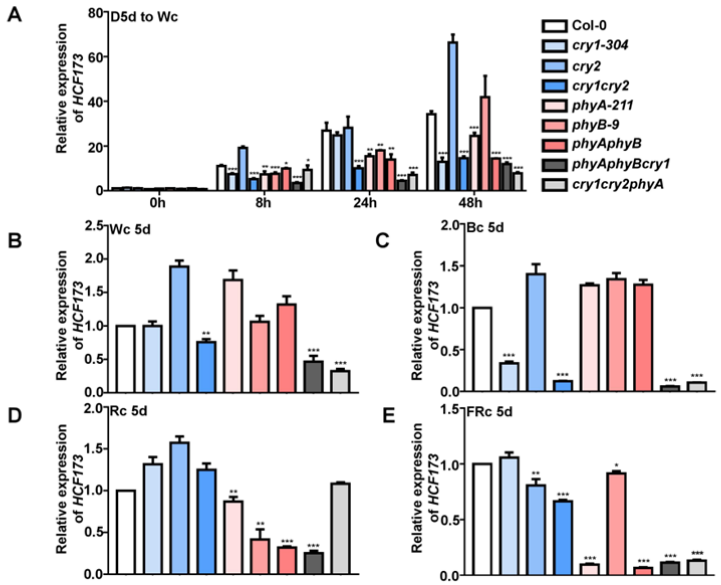

Figure 2. Both cryptochromes and phytochrome influence *HCF173* expression.

(A) RT-qPCR analysis of *HCF173* transcript levels after five-day-old etiolated Col-0 and different photoreceptor mutants were transferred to W light for the indicated periods.

(B–E) Analyses of *HCF173* expression under W, B, R, and FR light conditions in *phyA-211*, *phyB-9*, *cry1-304*, and *cry2* single and higher order mutants under five days of continuous light (Wc, Bc, Rc, and FRc) conditions as indicated. The expression levels were normalized to that of *ACTIN*. Data are represented as mean ± SEM (Three biological repeats). Asterisks represent statistically significant differences (*P < 0.05; **P < 0.01; ***P < 0.001; Student's t-test). All experiments were repeated at least three times with similar results. We did not perform statistical analyses on those mutants that showed up-regulation of *HCF173* transcripts.

expression was nearly abolished in the *cry1 cry2 phyA* and *phyA phyB cry1* triple mutants (Fig. 2B), further supporting that both phytochrome and cryptochrome mediate W-light-induced *HCF173* expression in a functionally redundant manner.

To further explore the role of different photoreceptors in regulation of the *HCF173* transcript levels in response to various monochromatic wavelengths of light, we quantified *HCF173* transcript levels in WT Col-0, cryptochrome mutants, and phytochrome mutants grown under continuous B, R, or FR light for five days. After induction by B light, *HCF173* expression was lower in the *cry1-304* single mutant and the *cry1 cry2* double mutant than in Col-0. The decrease in *HCF173* expression in the *cry1 cry2 phyA* and *phyA phyB cry1* triple mutants was more drastic (Fig. 2C), indicating that CRY1 is the major

regulator of *HCF173* expression under B light conditions. After induction by R light, *HCF173* expression was lower in the *phyA-211* and *phyB-9* single mutants and in the *phyA phyB* double mutant than in Col-0. The decrease in *HCF173* expression in the *phyB-9* mutant was greater than that in the *phyA-211* mutant. The decrease of *HCF173* expression in the *phyA phyB cry1* triple mutant was more drastic than that in the *cry1 cry2 phyA* triple mutant (Fig. 2D), indicating that PHYB is the major regulator of *HCF173* expression under R light conditions. After induction by FR light, the decrease in *HCF173* expression in the *phyA-211* mutant was greater than that in the *phyB-9* mutant (Fig. 2E), indicating that PHYA is the major regulator of *HCF173* expression under FR light conditions.

Collectively, these data indicate that *HCF173* expression is induced by various monochromatic wavelengths of light and is redundantly regulated by the phytochrome and cryptochrome photoreceptors.

HY5 Directly Binds to the Promoter of *HCF173* and Regulates Its Expression

As HY5 is activated by light signals sensed by upstream photoreceptors (Oyama et al., 1997; Ang et al., 1998), we next examined whether HY5 affects the transcript level of *HCF173* during W light-induced greening, using RT-qPCR analysis. *HCF173* transcript levels were markedly elevated after etiolated Col-0 and *hy5-215* mutant seedlings were transferred to W light for up to 48 h (Fig. 3A); however, *HCF173* expression was lower in the *hy5-215* mutant than in Col-0 at all time points examined, indicating that HY5 specifically positively regulates *HCF173* expression in response to W light during early plant development. Next, we examined whether *HCF173* transcript levels are regulated by HY5 during continuous W, B, R, or FR light conditions. *HCF173* transcript levels were dramatically lower in the *hy5-215* mutant than in the WT

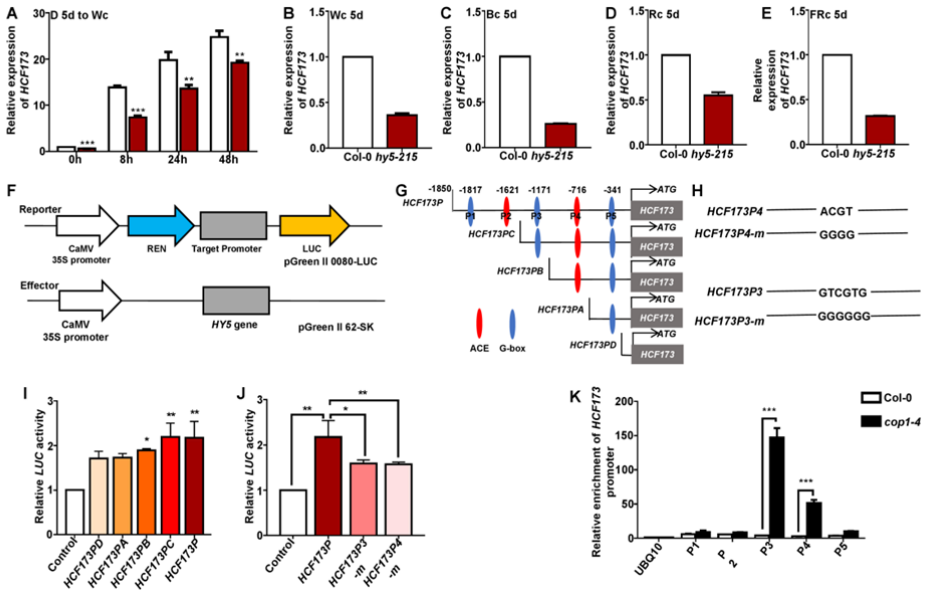

Figure 3. HY5 regulates the transcript level of *HCF173* through directly associating with its promoter.

(A) RT-qPCR analysis of the relative expression of *HCF173* after five-day-old etiolated Col-0 and *hy5-215* mutant seedlings were transferred to W light for the indicated time points. The expression levels were normalized to that of *ACTIN*.

(B–E) RT-qPCR analysis showing the expression levels of *HCF173* in five-day-old Col-0 and *hy5-215* mutant seedlings grown under continuous W, B, R, and FR light conditions.

(F) Schematic representation of various constructs used in the transient transfection assay in Arabidopsis protoplasts. Reporter, A schematic map of the transient expression vector pGreenII-0800-LUC. REN, Renilla luciferase; LUC, firefly luciferase. Effector, Diagram of the HY5 effector of the transient expression vector pGreenII 62-SK.

(G) Diagram representing different variants of the *HCF173* promoter. Part of the coding region of *HCF173* is shown. Positions of the putative G-boxes (blue) and ACE motifs (red) are numbered.

(H) DNA sequences of the wild-type *HCF173* promoter containing the ACE motifs (*HCF173P4*) or G-boxes (*HCF173P3*) and mutant *HCF173* promoters harboring a mutant ACE motif (*HCF173P4-m*) or G-box (*HCF173P3-m*).

(I) Effects of HY5 on *HCF173* transcriptional regulation using different *HCF173* promoter variants in Col-0 protoplasts.

(J) The roles of ACE motif or G-box for activity of *HCF173* promoter regulated by HY5. The protoplasts without the HY5 effector were used as a negative control. The relative LUC activities were normalized to REN activities.

(K) The analyses of association between HY5 and the *HCF173* promoter in vivo by ChIP-qPCR assays. Anti-HY5 antibody was used to immunoprecipitate the HY5-HCF173 complex. Col-0 and *UBQ10* were used as negative controls. Data are represented as mean ± SEM. Asterisks represent statistically significant differences (*P < 0.05; **P < 0.01; ***P < 0.001; Student's t-test). All experiments were repeated at least three times with similar results.

232 Col-0 when grown in continuous W, B, R, or FR light conditions for five days
233 (Fig. 3B–E), further demonstrating that HY5 indeed positively regulates the

transcript levels of *HCF173* in response to various wavelengths of light.

To establish whether HY5 affects HCF173 accumulation during light-induced greening, the WT Col-0 and *hy5-215* mutant seedlings were grown in darkness for five days, transferred to W light for up to 48 h, and then harvested and subjected to immunoblot analysis. HCF173 protein levels increased after illumination in both genotypes, consistent with the *HCF173* transcript levels, but were lower in the *hy5-215* mutant than in Col-0 (Supplemental Fig. S2A and B). We further examined whether HY5 affects HCF173 accumulation after induction by B, R, or FR light. The WT Col-0 and *hy5-215* mutant seedlings were grown under continuous B, R, or FR light for five days, harvested, and then subjected to immunoblot analysis. HCF173 protein levels were lower in the *hy5-215* mutant than in Col-0 under continuous B, R, or FR light conditions (Supplemental Fig. S3A-C), demonstrating that HCF173 accumulation is positively regulated by HY5 in response to various monochromatic wavelengths of light. Thus, HCF173 accumulation is positively regulated by HY5 in response to W light-induced greening, and single wavelengths of light can also induce the expression of HCF173 protein.

