Light Signaling-Dependent Regulation of PSII Biogenesis and Functional Maintenance

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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 PSII function in Arabidopsis (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.

Chloroplasts and nonphotosynthetic 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: PSII, cytochrome b6f, 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; Malnoë 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 EFFICIENCY1 (LPE1), a nuclear-encoded chloroplast-targeted pentatrichopeteptide 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 (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 UV-B light receptor UV RESISTANCE LOCUS8 (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 proteins photochrome-interacting factors (Leivar and Quail, 2011) and the bZIP protein ELONGATED HYCOTYL5 (HY5; Osterlund et al., 2011). Photoreceptors transmit light signals to downstream transcription factors, such as the basic helix-loop-helix proteins photochrome-interacting factors (Leivar and Quail, 2011) and the bZIP protein ELONGATED HYOCOTYL5 (HY5; Osterlund et al., 2011), 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 PHOTOMORPHOCGENIC1 (COP1) is enriched in the nucleus in D 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 expression are well understood, the relationship between light signaling pathways and PSII function remains largely unknown.

In this study, we demonstrate that the light signaling pathway is required for regulation of PSII biogenesis and functional maintenance. We found that various monochromatic wavelengths of light cooperate to regulate PSII function in Arabidopsis. Photoreceptor-mediated light signaling regulates PSII function, including PSII biogenesis, assembly, and repair, through the transcription factor HY5. HY5 directly binds to the promoter of genes related to PSII assembly and repair, which is required for the functional maintenance of PSII.

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 Columbia-0 (Col-0) seedlings were grown in D for 5 d, transferred to W light (100 μmol photons m⁻² s⁻¹), B light (58 μmol photons m⁻² s⁻¹), R light (44 μmol photons m⁻² s⁻¹), or FR light (10 μmol photons m⁻² s⁻¹) for 48 h (Fig. 1, A, 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. 1, B and C), both PSII activity and D1 protein levels increased substantially following exposure to all of the specific wavelengths of light (Fig. 1, F, G, J, K, N, 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 expression 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 2-d 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. 1, D, G, J, K, N, and O). 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 5 d 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 CRYs and PHYs Influence HCF173 Expression

To examine whether photoreceptors regulate HCF173 expression, we quantified HCF173 transcript levels in Col-0 and the PHY and CRY single, double, and triple mutants. The seedlings were grown in the D condition for 5 d 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

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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 PHY and CRY during light induction and that CRY2 has a minor role in this process.

To confirm this, we quantified HCF173 transcript levels in wild-type Col-0, CRY mutants, and PHY mutants grown under continuous W light for 5 d. After induction by W light, HCF173 expression in phyA, phyB, cry1, or cry2 single mutants and a phyA phyB phyA 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 expression was nearly abolished in the cry1 cry2 phyA and phyA phyB cry1 triple mutants (Fig. 2B), further supporting that both PHY and CRY 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 wild-type Col-0, CRY mutants, and PHY mutants grown under continuous B, R, or FR light for 5 d. 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 phyA double mutant than in Col-0. The

Figure 1. Single wavelengths of light regulate PSII activity, as well as D1 and HCF173 protein accumulation during greening. A, E, I, and M, Schematic representation of the experimental setup used with W, B, R, and 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, and N, False-color images representing the maximal photochemical efficiency of PSII (Fv/Fl) during W, B, R, and FR light-induced greening of etiolated wild-type Arabidopsis seedlings. After growth in darkness for 5 d, etiolated seedlings were illuminated for 0, 8, 24, or 48 h, and false-color images representing Fv/Fl were captured. C, G, K, and 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, and P, RT-qPCR analysis showing the relative expression of HCF173 after 5-d-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 5-d-old etiolated Col-0 and different photoreceptor mutants were transferred to W light for the indicated periods. B to 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 5 d 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 using Student’s t test (*P < 0.05; **P < 0.01; and ***P < 0.001). 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.

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 wild-type Col-0 when grown in continuous W, B, R, or FR light conditions for 5 d (Fig. 3, B–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 wild-type Col-0 and hy5-215 mutant seedlings were grown in D for 5 d, 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. S2, A and B). We further examined whether HY5 affects HCF173 accumulation after induction by B, R, or FR light. The wild-type Col-0 and hy5-215 mutant seedlings were grown under continuous B, R, or FR light for 5 d, 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. S3, A–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

