Light and Abscisic Acid Coordinately Regulate Greening of Seedlings[1][OPEN]

Di Xu,[a] Di Wu,[a,2] Xiao-han Li,[a,2] Yu'e Jiang,[a,2] Tian Tian,[a] Qingshuai Chen,[a,b] Lin Ma,[a,c] Haiyang Wang,[d] Xing Wang Deng,[e] and Gang Li[a,3,4]

[a]State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, Tai'an 271018, China
[b]Shandong Provincial Key Laboratory of Biophysics, Institute of Biophysics, Dezhou University, Dezhou 253023, China
[c]School of Biological Science and Technology, University of Jinan, Jinan 250022, China
[d]College of Life Sciences, State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, South China Agricultural University, Guangzhou 510642, China
[e]State Key Laboratory of Protein and Plant Gene Research, the Peking-Tsinghua Center for Life Sciences, School of Advanced Agricultural Sciences and School of Life Sciences, Peking University, Beijing 100871, China

ORCID IDs: 0000-0002-9665-8178 (D.X.); 0000-0003-3313-418X (T.T.); 0000-0001-8471-9036 (Q.S.C.); 0000-0001-9499-5878 (L.M.); 0000-0002-1302-5747 (H.Y.W.); 0000-0001-8709-1467 (X.W.D.); 0000-0002-0085-3252 (G.L.).

The greening of etiolated seedlings is crucial for the growth and survival of plants. After reaching the soil surface and sunlight, etiolated seedlings integrate numerous environmental signals and internal cues to control the initiation and rate of greening thus to improve their survival and adaption. However, the underlying regulatory mechanisms by which light and phytohormones, such as abscisic acid (ABA), coordinately regulate greening of the etiolated seedlings is still unknown. In this study, we showed that Arabidopsis (*Arabidopsis thaliana*) DE-ETIOLATED1 (DET1), a key negative regulator of photomorphogenesis, positively regulated light-induced greening by repressing ABA responses. Upon irradiating etiolated seedlings with light, DET1 physically interacts with FAR-RED ELONGATED HYPOCOTYL3 (FHY3) and subsequently associates to the promoter region of the FHY3 direct downstream target *ABA INSENSITIVE5* (*ABI5*). Further, DET1 recruits HISTONE DEACETYLASE6 to the locus of the *ABI5* promoter and reduces the enrichments of H3K27ac and H3K4me3 modification, thus subsequently repressing *ABI5* expression and promoting the greening of etiolated seedlings. This study reveals the physiological and molecular function of DET1 and FHY3 in the greening of seedlings and provides insights into the regulatory mechanism by which plants integrate light and ABA signals to fine-tune early seedling establishment.

Light serves as one of the most important environmental signals to modulate diverse aspects of plant growth and development. In most species, when a seed germinates in dark conditions (e.g. in soil), it exhibits an etiolated development, including an elongated hypocotyl, folded apical hook, and tightly closed cotyledons without chlorophyll biosynthesis. Once the etiolated seedling reaches the soil surface and perceives sunlight, light-induced photomorphogenesis is initiated, including inhibiting the elongation of the hypocotyl, opening the tightly closed cotyledons, and activating chlorophyll production, so the seedling turns green (Gommers and Monte, 2018). In natural conditions, when the etiolated seedlings grow close to or out of the soil surface, the dramatic environmental changes, including reduced moisture, increased temperature, and light intensity, all could affect photomorphogenesis. These external environmental changes mostly act through internal auxin, ethylene, cytokinin, abscisic acid (ABA), and salicylic acid signaling pathways to fine-tune the photomorphogenesis of etiolated seedlings and subsequently increase the acclimatization and survival of plants (Zhong et al., 2009; Guan et al., 2014; Abbas et al., 2015; Riber et al., 2015; Zhang et al., 2016, 2018; Xu et al., 2018; Yang et al., 2018; Huang et al., 2020). Although ABA has been known to play an essential role in seed germination and early seedling establishment (Chen et al., 2008, 2020; Tang et al., 2013; Fernando and Schroeder, 2015), the regulatory mechanisms

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[2]These authors contributed equally to this article.
[3]Author for contact: gangli@sdau.edu.cn.
[4]Senior author.
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Gang Li (gangli@sdau.edu.cn).
D.X. performed most of the experiments; D.W. identified the germination and root inhibition phenotypes; X.-H.L., and Y.E.J. identified the mutants and transgenic lines; T.T., Q.S.C., and L.M. analyzed the data; H.Y.W., X.W.D., and G.L. designed the experiments; D.X. and G.L. wrote the article.
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by which light and ABA coordinately regulate the greening of the etiolated seedlings remain largely unknown.