Previous studies revealed that HY5 can directly bind to the ACE motif or G-box *cis*-acting elements within the promoters of its target genes (Lee et al., 2007; Zhang et al., 2011). We therefore analyzed the 1850-bp *HCF173* promoter region directly upstream of the start codon (ATG) and identified two ACE motifs and three atypical G-box *cis*-elements. The basic domain/Leu zipper transcription factor HY5 acts downstream of COP1 and is degraded by COP1 in darkness (Osterlund et al., 2000). We thus analyzed the effect of HY5 on the activity of the *HCF173* promoter using the dual-luciferase (LUC) reporter system in Arabidopsis Col-0 protoplasts (Fig. 3F and G). The truncated promoter fragments (*HCF173PA-LUC*, *HCF173PB-LUC*, *HCF173PC-LUC*, and *HCF173PD-LUC*) and full-length promoter (*HCF173P-LUC*) were

transiently expressed in protoplasts with or without the HY5 effector. HY5 increased the activity of *HCF173PA*/*PB*/*PC*/*PD*/*P-LUC* approximately 1.5- to 2.2-fold, and the activity of the *HCF173* promoter fragments containing the ACE (*HCF173PB*) motif and G-box (*HCF173PC*) element were more significantly increased by HY5 (Fig. 3I). An analysis using point mutations that specifically disrupted the *P3* G-box or the *P4* ACE motif (Fig. 3J) revealed that the *P4* ACE motif and *P3* G-box in the *HCF173* promoter are required for HY5 binding.

Next, we performed a chromatin immunoprecipitation (ChIP)-qPCR analysis to determine whether HY5 binds to the *HCF173* promoter *in vivo*. Considering that HY5 accumulation is limited in etiolated WT seedlings illuminated for two days and that the *cop1-4* mutant accumulates more HY5 protein than the wild type (Supplemental Fig. S4B), we used etiolated *cop1-4* seedlings grown under continuous D for five days in this analysis. HCF173 protein accumulation and *HCF173* transcript levels were markedly higher in the *cop1-4* mutant than in Col-0 (Supplemental Fig. S4A and C). Under the D condition, HY5 levels increased to a greater extent in the *cop1-4* mutant than in Col-0 (Supplemental Fig. S4B), consistent with a previous report (Osterlund et al., 2000). ChIP-qPCR assays using etiolated Col-0 and *cop1-4* seedlings and an anti-HY5 antibody were employed to confirm the binding of HY5 to the *HCF173* promoters *in vivo*. HY5 could immunoprecipitate the *HCF173* promoter region containing the *P4* ACE motif and *P3* G-box (Fig. 3K), suggesting that HY5 associates with the *HCF173* promoter *in vivo*. To further examine the interaction between HY5 and the *HCF173* promoter, we conducted a ChIP-qPCR analysis using transgenic Arabidopsis protoplasts (Saleh et al., 2008) expressing *35S:HY5-HA*, and protoplasts without transfection and a *UBIQUITIN10* (*UBQ10*) promoter fragment were used as a negative control. DNA was immunoprecipitated using an anti-HA affinity gel. ChIP-qPCR results showed that HY5 could also recognize the chromatin region

290 containing the ACE motif (*P4*) and G-box (*P3*) of the *HCF173* promoter
291 (Supplemental Fig. S4D and E).

292 To determine whether HY5 directly binds to the promoter of *HCF173*, we
293 performed electrophoretic mobility shift assays (EMSA). CHIP assays indicated
294 that HY5 preferably binds to the *P3* region of the *HCF173* promoter (Figure 3K).
295 Thus, we chose the *P3* region of the *HCF173* promoter containing the G-box
296 *cis*-element as the DNA probe. We purified the HY5 proteins from Arabidopsis
297 plants by immunoprecipitation using HY5 antibody. The purified HY5 proteins
298 were incubated with the *HCF173* promoter DNA probe. The results showed that
299 the HY5–DNA complex was detected as a band that migrated more slowly than
300 the free probe in the gel; increasing retardation of the band was detected as the
301 amount of HY5 proteins was increased (Supplemental Fig. S5). The
302 association of HY5 with the *HCF173* promoter DNA probe was also confirmed
303 by competition experiments with an unlabeled *HCF173* promoter DNA probe
304 (Supplemental Fig. S5), indicating that HY5 directly binds to the *HCF173*
305 promoter. Together, these data suggest that HY5 directly binds to the promoter
306 of *HCF173* and regulates its expression.
307

308 **HY5 Deficiency Results in Decreased PSII Activity under Various**
309 **Monochromatic Wavelengths of Light**

310 Analyses of chlorophyll fluorescence parameters indicated that single
311 wavelengths of light can induce PSII activity. To determine whether HY5 is
312 involved in the functional maintenance of PSII during induction with various
313 monochromatic wavelengths of light, we established an induction system using
314 various monochromatic wavelengths of light to control PSII biogenesis in
315 Arabidopsis. After 5 d of growth in the D condition, etiolated seedlings were
316 exposed to W, B, R, and FR light for 0, 8, 24, or 48 h. The leaves of WT
317 seedlings gradually turned green when exposed to increasing periods of W and

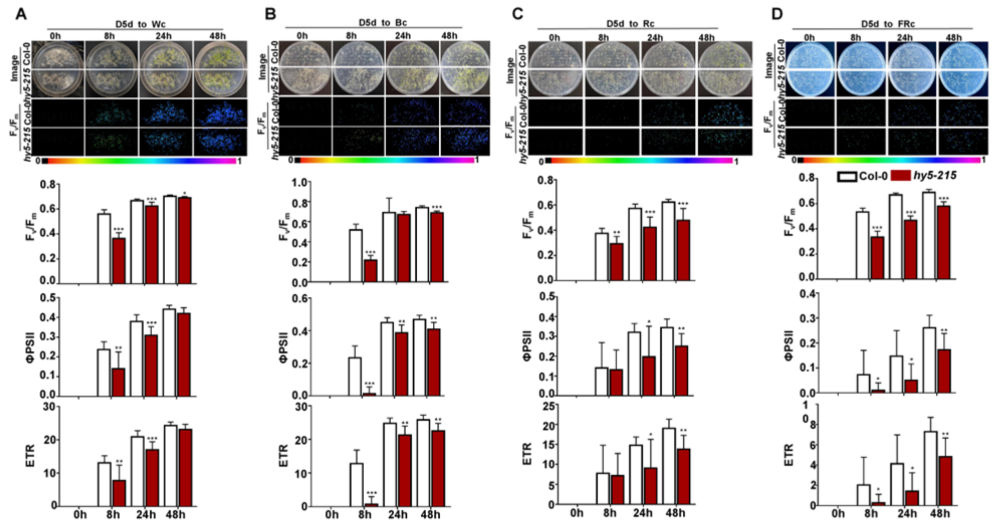

Figure 4. HY5 promoters PSII activity during light-induced greening with various monochromatic light. Photographs and false-color images representing F_v/F_m during W **(A)**, B **(B)**, R **(C)**, and FR **(D)** light-induced greening of etiolated wild-type and *hy5-215* Arabidopsis seedlings. After growth in darkness for 5 days, etiolated seedlings were illuminated for 0, 8, 24, or 48 h, and photographs and false-color images representing F_v/F_m were captured.

The maximal photochemical efficiency of PSII (F_v/F_m), PSII quantum yield (ΦPSII), and electron transport rate (ETR) of etiolated seedlings during W **(A)**, B **(B)**, R **(C)**, and FR **(D)** light-induced greening are shown below the images. After growth in darkness for 5 days, etiolated seedlings of the wild type and *hy5-215* mutant were illuminated for 0, 8, 24, or 48 h, and F_v/F_m, ΦPSII, and ETR were measured. Data are represented as mean ± SEM. Asterisks represent statistically significant differences (*P < 0.05; **P < 0.01; ***P < 0.001; Student's t-test). All experiments were repeated at least three times with similar results.

other specific wavelengths of light (Fig. 4A–D), and chlorophyll fluorescence parameters, including the maximum photochemical efficiency of PSII (F_v/F_m), the kinetics curves of PSII quantum yield (ΦPSII), and the electron transport rate (ETR), increased simultaneously (Fig. 4A–D). However, HY5-deficient *hy5-215* seedlings showed reduced F_v/F_m, ΦPSII, and ETR values when exposed to W and other various monochromatic wavelengths of light (Fig. 4A–D). In conclusion, these data suggest that HY5-mediated PSII biogenesis contributes to the increase in PSII activity during W, B, R, and FR-induced de-etiolation.