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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 PHY and CRY photoreceptors.
We thus analyzed the effect of HY5 on the activity of the \textit{HCF173} promoter using the dual-luciferase (LUC) reporter system in Arabidopsis Col-0 protoplasts (Fig. 3, F and G). The truncated promoter fragments (\textit{HCF173PA-LUC}, \textit{HCF173PB-LUC}, \textit{HCF173PC-LUC}, and \textit{HCF173PD-LUC}) and full-length promoter (\textit{HCF173P-LUC}) were transiently expressed in protoplasts with or without the HY5 effector. HY5 increased the activity of \textit{HCF173PA/PB/PC/PD/P-LUC} approximately 1.5- to 2.2-fold, and the activity of the \textit{HCF173} promoter fragments containing the ACE (\textit{HCF173PB}) motif and G-box (\textit{HCF173PC}) element were more significantly increased by HY5 (Fig. 3I). An analysis using point mutations that specifically disrupted the \textit{P3} G-box or the \textit{P4} ACE motif (Fig. 3J) revealed that the \textit{P4} ACE motif and \textit{P3} G-box in the \textit{HCF173} promoter are required for HY5 binding.
mutant accumulates more HY5 protein than the wild type (Supplemental Fig. S4B), we used etiolated *cop1-4* seedlings grown under continuous D for 5 d in this analysis. HCF173 protein accumulation and HCF173 transcript levels were markedly higher in the *cop1-4* mutant than in Col-0 (Supplemental Fig. S4, A 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 UBQUITIN10 (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 containing the ACE motif (P4) and G-box (P3) of the HCF173 promoter (Supplemental Fig. S4, D and E).

To determine whether HY5 directly binds to the promoter of HCF173, we performed electrophoretic mobility shift assays (EMSAs). ChiP assays indicated that HY5 preferably binds to the P3 region of the HCF173 promoter (Fig. 3K). Thus, we chose the P3 region of the HCF173 promoter containing the G-box cis-element as the DNA probe. We purified the HY5 proteins from Arabidopsis plants by immunoprecipitation using HY5 antibody. The purified HY5 proteins were incubated with the HCF173 promoter DNA probe. The results showed that the HY5-DNA complex was detected as a band that migrated more slowly than the free probe in the gel; increasing retardation of the band was detected as the amount of HY5 proteins was increased (Supplemental Fig. S5). The association of HY5 with the HCF173 promoter DNA probe was also confirmed by competition experiments with an unlabeled HCF173 promoter DNA probe (Supplemental Fig. S5), indicating that HY5 directly binds to the HCF173 promoter. Together, these data suggest that HY5 directly binds to the promoter of HCF173 and regulates its expression.

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

Analyses of chlorophyll fluorescence parameters indicated that single wavelengths of light can induce PSII activity. To determine whether HY5 is involved in the functional maintenance of PSII during induction with various monochromatic wavelengths of light, we established an induction system using various monochromatic wavelengths of light to control PSII biogenesis in Arabidopsis. After 5 d of growth in the D condition, etiolated seedlings were exposed to W, B, R, and FR light for 0, 8, 24, or 48 h. The leaves of wild-type seedlings gradually turned green when exposed to increasing periods of W and other specific wavelengths of light (Fig. 4), and chlorophyll fluorescence parameters, including the $F_v/F_m$, the kinetics curves of PSII quantum yield (ΦPSII), and the electron transport rate (ETR), increased simultaneously (Fig. 4). 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. 4). 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, ALBINO3 (ALB3), and PHOTOSYNTHESIS AFFECTED MUTANT68 (PAM68), was lower in the *hy5-215* mutant than in the wild type during W light-induced greening (Fig. 5A). The expression of HYPERSENSITIVE TO HIGH LIGHT1 (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 HHLL1 is positively regulated by HY5 (Fig. 5A). In addition, the expression of a gene encoding a chloroplast protease, FILAMENTATION-TEMPERATURE-SENSITIVE PROTEIN H8 (FtsH8), involved in the degradation of photodamaged D1, was lower in the *hy5-215* mutant than in the wild type 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 HHLL1 promoter region containing a CG-box, an A-box, and a G-box (Fig. 5, B and C), the PAM68 promoter region containing an ACE-box, the LOW QUANTUM YIELD OF PHOTOSYSTEM II (LQY1) promoter region containing two A-boxes and a G-box, the FtsH8 promoter region containing an A-box, and the DEGRADATION OF PERIPLASMIC PROTEINS1 (DEG1) promoter region containing an A-box (Fig. 5, B 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 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 5-d-old Col-0 seedlings had a higher Fv/Fm in growth light conditions 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. After a short-term high-light treatment (3 h), Col-0 and hy5-215 seedlings had a much lower Fv/Fm and ETR than after a 3-h treatment under growth light conditions. Furthermore, hy5-215 seedlings had significantly reduced Fv/Fm, ΦPSII, and ETR values compared with those in Col-0 before and after the high-light treatment (Fig. 5, D 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 wild-type and hy5-215 plants. Thylakoid membranes were solubilized in 2%...
dodecylmaltoside, membrane protein complexes were separated by blue native PAGE (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 wild-type 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 wild type after a 3-h high-light treatment, but the increase of their expression levels was also suppressed in the hy5-215 mutant. The difference in gene expression between the wild type and the hy5-215 mutant was greater under the high-light treatment compared to growth light conditions.
These results suggest that HY5 is required for the functional maintenance of PSII under photodamaging conditions.