HY5 Regulates the Expression of PSII Assembly- and Repair-Associated Genes and the Functional Maintenance of PSII

As our results indicated that HY5 functions in PSII biogenesis under various wavelengths of light, we next investigated whether PSII assembly and repair are regulated by HY5. The expression of several types of PSII assembly- and repair-associated genes, including *HCF136*, *ALBINO 3* (*ALB3*), and *PHOTOSYNTHESIS AFFECTED MUTANT 68* (*PAM68*), was lower in the *hy5-215* mutant than in the WT during W light-induced greening (Fig. 5A). The expression of *HYPERSENSITIVE TO HIGH LIGHT 1* (*HHL1*), encoding a thylakoid protein and an important regulator of PSII repair (Jin et al., 2014), was induced by light in both genotypes, but to a lesser extent in the *hy5-215* mutant, suggesting that *HHL1* is positively regulated by HY5 (Fig. 5A). In addition, the expression of a gene encoding a chloroplast protease, *FILAMENTATION-TEMPERATURE-SENSITIVE PROTEIN H 8* (*FtsH8*), involved in the degradation of photodamaged D1, was lower in the *hy5-215* mutant than in the WT during de-etiolation (Fig. 5A). Collectively, these data indicate that HY5 positively regulates the expression of several light-responsive genes involved in PSII assembly and repair.

Next, we conducted a ChIP-qPCR assay to examine whether HY5 directly binds to the promoters of light-responsive genes and accelerates their transcription. HY5 immunoprecipitated the promoter region of *HCF136* containing a G-box and an A-box, the *ALB3* promoter region containing a C-box and a G-box, the *HHL1* promoter region containing a CG-box, A-box, and G-box (Fig. 5B and C), the *PAM68* promoter region containing an ACE-box, the *LOW QUANTUM YIELD OF PHOTOSYSTEM II 1* (*LQY1*) promoter region containing two A-boxes and a G-box, the *FtsH8* promoter region containing an A-box, and the *DEGRADATION OF PERIPLASMIC PROTEINS 1* (*DEG1*) promoter region containing an A-box (Fig. 5B and C), suggesting that HY5 associates with the promoters of genes encoding PSII assembly and repair factors *in vivo*. To verify these results, we then performed a LUC reporter

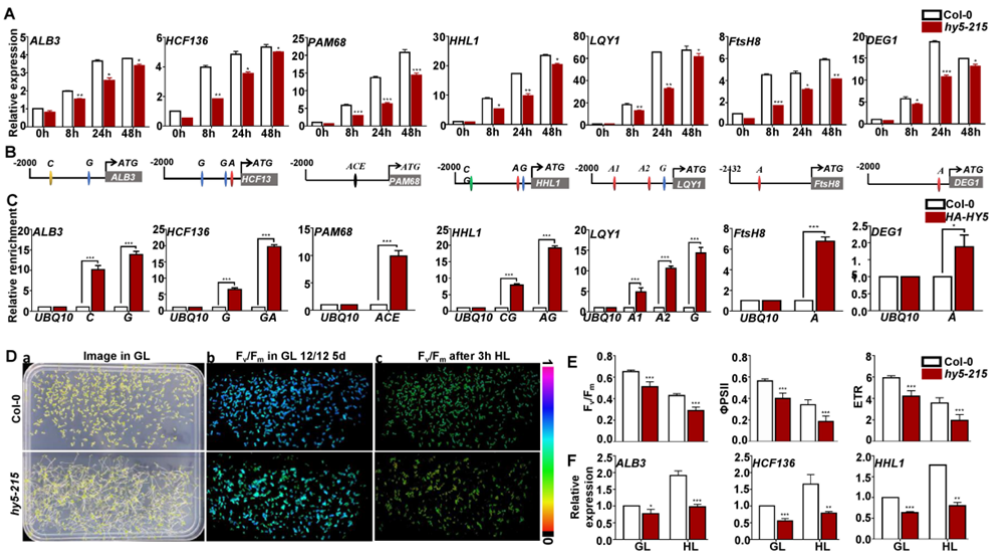

Figure 5. HY5 accelerates the expression of PSII assembly- and repair-associated genes and PSII activity.

(A) RT-qPCR analysis showing the relative expression of *ALB3*, *HCF136*, *PAM68*, *HHL1*, *LQY1*, *FtsH8*, and *DEG1* after five-day-old etiolated Col-0 and *hy5-215* mutant seedlings were transferred to W light for the indicated periods.

(B) Diagram of the promoter structure of *ALB3*, *HCF136*, *PAM68*, *HHL1*, *LQY1*, *FtsH8*, and *DEG1*. Part of the coding region of each gene is shown. Positions of the putative G-box (G, blue), C-box (C, yellow), CG-box (green), ACE-box (black), or A-boxes (A1, A2, red) fragments amplified in the ChIP-qPCR assay are shown.

(C) ChIP-qPCR results showing that HY5 binds to the promoters of *ALB3*, *HCF136*, *PAM68*, *HHL1*, *LQY1*, *FtsH8*, and *DEG1* in vivo. Anti-HA antibody was used to immunoprecipitate the above-mentioned genes. Col-0 and *UBQ10* were used as negative controls.

(D) Photographs in (a) are of five-day-old Col-0 and *hy5-215* plants under growth light (12 h light /12h dark) conditions. (b) False-color images representing F_v/F_m under growth light conditions in five-day-old Col-0 and *hy5-215* seedlings. (c) False-color images representing F_v/F_m after a 3-h high light treatment in five-day-old Col-0 and *hy5-215* plants. GL, growth light (~100 μmol photons m^{-2} s^{-1}); HL, high light (~1200 μmol photons m^{-2} s^{-1}).

(E) The maximal photochemical efficiency of PSII (F_v/F_m), PSII quantum yield (ΦPSII), and electron transport rate (ETR) were measured in Col-0 and *hy5-215* seedlings after 5 d of growth under light conditions and again after a 3-h high light treatment.

(F) RT-qPCR analysis showing the relative expression of *ALB3*, *HCF136*, and *HHL1*. After growth for 5 d in growth light conditions (GL), Col-0 and *hy5-215* mutant seedlings were transferred to a 3-h high light (HL) treatment. The gene expression levels were normalized to that of *ACTIN*. Data are represented as mean ± SEM. Asterisks represent statistically significant differences (*P < 0.05; **P < 0.01; ***P < 0.001; Student's t-test). All experiments were repeated at least three times with similar results.

plasmid assay. Indeed, HY5 activates *proALB3:LUC*, *proHCF136:LUC*, and *proHHL1:LUC* when transiently expressed in Arabidopsis protoplasts (Supplemental Fig. S6). Together, these results suggest that HY5 accelerates

the transcriptional activation activity of genes encoding PSII assembly and repair factors through direct binding to their promoters and thereby maintains efficient photosynthesis.

PSII performs the energy-demanding chemical reaction of water oxidation and thereby renders its own protein components at risk of photodamage. PSII repair is required to maintain efficient photosynthesis (Mulo et al., 2008). Our data showed that five-day-old Col-0 seedlings had a higher F_v/F_m in growth light conditions (~100 μmol photons m^{-2} s^{-1}) than the *hy5-215* mutant, suggesting that PSII activity was disturbed in the *hy5-215* mutants. High intensity light energy can induce greater photodamage of PSII (Takahashi and Badger, 2011). We further examined whether HY5 is involved in the high light-induced PSII damage repair process. High light treatment (3 h) (~1200 μmol photons m^{-2} s^{-1}) caused substantially greater photodamage via reducing F_v/F_m values in the *hy5-215* mutant than in Col-0 (Fig. 5D and E), suggesting that HY5 participates in the functional maintenance of PSII under both growth light and high light conditions.

To further characterize the photosynthetic apparatus, we analyzed the light intensity dependence of two chlorophyll fluorescence parameters, ΦPSII and the ETR, before and after a 3-h high light treatment. After a short-term high light treatment (3 h), Col-0 and *hy5-215* seedlings had a much lower ΦPSII and ETR than after a 3-h treatment under growth light conditions. Furthermore, *hy5-215* seedlings had significantly reduced F_v/F_m, ΦPSII, and ETR values compared with those in Col-0 before and after the high light treatment (Fig. 5D and E), indicating that HY5 might regulate PSII repair after exposure to high light.

It was previously reported that high light-induced damage of the PSII core protein D1 can be rapidly repaired and reassembled to maintain photosynthetic electron transport (Nickelsen and Rengstl, 2013). The defects in PSII activity displayed by the *hy5-215* mutants were possibly caused by a decreasing level

or malfunction of thylakoid protein supercomplexes in the electron transport chain. To further investigate the effects of a lack of functional HY5 on PSII structure and function, we analyzed the accumulation of various thylakoid complexes in WT and *hy5-215* plants. Thylakoid membranes were solubilized in 2% dodecylmaltoside (DM), membrane protein complexes were separated by blue native polyacrylamide gel electrophoresis (BN-PAGE) (Supplemental Fig. S7A), and the complexes were analyzed by immunoblotting with antibodies specific for PSII core proteins. Analysis with antisera against PSII core antenna proteins, anti-CP47, showed that *hy5-215* mutants contain lower levels of the PSII-light harvesting complex II (LHCII) supercomplex, PSII dimer, and PSII core monomer than WT thylakoid membranes, especially after high light treatment (Supplemental Fig. S7C), suggesting that the absence of HY5 may affect the formation and stability of the PSII supercomplex after high light treatment. Moreover, the reduction in PSI monomer in the *hy5-215* mutants was confirmed by immunoblot analysis with anti-PsaD antisera before and after the high light treatment, suggesting that the absence of HY5 may affect PSI monomer formation before and after the high light treatment (Supplemental Fig. S7D).

Next, we determined whether high light-induced damage induced the transcription of PSII assembly- and repair-associated genes. Col-0 and *hy5-215* mutant seedlings were grown for 5 d in growth light conditions and transcript levels were measured by RT-qPCR analysis. The seedlings were transferred to high light conditions for 3 h and gene expression was measured again. The expression levels of *PAM68*, *ALB3*, *HCF136*, *HHL1*, *LQY1*, *FtsH8*, and *DEG1* were dramatically increased in the WT after a 3-h high light treatment in wild type plants, but the increase of their expression levels was also suppressed in the *hy5-215* mutant. The difference in gene expression between the WT and the *hy5-215* mutant was greater under the high light

416 treatment compared to growth light conditions (Fig. 5F; Supplemental Fig.
417 S8A–D). These results suggest that HY5 is required for the functional
418 maintenance of PSII under photodamaging conditions.
419
420 **HY5 Broadly Regulates the Protein Accumulation of Photosystem**
421 **Subunits**
422 D1 protein accumulation was induced after etiolated Arabidopsis seedlings
423 perceived light (Fig. 1C), which prompted us to question whether the D1 protein
424 level was also regulated by HY5 during de-etiolation. Etiolated Col-0 and
425 *hy5-215* mutant seedlings were grown in darkness for five days followed by
426 exposure to W light for 0, 8, 24, and 48 h, and then harvested. Immunoblot
427 analysis showed that D1 also accumulated after light perception in the *hy5-215*
428 mutant, but the D1 protein level was lower in the *hy5-215* mutant than in Col-0
429 (Fig. 6A and B), indicating that D1 accumulation was positively regulated by
430 HY5 during de-etiolation.
431 We also examined if specific wavelengths of light (B, R, and FR) mediated
432 the regulation of D1 protein levels by HY5. Col-0 and the *hy5-215* mutant were
433 grown in continuous B, R, or FR light conditions for five days and then
434 harvested. Immunoblot analysis showed that the D1 protein level was lower in
435 the *hy5-215* mutant than in Col-0 under all specific wavelengths of light tested,
436 indicating that HY5 positively regulates D1 accumulation in response to B, R,
437 and FR light (Supplemental Fig. S3A-C).
438 Next, we examined whether the protein levels of other plastid-encoded and
439 nuclear-encoded photosystem subunits were regulated by HY5 during
440 light-induced greening. Immunoblot analysis indicated that the levels of the
441 PSII proteins D2, CP43, CP47, PsbO, PsbE, and PsbS were dramatically lower
442 in the *hy5-215* mutant than in Col-0 following exposure to light. In addition, the
443 levels of the PSI proteins PsaC and Cytf were also dramatically lower in the

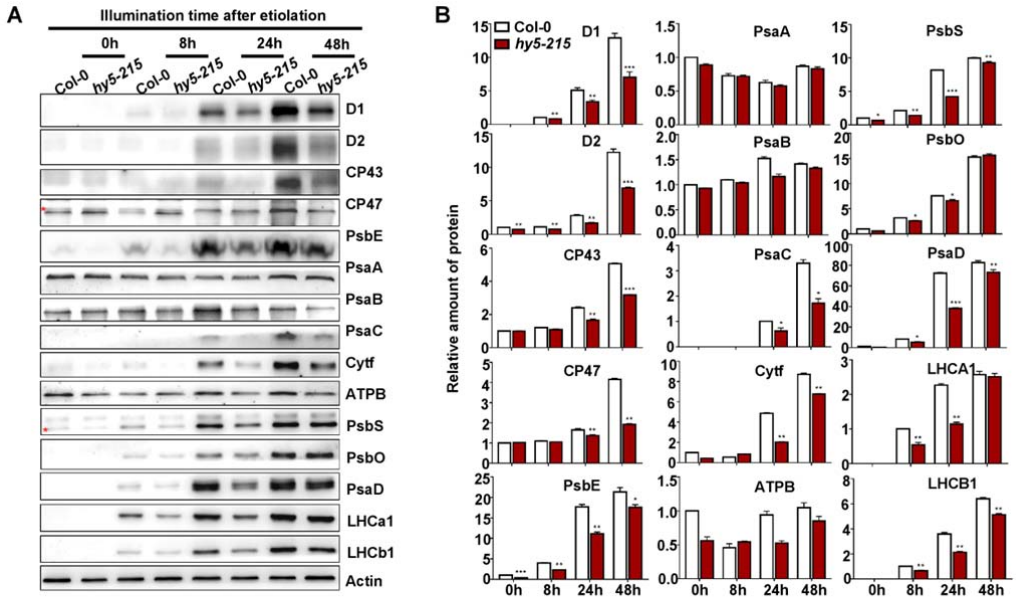

Figure 6. HY5 promotes the synthesis of thylakoid subunits.

(A) Immunoblots showing D1 protein and other photosystem proteins isolated from etiolated WT Col-0 and *hy5-215* mutant seedlings during light-induced greening. The asterisk represent PsbS protein. Anti-Actin was used as a sample loading control.

(B) The relative abundance of PSII proteins was analyzed with Phoretix 1D Software (Phoretix International, UK). Data are represented as mean ± SEM. Asterisks represent statistically significant differences (*P < 0.05; **P < 0.01; ***P < 0.001; Student's t-test). All experiments were repeated at least three times with similar results.

444 *hy5-215* mutant than in Col-0 (Fig. 6A and B). These observations indicate that
445 HY5 systematically and broadly regulates the protein accumulation of
446 photosystem subunits in response to W light-induced greening.
447
448

DISCUSSION

Light is required for photosynthesis and is a key environmental signal regulating the biosynthesis, assembly, and repair of the photosystem (Nickelsen and Rengstl, 2013; Lu, 2016). Photoreceptors, including cryptochromes and phytochrome, sense light and regulate plant growth and development. However, the crosstalk of light signaling and photosystem function remains largely unknown. In this study, we confirmed that cryptochromes and phytochrome cooperatively regulate PSII biogenesis and maintenance of its function through HY5 in Arabidopsis.

PSII plays a critical role in water splitting, oxygen evolution, and plastoquinone reduction. PSII biogenesis refers to the synthesis of its protein subunits and the concerted assembly as well as the incorporation of various auxiliary proteins (Nickelsen and Rengstl, 2013; Lu, 2016). Plastid-encoded PSII proteins are synthesized on thylakoid-bound ribosomes and inserted into the thylakoid membrane, and the core protein D1 is co-translationally incorporated into PSII, not only for D1 assembly but also for photodamage repair (Klein et al. 1988; Keegstra and Cline 1999; Zhang et al. 1999). HCF173, a short-chain dehydrogenases/reductase-like protein, plays critical roles in PSII biogenesis. *HCF173* deficiency results in impaired *psbA* mRNA stability as well as decreased synthesis of the PSII reaction center protein D1 (Schult et al., 2007). We recently revealed that a newly identified regulator of *psbA* mRNA translation, LPE1, could interact with HCF173 to regulate D1 synthesis (Jin et al., 2018). Furthermore, we showed that *HCF173* but not *LPE1* expression was greatly induced by light during de-etiolation (Jin et al., 2018), an observation confirmed in our present study (Fig. 1D). In addition, our data indicated that various monochromatic wavelengths of light, including B, R, and FR light, cooperatively regulate *HCF173* expression, D1 accumulation, and PSII activity (Fig. 1H, L and P, Supplemental Fig. S1; Fig. 1G, K and O; Fig. 1F, J and N).

Moreover, HCF173 expression, D1 accumulation, and PSII activity are positively regulated by HY5 in response to specific wavelengths of light (B, R, and FR light) (Supplemental Fig. S3; Fig. 4B–D), and this redundancy probably ensures the functional maintenance of the photosystem in plants under various light-quality conditions. Partial PSII activity still occurs in mutant plants lacking HY5 (Fig. 4), suggesting that other transcription factors may also regulate PSII function.

Light signal transduction pathways refer to a series of specific photoreceptors that regulate critical developmental processes such as skotomorphogenesis and photomorphogenesis (Chen et al., 2004; Kami et al., 2010; Von Arnim and Deng, 1996). Our results indicated that both cryptochromes and phytochrome influence *HCF173* expression (Fig. 2), suggesting that cryptochromes and phytochrome cooperate to regulate the expression of *HCF173*. As partial PSII activity still occurs in mutants lacking cryptochromes and/or phytochromes (Fig. 2), other wavelengths of light besides R, FR, and B light may also contribute to PSII function. A previous study reported that CRY2 is more quickly degraded in blue light and its role is a bit more complicated than that of CRY1 (Zuo et al., 2012). Our results show that many PSII-related regulatory genes including *HCF173*, *HCF136*, *ALB3*, *DEG1*, *HHL1* and *PAM68* also show higher expression levels in the *cry2* monogenic mutant compared to the wild type after 48 h white light exposure (Fig. 2A, Supplemental Fig. S9A-E), and most of these genes show increased expression after five days of continuous W, B, and R light conditions but deceased expression after five days of continuous FR light conditions (Fig. 2B-E, Supplemental Fig. S9H-L). However, the expression level of *FtsH8* and *LQY1* was much lower in the *cry2* monogenic mutant than in the wild type after 48 h of W light exposure (Supplemental Fig. S9F and G), or five days of continuous W, B, R, and FR light conditions (Supplemental Fig. S9M and N).

These results suggest that CRY2 can act as a positive or negative regulator to regulate different gene expression under different light conditions or during different developmental stages, implying a bit more complicated roles in the regulation of light signaling.