**HY5 Broadly Regulates the Protein Accumulation of Photosystem Subunits**

D1 protein accumulation was induced after etiolated Arabidopsis seedlings perceived light (Fig. 1C), which prompted us to question whether the D1 protein level was also regulated by HY5 during de-etiolation. Etiolated Col-0 and \( hy5-215 \) mutant seedlings were grown in D for 5 d followed by exposure to W light for 0, 8, 24, and 48 h, and then harvested. Immunoblot analysis showed that D1 also accumulated after light perception in the \( hy5-215 \) mutant, but the D1 protein level was lower in the \( hy5-215 \) mutant than in Col-0 (Fig. 6), indicating that D1 accumulation was positively regulated by HY5 during de-etiolation.

We also examined if specific wavelengths of light (B, R, and FR) mediated the regulation of D1 protein levels by HY5. Col-0 and the \( hy5-215 \) mutant were grown in continuous B, R, or FR light conditions for 5 d and then harvested. Immunoblot analysis showed that the D1 protein level was lower in the \( hy5-215 \) mutant than in Col-0 under all specific wavelengths of light tested, indicating that HY5 positively regulates D1 accumulation in response to B, R, and FR light (Supplemental Fig. S3, A–C).

Next, we examined whether the protein levels of other plastid-encoded and nuclear-encoded photosystem subunits were regulated by HY5 during light-induced greening. Immunoblot analysis indicated that the levels of the PSII proteins D2, CP43, CP47, PsbO, PsbE, and PsbS were dramatically lower in the \( hy5-215 \) mutant than in Col-0 following exposure to light. In addition, the levels of the PSI proteins PsaC and Cytf were also dramatically lower in the \( hy5-215 \) mutant than in Col-0 (Fig. 6). These observations indicate that HY5 systematically and broadly regulates the protein accumulation of photosystem subunits in response to W light-induced greening.

**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 CRYs and PHYs, sense light and regulate plant growth...
and development. However, the cross talk of light signaling and photosystem function remains largely unknown. In this study, we confirmed that CRYs and PHYs 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 cotranslationally 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 this 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. 1, F–H, J–L, and N–P; Supplemental Fig. S1). 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; Fig. 4, B–D; Supplemental Fig. S3), 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 (Von Arnim and Deng, 1996; Chen et al., 2004; Kami et al., 2010). Our results indicate that both CRYs and PHYs influence HCF173 expression (Fig. 2), suggesting that CRYs and PHYs cooperate to regulate the expression of HCF173. As partial PSII activity still occurs in mutants lacking CRY1 and/or PHYs (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 B light, and its role is a bit more complicated than that of CRY1 (Zao 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 W light exposure (Fig. 2A; Supplemental Fig. S9, A–E), and most of these genes show increased expression after 5 d of continuous W, B, and R light conditions but decreased expression after 5 d of continuous FR light conditions (Fig. 2, B–E; Supplemental Fig. S9, H–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. S9, F and G) or 5 d of continuous W, B, R, and FR light conditions (Supplemental Fig. S9, M 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 D 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 (Figs. 2 and 3, A–E; Supplemental Figs. S2 and S4, A and C). When grown in D, 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 promoter 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 (Figs. 4 and 5, D 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, 2010; Huesgen et al., 2009; Kato et al., 2012; Komenda et al., 2012) are involved in the degradation of photodamaged D1. LQY1 (Lu, 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. 5, A–C; Supplemental Fig. S6, A–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 show 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 CRYs and PHYs 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 photoreceptors and HY5, providing insight into the light-mediated regulation of PSII function in land plants.

MATERIALS AND METHODS

Plant Materials and Growth Conditions
The Arabidopsis (Arabidopsis thaliana) mutants phyA-211, phyB-9 (Zhang et al., 2017), cry1-304, cry2 (Liu et al., 2013), phyA phyB (Chen et al., 2015), cry1 cry2 (Liu et al., 2013), phyA phyB cry1 (Kang et al., 2009), cry1 cry2 phyE (Liu et al., 2013), hsp-215, and cry1-4 (Zhang et al., 2017), as well as the HA-HY5:hsp-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 one-half strength Murashige and Skoog media with 1% (w/v) Suc and 0.8% (w/v) agar. After vernalization in D at 4°C for 2 d, the plates were placed in W light with an irradiance of 100 μmol photons m⁻² s⁻¹. R light with an irradiance of 44 μmol photons m⁻² s⁻¹, B light with an irradiance of 58 μmol photons m⁻² s⁻¹, or 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 ResH1/EcoRI sites of the pGreen II 62-SK vector under the 35S promoter. Then, 297-, 664-, 1107-, 1611-, 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/ResHI 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 (Heine-Walz Instruments). The Fv/Fm, ΦPSII, and the ETR were measured in 5-d-old Col-0 and hsp-215 plants after irradiation with 100 μmol photons m⁻² s⁻¹ under a growth light and a 3-h light-dark treatment (∼1200 μmol photons m⁻² s⁻¹).