A previous study reported that HY5 constitutively binds to its target sites in both darkness and light and affects the expression of its target genes (Lee et al., 2007; Zhang et al., 2011). COP1 is inactivated upon light irradiation and acts in the dark as an E3 ubiquitin ligase as well as the central repressor of light signaling through ubiquitinating and degrading downstream substrates including HY5 (Lau and Deng, 2012; Huang et al., 2014). Hence, the COP1–HY5 regulatory module plays a central role in the light signal transduction pathway between transcriptional and posttranslational network hubs. However, the role of the COP1-HY5 regulatory module in the functional maintenance of the photosystem was largely unknown. Here, we verified that a variety of wavelengths of light induce *HCF173* expression, but also function through the COP1–HY5 regulatory module (Fig. 2, 3A–E, Supplemental Fig. S2; Supplemental Fig. S4A and C). When grown in darkness, *cop1* mutant seedlings had increased *HCF173* transcript and protein levels, due to the accumulation of HY5, and HY5 directly bound to the ACE motif and G-box *cis*-element present in the promoter of *HCF173*, activating its transcription (Fig. 3K). Light triggered the accumulation of HY5 due to the light-controlled inactivation of COP1 (Supplemental Fig. S4B), and HY5 associated directly with the *HCF173* promoter (Supplemental Fig. S4E). These findings suggest that HY5, a positive regulator of light signaling, affects *HCF173* expression by binding to the *HCF173* promoter *in vivo* both in dark and light conditions, thus providing a transcriptional regulatory mechanism that fine-tunes translational regulator-based PSII biogenesis in response to changing light conditions.

In addition, the induction of *HCF173* expression by light may also be associated with the enrichment of histone H2B ubiquitination in its promotor region (Bourbousse et al., 2012). However, *LPE1*, encoding another regulator of D1 translation, is not obviously regulated by light (Supplemental Fig. S10), which is consistent with the results of a previous study (Jin et al., 2018). Furthermore, we found that the expression of *LPE1* is not affected obviously in the *hy5* mutant compared to the wild type during the light induction process overall, and is only decreased slightly at 8 h (Supplemental Fig. S10), suggesting that *LPE1* is not HY5-dependent. A previous study reported that light triggers D1 synthesis to contribute to PSII biogenesis through inducing LPE1 and *psbA* mRNA association based on a redox mechanism (Jin et al., 2018), suggesting that PSII biogenesis is controlled by dual strategies of transcriptional regulation in the nucleus and translational regulation in the chloroplasts.

Although light energy powers photosynthesis, excessive light causes photoinhibition and damage to the photosynthetic apparatus, especially to PSII (Takahashi and Badger, 2011). D1 is the main target of photodamage and has an unusually high turnover rate. Photodamaged D1 is rapidly removed and replaced by newly synthesized D1 (Mulo et al., 2012). Given that HY5 functions in D1 synthesis, this transcription factor may also function in PSII repair. The decreased photosynthetic efficiency during light-induced greening by various monochromatic wavelengths of light and increased high light-sensitive phenotype in the *hy5-215* mutant support this hypothesis (Fig. 4; Fig. 5D and E). The PSII repair cycle involves the phosphorylation, disassembly, and reassembly of the PSII-LHCII supercomplex from grana stacks to the stroma lamellae. Several assembly and repair factors important for the biogenesis of the PSII core complex have been identified, including HCF136 (Mabbitt et al., 2014), ALB3 (Walter et al., 2015), and PAM68 (Armbruster et al., 2010). Deg

proteases (Schuhmann and Adamska, 2012) and FtsH proteases (Silva et al., 2003; Nixon et al., 2005; Nixon et al., 2010; Huesgen et al., 2009; Kato et al., 2012; Komenda et al., 2012) are involved in the degradation of photodamaged D1. LQY1 (Lu et al., 2011) and HHL1 (Jin et al., 2014) are involved in the repair cycle and reassembly of PSII. We demonstrated that HY5 directly associates with the promoters of assembly- and repair-related genes to regulate their expression (Fig. 5A–C, Supplemental Fig. S6A–C). Furthermore, a deficiency of HY5 results in reduced expression of these genes, particularly after exposure to high light (Fig. 5F; Supplemental Fig. S8), suggesting that HY5 is involved in the functional maintenance of PSII through regulating the expression of PSII assembly- and repair-associated genes. Although HY5 can associate with numerous PSII-related genes, the associated *cis*-acting element varies for different genes. More interestingly, our results showed that HY5 also affects the protein accumulation of other photosynthetic complexes besides PSII (Fig. 6), suggesting that HY5 may act as a hub to control the expression of regulators of other photosynthetic complex-related genes besides PSII.

In summary, our data indicate that various monochromatic wavelengths of light cooperate to regulate the biogenesis and functional maintenance of PSII. The photoreceptors cryptochromes and phytochromes and the transcription factor HY5 are key regulators of PSII function. During de-etiolation, light-induced HY5 directly binds to the promoter of *HCF173* or of other PSII-associated translation factor genes, thereby promoting their expression, the synthesis of PSII subunits, and the biogenesis of the PSII complex. Under high light conditions, light-induced HY5 directly binds to the promoters of PSII repair factor genes, thereby promoting their expression and PSII repair and maintaining efficient photosynthesis (Fig. 7). This study establishes a relationship between PSII function and light signaling mediated by

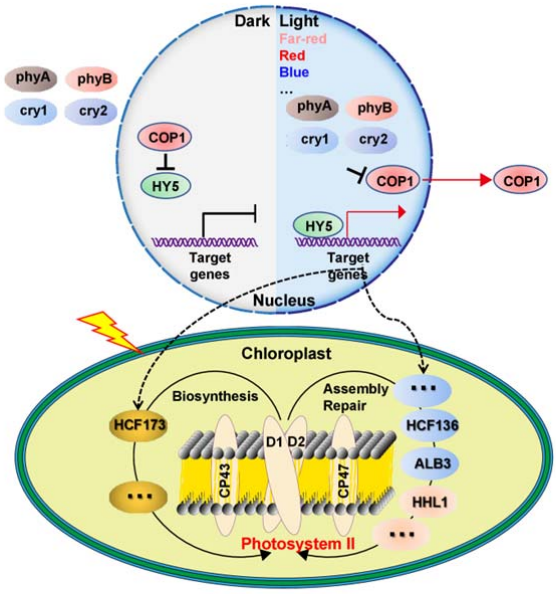

Figure 7. Proposed working model depicting how photoreceptor–HY5 regulatory modules mediate PSII biogenesis and functional maintenance.

During de-etiolation, light-induced HY5 directly binds to the promoter of *HCF173* or of other PSII-associated translation factor genes, thereby accelerating their expression and promoting the synthesis of PSII subunits and the biogenesis of the PSII complex. Under photodamaging conditions, light-induced HY5 directly binds to the promoters of PSII assembly and repair factor-related genes, including *HCF136*, *ALB3*, and *HHL1*, thereby accelerating their expression and promoting PSII assembly and repair and maintaining efficient photosynthesis.

587 photoreceptors and HY5, providing insight into the light-mediated regulation of
588 PSII function in land plants.
589
590

METHODS

Plant Materials and Growth Conditions

The *Arabidopsis thaliana* mutants *phyA-211*, *phyB-9* (Zhang et al., 2017), *cry1-304, cry2* (Liu et al., 2013), *phyA phyB* (Chen et al., 2013), *cry1 cry2* (Liu et al., 2013), *phyA phyB cry1* (Kang et al., 2009), *cry1 cry2 phyA* (Liu et al., 2013), *hy5-215*, and *cop1-4* (Zhang et al., 2017), as well as the *HA-HY5 hy5-215* transgenic line (Heng et al., 2019), are all in the Columbia-0 (Col-0) background. All seeds were surface sterilized with 20% (v/v) bleach for 20 min and sown on ½ Murashige and Skoog (MS) media with 1% (w/v) sucrose and 0.8% (w/v) agar. After vernalization in darkness at 4°C for 2 d, the plates were placed in white (W) light with an irradiance of 100 μmol photons m^{-2} s^{-1}, red (R) light with an irradiance of 44 μmol photons m^{-2} s^{-1}, blue (B) light with an irradiance of 58 μmol photons m^{-2} s^{-1}, or far-red (FR) light with an irradiance of 10 μmol photons m^{-2} s^{-1} at 22°C.

Plasmid Construction

The full-length *HY5* open reading frame was cloned into the BamHI/EcoRI sites of the pGreen II-62-SK vector under the 35S promoter. Then, 297-bp, 664-bp, 1107-bp, 1611-bp, and 1850-bp fragments of the *HCF173* promoter upstream of the ATG start site were amplified by PCR using primer pairs listed in Supplemental Table S1 and then cloned into the HindIII/BamHI sites of the pGreen II 0800-LUC vector.

Chlorophyll Fluorescence Analysis

Chlorophyll fluorescence parameters were measured with the MAXI version of the Imaging-PAM M-Series chlorophyll fluorescence system (Heinz-Walz Instruments). The maximal photochemical efficiency of PSII (F_v/F_m), the kinetics curves of PSII quantum yield (ΦPSII), and the electron transport rate

(ETR) were measured in five-day-old Col-0 and *hy5-215* plants after irradiation with 100 μmol photons m^{-2} s^{-1} under a growth light and a 3-h high light treatment (~1200 μmol photons m^{-2} s^{-1}).

RNA Isolation and RT-qPCR Assays

Total RNA was extracted from Arabidopsis seedlings using an RNAeasy Plant Mini Kit (MAGEN). The RNA samples were reverse transcribed into first strand cDNA using the PrimeScript RT Reagent Kit (TaKaRa). Quantitative PCR was carried out using gene-specific primers, SYBR Premix ExTaq reagent (Takara), and a real-time PCR system (RoChe-LC480). *UBQ4* and *ACTIN2* were used as internal controls. Primer sequences of genes tested in RT-qPCR are listed in Supplemental Table S1.

Thylakoid Membrane Isolation

Thylakoid membranes were prepared as previously described **(**Robinson and Yocum, 1980). Isolated thylakoid membranes were quantified based on total chlorophyll as previously described (Porra et al., 1989). Total proteins extracted from leaf thylakoid membrane preparations were prepared as described (Liu et al., 2012). Protein concentrations were determined using the Bio-Rad detergent-compatible colorimetric protein assay according to the manufacturer's protocol (Bio-Rad).

BN-PAGE and Immunoblot Analyses

BN-PAGE and immunodetection of proteins on a polyvinylidene difluoride (PVDF) membrane were performed as previously described (Schägger et al., 1994) with the modifications described (Peng et al., 2006). For quantification of thylakoid proteins, gels were loaded on an equivalent chlorophyll basis, in amounts ensuring that immunodetection was in the linear range. All primary

antibodies and antisera were raised in rabbits. Antisera against photosynthetic proteins were purchased from Agrisera.

Immunoblot Assay

Total proteins were extracted with protein extraction buffer containing 50 mM Tris-HCl at pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.1% (v/v) Triton X-100, and protease inhibitor cocktail (Roche). The extracts were subsequently centrifuged at 18,000 x g for 10 min at 4°C to collect the supernatants for immunoblot analyses. Total proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, the proteins were transferred to PVDF membranes (Millipore) and probed using specific antibodies. Primary antibodies used in this study were anti-Actin (which were raised in mice), anti-HY5 (which were raised in rabbits), and antisera against photosynthetic proteins (which were raised in rabbits). Antisera against HY5 and photosynthetic proteins were purchased from Agrisera. D1, AS05084; D2, AS06146; CP43, AS111787; CP47, AS10939; PsbE, AS06112; PsbF, AS06113; PsbO, AS05092; PsbS, AS09533; Cytf, AS08306; PsaA, AS06172; PsaB, AS10695; PsaC, AS10939; PsaD, AS09461; LHCa1, AS01005; LHCb1, AS01004; ATPB, AS05085; HY5, AS121867.

LUC Assay

To explore the transcriptional activation of the *HCF173* promoter by HY5, truncated and site-specific mutant sequences of the *HCF173* promoter were each cloned into the pGreen II 0800-LUC vector to generate reporter constructs. Each reporter construct was then co-transformed with the HY5 effector into Col-0 protoplasts for the transcriptional activity assay. The LUC activity was normalized to Renilla luciferase (REN). Firefly and Renilla

674 luciferase signals were assayed using the dual-luciferase assay reagents,
675 according to the manufacturer's instructions (Promega).
676
677 **ChIP Assay**
678 Chromatin was isolated from Col-0 and the *cop1-4* mutant grown under
679 constant dark conditions for five days. Col-0 and *HA-HY5 hy5-215* transgenic
680 plants grown under constant W light for five days and an anti-HA antibody were
681 used to confirm the binding of HY5 to the targeted-gene promoters *in vivo*. The
682 chromatin was sonicated to 250- to 500-bp fragments in an ice-water bath.
683 About 10% of the sonicated chromatin was used as an input DNA control. The
684 sheared chromatin was immunoprecipitated by anti-HY5 (Cat# AS121867,
685 Agrisera) antibody and anti-HA (Cat# H3663, Sigma). Both immunoprecipitated
686 DNA and input DNA were analyzed by RT-qPCR. All primers used for this
687 assay are listed in Supplemental Table S1.
688
689 **EMSA Assays**
690 The HY5 protein was purified from soluble extracts of *cop1-4* mutant plants by
691 immunoprecipitation of anti-HY5 antibody. For labeling of the synthetic
692 nucleotides of the *P3* promoter of *HCF173* DNA from -1225 bp upstream to
693 -1076 bp upstream containing the G-box *cis*-element, the DIG DNA Labeling
694 and Detection Kit (Roche) was employed. The purified HY5 was incubated
695 with DNA probes in binding buffer at room temperature for 30 min. The DNA–
696 protein complexes were separated on 10% native polyacrylamide gels. After
697 electrophoresis, the DNA was transferred onto a nylon membrane. The signals
698 from the labeled DNA were detected using DIG DNA Labeling and Detection
699 Kit (Roche).
700
701 **Statistical Analyses**

For RT-qPCR, CHIP-qPCR, and LUC/REN, Student's *t*-test was used to determine significant differences. * $P < 0.05$, was considered to indicate statistical significance, ** $P < 0.01$, was considered highly significant, and *** $P < 0.001$, was considered extremely significant.

ACCESSION NUMBERS

Sequence data from this article can be found in the Arabidopsis Genome Initiative data library under the following accession numbers: *HY5* (AT5G11260), *HCF173* (AT1G16720), *ALB3* (AT2G28800), *HCF136* (AT5G23120), *HHL1* (AT1G67700), *LQY1* (AT1G75690), *FtsH8* (AT1G06430), *DEG1* (AT3G27925), *PAM68* (AT4G19100), *LPE1* (AT3G46610), *ACTIN* (AT3G18780), and *UBQ10* (AT4G05320).

SUPPLEMENTAL INFORMATION

The following supplemental information is available.

Supplemental Figure S1. The expression of *HCF173* is induced by single wavelengths of light.

Supplemental Figure S2. HY5 regulates the expression of *HCF173* under light-induced greening.

Supplemental Figure S3. HY5 regulates the protein abundance of D1 and HCF173 in continuous B, R, and FR light.

Supplemental Figure S4. HY5 associates with the *HCF173* promoter.

Supplemental Figure S5. Electrophoretic mobility shift assays (EMSAs) containing HY5 protein and *HCF173* promotor probe.

Supplemental Figure S6. HY5 regulates the promoter activity of the genes related to PSII assembly and repair.

Supplemental Figure S7. Immunological analysis of PSII complexes separated by BN-PAGE.

Supplemental Figure S8. Transcript levels of the assembly- and repair factor-related genes in the wild type and the *hy5-215* mutants.

Supplemental Figure S9. Transcript levels of PSII assembly- and repair factor-related genes in the wild type and *cry2* mutant.

Supplemental Figure S10. Transcript levels of *HCF173* and *LPE1* during white light-induced greening in the wild type and *hy5-215* mutant.

Supplemental Table S1. Sequence of Primers Used in this Study.

ACKNOWLEDGMENTS

We are grateful to Prof. Xing Wang Deng from Peking University for kindly providing Arabidopsis *HA-HY5 hy5-215* seeds, Prof. Rongcheng Lin from the Institute of Botany of CAS for kindly providing Arabidopsis mutants *phyA-211*, *phyB-9*, *phyA phyB*, *hy5-215*, and *cop1-4* seeds, Prof. Hongtao Liu from Institute of Plant Physiology and Ecology of CAS for kindly providing *cry1-304*, *cry2*, *cry1 cry2*, and *cry1 cry2 phyA* seeds, and Prof. Hongquan Yang from Shanghai Normal University for kindly providing *phyA phyB cry1* seeds.

FIGURE LEGENDS

Figure 1. Single wavelengths of light regulate PSII activity, as well as D1 and HCF173 protein accumulation during greening.

(A, E, I, M) Schematic representation of the experimental set-up used with white (W), blue (B), red (R), and far-red (FR) light during greening. Five-day-old Arabidopsis Col-0 seedlings were maintained under darkness and were then transferred to different light conditions and harvested at 0, 8, 24, and 48 h for analysis.

(B, F, J, N) False-color images representing the maximal photochemical efficiency of PSII (F_v/F_m) during W, B, R, and FR light-induced greening of etiolated wild-type Arabidopsis seedlings. After growth in darkness for 5 days,

etiolated seedlings were illuminated for 0, 8, 24, or 48 h, and false-color images representing F_v/F_m were captured.

(C, G, K, O) Western blot analysis of D1 proteins isolated from 5-d-old etiolated Arabidopsis Col-0 seedlings during W, B, R, or FR light-induced greening for the indicated periods. Anti-Actin was used as a sample loading control.

(D, H, L, P) RT-qPCR analysis showing the relative expression of *HCF173* after five-day-old etiolated Arabidopsis Col-0 seedlings were transferred to W, B, R, or FR light conditions for the indicated periods.

Data are represented as mean ± SEM. All experiments were repeated at least three times with similar results.

Figure 2. Both cryptochromes and phytochrome influence *HCF173* expression.

(A) RT-qPCR analysis of *HCF173* transcript levels after five-day-old etiolated Col-0 and different photoreceptor mutants were transferred to W light for the indicated periods.

(B–E) Analyses of *HCF173* expression under W, B, R, and FR light conditions in *phyA-211*, *phyB-9*, *cry1-304*, and *cry2* single and higher order mutants under five days of continuous light (Wc, Bc, Rc, and FRc) conditions as indicated. The expression levels were normalized to that of *ACTIN*. Data are represented as mean ± SEM (three biological repeats). Asterisks represent statistically significant differences (*P < 0.05; **P < 0.01; ***P < 0.001; Student's *t*-test). All experiments were repeated at least three times with similar results. We did not perform statistical analyses on those mutants that showed up-regulation of *HCF173* transcripts.

Figure 3. HY5 regulates the transcript level of *HCF173* through directly associating with its promoter.