RNA Isolation and RT-qPCR Assays
Total RNA was extracted from Arabidopsis seedlings using an RNAasy mini plant mini kit (MAGEN). The RNA samples were reverse transcribed into first strand cDNA using the PrimeScript RT reagent kit (TaKaRa). qPCR was carried out using gene-specific primers, SYBR Premix ExTaq reagent (Takara), and a real-time PCR system (Roche-LC480). LBD4 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).

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 cotransformed with the HY5 effector into Col-0 protoplasts for the transcriptional activity assay. The LUC activity was normalized to Benillia luciferase (REN). Firefly and REN signals were assayed using dual-luciferase assay reagents, according to the manufacturer’s instructions (Promega).

ChIP Assay
Chromatin was isolated from Col-0 and the cry1-4 mutant grown under constant dark conditions for 5 d. Col-0 and HA-HY5 hsp-215 transgenic plants grown under constant W light for 5 d and an anti-HA antibody were used to confirm the binding of HY5 to the targeted gene promoters in vivo. The chromatin was sonicated to 250- to 500-bp fragments in an ice-water bath. About 10% of the sonicated chromatin was used as an input DNA control. The sheared chromatin was immunoprecipitated by anti-HY5 (catalog no. ASI12167, Agrisera) antibody and anti-HA (catalog no. H3636, Sigma). Both immunoprecipitated DNA and input DNA were analyzed by RT-qPCR. All primers used for this assay are listed in Supplemental Table S1.

EMSA Assays
The HY5 protein was purified from soluble extracts of cry1-4 mutant plants by immunoprecipitation of anti-HY5 antibody. For labeling of the synthetic nucleotides of the F3 promoter of HCF173 DNA from ∼1225 bp upstream to ∼1076 bp upstream containing the G-box cis-element, the DIG DNA labeling and detection kit (Roche) was employed. The purified HY5 was incubated with DNA probes in binding buffer at room temperature for 30 min. The DNA-protein complexes were separated on 10% native polyacrylamide gels. After electrophoresis, the DNA was transferred onto a nylon membrane. The signals from the labeled DNA were detected using the DIG DNA labeling and detection kit (Roche).

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.

BN-PAGE and Immunoblot Analyses
BN-PAGE and immunodetection of proteins on a polyvinylidene difluoride membrane were performed as previously described in Schägger et al. (1994) with the modifications as described in 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 of Tris-HCl at pH 7.5, 150 mM of NaCl, 5 mM of EDTA, 0.1% (v/v) Triton X-100, and protease inhibitor cocktail (Roche). The extracts were subsequently centrifuged at 18,000 for 10 min at 4°C to collect the supernatants for immunoblot analyses. Total proteins were separated by SDS-PAGE. After electrophoresis, the proteins were transferred to polyvinylidene difluoride membranes (Millipore) and probed using specific antibodies. Primary antibodies used in this study were anti-Actin (which were raised in mouse), 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 as follows: D1, AS50584; D2, AS60146; CP43, AS111787; CP47, AS10939; PsbE, AS60112; PsbF, AS06113; PsbO, AS05092; PsbS, AS05933; Cryf, AS08306; PsaA, AS06172; PsaB, AS10695; PsaC, AS10939; PsaD, AS09461; LHCa1, AS01005; LHCb1, AS01004; ATPB, AS05085; HY5, AS121867; Actin, AS163141.

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Regulation of PSII Function by Light Signaling

Accession Numbers
Sequence data from this article can be found in the Arabidopsis Genome Initiative data library under the following accession numbers: HYS (AT5G1260), HCF173 (AT1G16720), ALB3 (AT2G28800), HCF136 (AT5G23120), HHL1 (AT1G87000), LQY1 (AT1G75960), FhsHB (AT1G86340), DEG1 (AT5G2925), PAM68 (AT1G19100), LPE1 (AT3G46610), ACTIN (AT3G16780), and UBQ10 (AT4G03520).

Supplemental Information
The following supplemental materials are available.

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

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

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

Supplemental Figure S4. HYS associates with the HCF173 promoter.

Supplemental Figure S5. EMSAs containing HYS protein and HCF173 promoter probe.

Supplemental Figure S6. HYS regulates the protein 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 cry2 mutant.

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

Supplemental Table S1. Sequence of primers used in this study.

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