(A) RT-qPCR analysis of the relative expression of *HCF173* after five-day-old etiolated Col-0 and *hy5-215* mutant seedlings were transferred to W light for the indicated time points. The expression levels were normalized to that of *ACTIN*.

(B–E) RT-qPCR analysis showing the expression levels of *HCF173* in five-day-old Col-0 and *hy5-215* mutant seedlings grown under continuous W, B, R, and FR light conditions.

(F) Schematic representation of various constructs used in the transient transfection assay in Arabidopsis protoplasts. Reporter, A schematic map of the transient expression vector pGreenII-0800-LUC. REN, Renilla luciferase; LUC, firefly luciferase. Effector, Diagram of the HY5 effector of the transient expression vector pGreenII 62-SK.

(G) Diagram representing different variants of the *HCF173* promoter. Part of the coding region of *HCF173* is shown. Positions of the putative G-boxes (blue) and ACE motifs (red) are numbered.

(H) DNA sequences of the wild-type *HCF173* promoter containing the *ACE* motifs (*HCF173P4*) or G-boxes (*HCF173P3*) and mutant *HCF173* promoters harboring a mutant ACE motif (*HCF173P4-m*) or G-box (*HCF173P3-m*).

(I) Effects of HY5 on *HCF173* transcriptional regulation using different *HCF173* promoter variants in Col-0 protoplasts.

(J) The roles of the *ACE* motif or G-box for activity of the *HCF173* promoter regulated by HY5. The protoplasts without the HY5 effector were used as a negative control. The relative LUC activities were normalized to REN activities.

(K) The analyses of association between HY5 and the *HCF173* promoter *in vivo* by ChIP-qPCR assays. Anti-HY5 antibody was used to immunoprecipitate the HY5-HCF173 complex. Col-0 and *UBQ10* were used as negative controls. Data are represented as mean ± SEM. Asterisks represent statistically

significant differences (*P < 0.05; **P < 0.01; ***P < 0.001; Student's t-test). All experiments were repeated at least three times with similar results.

Figure 4. HY5 promoters PSII activity during light-induced greening with various monochromatic wavelengths of light.

Photographs and false-color images representing F_v/F_m during W **(A)**, B **(B)**, R **(C)**, and FR **(D)** light-induced greening of etiolated wild-type and *hy5-215* Arabidopsis seedlings. After growth in darkness for 5 days, etiolated seedlings were illuminated for 0, 8, 24, or 48 h, and photographs and false-color images representing F_v/F_m were captured.

The maximal photochemical efficiency of PSII (F_v/F_m), PSII quantum yield (ΦPSII), and electron transport rate (ETR) of etiolated seedlings during W **(A)**, B **(B)**, R **(C)**, and FR **(D)** light-induced greening are shown below the images. After growth in darkness for 5 days, etiolated seedlings of the wild type and *hy5-215* mutant were illuminated for 0, 8, 24, or 48 h, and F_v/F_m, ΦPSII, and ETR were measured. Data are represented as mean ± SEM. Asterisks represent statistically significant differences (*P < 0.05; **P < 0.01; ***P < 0.001; Student's t-test). All experiments were repeated at least three times with similar results.

Figure 5. HY5 accelerates the expression of PSII assembly- and repair-associated genes and PSII activity.

(A) RT-qPCR analysis showing the relative expression of *ALB3*, *HCF136*, *PAM68*, *HHL1*, *LQY1*, *FtsH8*, and *DEG1* after five-day-old etiolated Col-0 and *hy5-215* mutant seedlings were transferred to W light for the indicated periods. **(B)** Diagram of the promoter structure of *ALB3*, *HCF136*, *PAM68*, *HHL1*, *LQY1*, *FtsH8*, and *DEG1*. Part of the coding region of each gene is shown. Positions of the putative G-box (G, blue), C-box (C, yellow), CG-box (green), ACE-box

(black), or A-boxes (A1, A2, red) fragments amplified in the ChIP-qPCR assay are shown.

(C) ChIP-qPCR results showing that HY5 binds to the promoters of *ALB3*, *HCF136*, *PAM68*, *HHL1*, *LQY1*, *FtsH8*, and *DEG1 in vivo*. Anti-HA antibody was used to immunoprecipitate the above-mentioned genes. Col-0 and *UBQ10* were used as negative controls.

(D) Photographs in (a) are of five-day-old Col-0 and *hy5-215* plants under growth light (12 h light /12h dark) conditions. (b) False-color images representing F_v/F_m under growth light conditions in five-day-old Col-0 and *hy5-215* seedlings. (c) False-color images representing F_v/F_m after a 3-h high light treatment in five-day-old Col-0 and *hy5-215* plants. GL, growth light (~100 μmol photons m^{-2} s^{-1}); HL, high light (~1200 μmol photons m^{-2} s^{-1}).

(E) The maximal photochemical efficiency of PSII (F_v/F_m), PSII quantum yield (ΦPSII), and electron transport rate (ETR) were measured in Col-0 and *hy5-215* seedlings after 5 d of growth under light conditions and again after a 3-h high light treatment.

(F) RT-qPCR analysis showing the relative expression of *ALB3*, *HCF136*, and *HHL1.* After growth for 5 d in growth light conditions (GL), Col-0 and *hy5-215* mutant seedlings were transferred to a 3-h high light (HL) treatment. The gene expression levels were normalized to that of ACTIN. Data are represented as mean ± SEM. Asterisks represent statistically significant differences (*P < 0.05; **P < 0.01; ***P < 0.001; Student's *t*-test). All experiments were repeated at least three times with similar results.

Figure 6. HY5 promotes the synthesis of thylakoid subunits.

(A) Immunoblots showing D1 protein and other photosystem proteins isolated from etiolated WT Col-0 and *hy5-215* mutant seedlings during light-induced greening. Anti-Actin was used as a sample loading control.

868 **(B)** The relative abundance of PSII proteins was analyzed with Phoretix 1D
869 Software (Phoretix International, UK). Data are represented as mean ± SEM.
870 Asterisks represent statistically significant differences (*P < 0.05; **P < 0.01;
871 ***P < 0.001; Student's t-test). All experiments were repeated at least three
872 times with similar results.
873
874 **Figure 7. Proposed working model depicting how photoreceptor–HY5**
875 **regulatory modules mediate PSII biogenesis and functional maintenance.**
876 During de-etiolation, light-induced HY5 directly binds to the promoter of
877 *HCF173* or of other PSII-associated translation factor genes, thereby
878 accelerating their expression and promoting the synthesis of PSII subunits and
879 the biogenesis of the PSII complex. Under photodamaging conditions,
880 light-induced HY5 directly binds to the promoters of PSII assembly and repair
881 factor-related genes, including *HCF136*, *ALB3*, and *HHL1*, thereby accelerating
882 their expression and promoting PSII assembly and repair and maintaining
883 efficient photosynthesis.
884
885

Parsed Citations

Armbruster U, Zuhlke J, Rengstl B, Kreller R, Makarenko E, Ruhle T, Schunemann D, Jahns P, Weisshaar B, Nickelsen J, Leister D (2010). The Arabidopsis thylakoid protein PAM68 is required for efficient D1 biogenesis and photosystem II assembly. Plant Cell, 22: 3439-3460.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ang LH, Chattopadhyay S, Wei N, Oyama T, Okada K, Batschauer A, Deng XW (1998). Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of Arabidopsis development. Mol Cell, 1: 213-222.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Barkan A (2011). Expression of plastid genes: organelle-specific elaborations on a prokaryotic scaffold. Plant Physiol, 155: 1520-1532.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bourbousse C, Ahmed I, Roudier F, Zabulon G, Blondet E, et al. (2012) Histone H2B Monoubiquitination Facilitates the Rapid Modulation of Gene Expression during Arabidopsis Photomorphogenesis. PLoS Genet, 8: e1002825. doi:10.1371/journal.pgen.1002825.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Briggs WR, Christie JM (2002). Phototropins 1 and 2: versatile plant blue-light receptors. Trends Plant Sci, 7: 204-210.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Cashmore AR, Jarillo JA, Wu YJ, Liu D (1999). Cryptochromes: blue light receptors for plants and animals. Science, 284: 760-765.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chen D, Xu G, Tang W, Jing Y, Ji Q, Fei Z, Lin R (2013). Antagonistic basic helix-loop-helix/bZIP transcription factors form transcriptional modules that integrate light and reactive oxygen species signaling in Arabidopsis. Plant Cell, 25: 1657-1673.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chen M, Chory J, Fankhauser C (2004). Light signal transduction in higher plants. Annu Rev Genet, 38: 87-117.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Fromm H, Devic M, Fluhr R, Edelman M (1985). Control of psbA gene expression: in mature Spirodela chloroplasts light regulation of 32-kd protein synthesis is independent of transcript level. EMBO J, 4: 291-295.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gangappa SN, Botto JF (2016). The Multifaceted Roles of HY5 in Plant Growth and Development. Mol Plant, 9: 1353-1365.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Heng Y, Lin F, Jiang Y, Ding M, Yan T, Lan H, Zhou H, Zhao X, Xu D, Deng XW (2019). B-Box Containing Proteins BBX30 and BBX31, Acting Downstream of HY5, Negatively Regulate Photomorphogenesis in Arabidopsis. Plant Physiol, 180: 497-508.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Huang X, Yang P, Ouyang X, Chen L, Deng XW (2014). Photoactivated UVR8-COP1 module determines photomorphogenic UV-B signaling output in Arabidopsis. Plos Genet, 10, e1004218.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Huesgen PF, Schuhmann H, Adamska I (2009). Deg/HtrA proteases as components of a network for photosystem II quality control in chloroplasts and cyanobacteria. Res Microbiol, 160: 726-732.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Jin H, Fu M, Duan Z, Duan S, Li M, Dong X, Liu B, Feng D, Wang J, Peng L (2018). Low photosynthetic efficiency 1 is required for light-regulated photosystem II biogenesis in Arabidopsis. Proc Natl Acad Sci U S A, 115: E6075-E6084.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Jin H, Liu B, Luo L, Feng D, Wang P, Liu J, Da Q, He Y, Qi K, Wang J, Wang HB (2014). HYPERSENSITIVE TO HIGH LIGHT1 interacts with LOW QUANTUM YIELD OF PHOTOSYSTEM II1 and functions in protection of photosystem II from photodamage in Arabidopsis. Plant Cell, 26: 1213-1229.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kami C, Lorrain S, Hornitschek P, Fankhauser C (2010). Light-regulated plant growth and development. Curr Top Dev Biol, 91: 29-66.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kang CY, Lian HL, Wang FF, Huang JR, Yang HQ (2009). Cryptochromes, Phytochromes, and COP1 Regulate Light-Controlled Stomatal Development in "Arabidopsis". Plant Cell, 21: 2624-2641.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kato Y, Sun XW, Zhang LX, Sakamoto W (2012). Cooperative D1 Degradation in the Photosystem II Repair Mediated by Chloroplastic Proteases in Arabidopsis. Plant Physiol, 159: 1428-1439.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Keegstra K, Cline K (1999). Protein import and routing systems of chloroplasts. Plant Cell, 11: 557-570.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Klein RR, Mason HS, Mullet JE (1988). Light-regulated translation of chloroplast proteins. I. Transcripts of psaA-psaB, psbA, and rbcL are associated with polysomes in dark-grown and illuminated barley seedlings. J Cell Biol, 106: 289-301.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Komenda J, Sobotka R, Nixon PJ (2012). Assembling and maintaining the Photosystem II complex in chloroplasts and cyanobacteria. Curr Opin Plant Biol, 15: 245-251.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Krupinska K, Apel K (1989). Light-induced transformation of etioplasts to chloroplasts of barley without transcriptional control of plastid gene expression. Mol Genet Genomics, 219: 467-473.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lau OS, Deng XW (2012). The photomorphogenic repressors COP1 and DET1: 20 years later. Trends Plant Sci, 17: 584-593.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

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

Leivar P, Quail PH (2011). PIFs: pivotal components in a cellular signaling hub. Trends Plant Sci, 16: 19-28.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Liere K, Börner T (2007). Transcription and transcriptional regulation in chloroplasts. In R Bock, ed, Cell and Molecular Biology of Plastids. Springer-Verlag, Heidelberg, pp 121–174.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Liu H, Wang Q, Liu Y, Zhao X, Imaizumi T, Somers DE, Tobin EM, Lin C (2013). Arabidopsis CRY2 and ZTL mediate blue-light regulation of the transcription factor CIB1 by distinct mechanisms. Proc Natl Acad Sci U S A, 110: 17582-17587.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Liu J, Yang H, Lu Q, Wen X, Chen F, Peng L, Zhang L, Lu C (2012). PSBP-DOMAIN PROTEIN1, a Nuclear-Encoded Thylakoid Lumenal Protein, Is Essential for Photosystem I Assembly in Arabidopsis. Plant Cell, 24: 4992-5006.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Loll B, Kern J, Saenger W, Zouni A, Biesiadka J (2005). Towards complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II. Nature, 438: 1040-1044.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lu Y (2011). The occurrence of a thylakoid-localized small zinc finger protein in land plants. Plant Signal Behav, 6: 1881-1885.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lu Y (2016). Identification and Roles of Photosystem II Assembly, Stability, and Repair Factors in Arabidopsis. Front Plant Sci, 7: 168.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ma L, Li J, Qu L, Hager J, Chen Z, Zhao H, Deng XW (2001). Light control of Arabidopsis development entails coordinated regulation of

genome expression and cellular pathways. Plant Cell, 13: 2589-2607.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mabbitt PD, Wilbanks SM, Eaton-Rye JJ (2014). Structure and function of the hydrophilic Photosystem II assembly proteins: Psb27, Psb28 and Ycf48. Plant Physiol Biochem, 81: 96-107.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Malnoe P, Mayfield SP, Rochaix JD (1988). Comparative analysis of the biogenesis of photosystem II in the wild-type and Y-1 mutant of Chlamydomonas reinhardtii. J Cell Biol, 106: 609-616.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mulo P, Sirpio S, Suorsa M, Aro EM (2008). Auxiliary proteins involved in the assembly and sustenance of photosystem II. Photosynth Res, 98: 489-501.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mulo P, Sakurai I, Aro EM (2012). Strategies for psbA gene expression in cyanobacteria, green algae and higher plants: from transcription to PSII repair. Biochim Biophys Acta, 1817: 247-257.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nickelsen J, Rengstl B (2013). Photosystem II assembly: from cyanobacteria to plants. Annu Rev Plant Biol, 64: 609-635.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nixon PJ, Barker M, Boehm M, de Vries R, Komenda J (2005). FtsH-mediated repair of the photosystem II complex in response to light stress. J Exp Bot, 56: 357-363.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nixon PJ, Michoux F, Yu J, Boehm M, Komenda J (2010). Recent advances in understanding the assembly and repair of photosystem II. Ann. Bot. 106: 1-16.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Osterlund MT, Hardtke CS, Wei N, and Deng XW (2000). Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature, 405: 462-466.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Oyama T, Shimura Y, Okada K (1997). The Arabidopsis HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. Gene Dev, 11: 2983-2995.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Peng L, Ma J, Chi W, Guo J, Zhu S, Lu Q, Lu C, Zhang L (2006). LOW PSII ACCUMULATION1 is involved in efficient assembly of photosystem II in Arabidopsis thaliana. Plant Cell, 18: 955-969.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Porra RJ, Thompson WA, Kriedemann PE (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta, 975: 0-394.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Quail PH (2002) Photosensory perception and signalling in plant cells: new paradigms? Curr Opin Cell Biol, 14: 180–188.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Rizzini L. et al. (2011) Perception of UV-B by the Arabidopsis UVR8 protein. Science, 332: 103–106.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Robinson HH, Yocum CF (1980). Cyclic photophosphorylation reactions catalyzed by ferredoxin, methyl viologen and anthraquinone sulfonate. Use of photochemical reactions to optimize redox poising. BBA Bioenergetics, 590: 97-106.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Saleh A, Alvarez-Venegas R, Avramova Z (2008). An efficient chromatin immunoprecipitation (ChIP) protocol for studying histone modifications in Arabidopsis plants. Nat Protoc, 3: 1018–1025.
Pubmed: Author and Title

Google Scholar: Author Only Title Only Author and Title

Schagger H, Cramer WA, Vonjagow G (1994). Analysis of Molecular Masses and Oligomeric States of Protein Complexes by Blue Native Electrophoresis and Isolation of Membrane Protein Complexes by Two-Dimensional Native Electrophoresis. Anal Biochem, 217: 220-230.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Schuhmann H, Adamska I (2012). Deg proteases and their role in protein quality control and processing in different subcellular compartments of the plant cell. Physiol Plantarum, 145: 224-234.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Schult K, Meierhoff K, Paradies S, Toller T, Wolff P, Westhoff P (2007). The Nuclear-Encoded Factor HCF173 Is Involved in the Initiation of Translation of the psbA mRNA in Arabidopsis thaliana. Plant Cell, 19: 1329-1346.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Silva P, Thompson E, Bailey S, Kruse O, Mullineaux CW, Robinson C, Mann NH, Nixon PJ (2003). FtsH is involved in the early stages of repair of photosystem II in Synechocystis sp PCC 6803. Plant Cell, 15: 2152-2164.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Takahashi S, and Badger, MR (2011). Photoprotection in plants: a new light on photosystem II damage. Trends Plant Sci, 16: 53-60.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Timmis JN, Ayliffe MA, Huang CY, Martin W (2004). Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. Nat Rev Genet, 5: 123-135.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Von Arnim A, Deng XW (1996). Light Control of Seedling Development. Annu Rev Plant Physiol Plant Mol Biol, 47: 215-243.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Walter B, Hristou A, Nowaczyk MM, Schunemann D (2015). In vitro reconstitution of co-translational D1 insertion reveals a role of the cpSec-Alb3 translocase and Vipp1 in photosystem II biogenesis. Biochem J, 468: 315-324.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhang H, He H, Wang X, Wang X, Yang X, Li L, Deng XW (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.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhang L, Paakkarinen V, Wijk KJ, Van, Aro EM (1999). Co-translational assembly of the D1 protein into photosystem II. J Biol Chem, 274: 16062-16067.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhang X, Shang F, Huai J, Xu G, Tang W, Jing Y, Lin R (2017). A PIF1/PIF3-HY5-BBX23 Transcription Factor Cascade Affects Photomorphogenesis. Plant Physiol, 174: 2487-2500.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zouni A, Witt HT, Kern J, Fromme P, Krauss N, Saenger W, Orth P (2001). Crystal structure of photosystem II from Synechococcus elongatus at 3.8 Å resolution. Nature, 409: 739-743.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zuo ZC, Meng YY, Yu XH, Zhang ZL, Feng DS, Sun SF, Liu B, Lin CT (2012). A study of the blue-light-dependent phosphorylation, degradation, and photobody formation of Arabidopsis CRY2. Mol Plant, 5: 726-733.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title