The Arabidopsis (*Arabidopsis thaliana*) transcription factor FAR-RED ELONGATED HYPOCOTYL3 (FHY3) was originally identified in the phytochrome A-mediated far-red light signaling pathway (Lin et al., 2007; Wang and Wang, 2015; Ma and Li, 2018). FHY3 also acts as a crucial regulator of multiple cellular processes, including the circadian clock, chloroplast division, chlorophyll biosynthesis, ABA and stress responses, oxidative stress and cell death, shade avoidance responses, leaf senescence, and plant defense (Li et al., 2011; Ouyang et al., 2011; Tang et al., 2012, 2013; Ma et al., 2016, 2017, 2019; Liu et al., 2019, 2020; Tian et al., 2020). FHY3 positively regulates chlorophyll biosynthesis by activating the transcription of *HEMB1*, which encodes 5-aminolevulinic acid dehydratase, and mutation of *FHY3* decreased the accumulation of Pchlide and subsequently promoted the greening of etiolated seedlings (Tang et al., 2012). Moreover, FHY3 positively regulates ABA and stress responses by directly activating the transcription of *ABA INSENSITIVE5* (*ABI5*), which encodes a crucial transcription factor in the ABA signaling pathway (Finkelstein and Lynch, 2000; Tang et al., 2013). Mutation of *ABI5* or reduction of its protein abundance promoted seedling greening and early seedling establishment, indicating its important regulatory roles in these processes (Lopez-Molina et al., 2001; Guan et al., 2014). However, the underlying regulatory mechanisms by which FHY3 and its direct target *ABI5* mediate light and ABA signals to regulate the greening of etiolated seedlings remain unknown.

Arabidopsis DE-ETIOLATED1 (DET1) is a key negative regulator in photomorphogenesis. In Arabidopsis, the null allele of *det1* is lethal, and the weak allele *det1-1* exhibits a continuous photomorphogenesis phenotype in darkness (Pepper et al., 1994). DET1 interacts with DAMAGED DNA BINDING PROTEIN1 (DDB1) and CONSTITUTIVE PHOTOMORPHOGENIC10 (COP10), forming the COP10–DET1–DDB1 (CDD) complex and facilitating the protein degradation of the positive regulators of photomorphogenesis (e.g. ELONGATED HYPOCOTYL5), thus repressing photomorphogenesis in darkness (Yanagawa et al., 2004). Meanwhile, DET1 mediates the stabilization of PHYTOCHROME-INTERACTING FACTORS (PIFs, including PIF1, PIF3, PIF4, and PIF5), and thus promotes the elongation of the etiolated seedling (Dong et al., 2014). Besides its key negative role in light signal transduction, the CDD complex negatively regulates ABA responses. The CDD complex interacts with DET1-, DDB1-ASSOCIATED1, which acts as a substrate adaptor to promote the degradation of ABA receptors (e.g. PYRABACTIN RESISTANCE 1-LIKE8), thus, to negatively regulate ABA signal transduction (Irigoyen et al., 2014). Meanwhile, DET1 affects the transcription of a set of dark-to-light transition-related genes by controlling the monoubiquitination of histone 2B or other modifications (Nassrallah et al., 2018). Although DET1 has been shown to play essential roles in light and ABA signaling, the molecular mechanisms by which DET1 integrates light and ABA signals to regulate greening of seedlings remain unknown.

In this study, we found that although *det1-1* plants exhibit a constitutive photomorphogenic phenotype, their light-induced greening of seedlings is significantly inhibited, especially in ABA-treated seedlings. Further, we showed that DET1 interacted with FHY3 and repressed its transcriptional activation to *ABI5*. Finally, we demonstrated that DET1 recruits HISTONE DEACETYLASE6 (HDA6) to the promoter region of *ABI5* and subsequently represses the FHY3-mediated transcriptional activation of *ABI5* in light- or ABA-treated seedlings. Our finding identified the molecular regulatory mechanisms by which light and ABA coordinately regulate greening of seedlings and ABA responses through DET1, HDA6, and FHY3.

RESULTS

ABA Inhibits Light-Induced Greening of Etiolated Seedlings through DET1

To investigate the relationship between light signaling and the ABA pathway in controlling the greening of etiolated seedlings in Arabidopsis, we measured the greening rates of a set of well-known light-signaling related mutants that were germinated and grown under continuous darkness for 4 d. As shown in Figure 1, A and B, we found that seedlings of the *det1-1* mutant (a weak allele) showed a slower greening rate than wild-type Col-0 plants after the seedlings were irradiated with light, although the *det1-1* plants exhibited a constitutive photomorphogenic phenotype in darkness. In wild-type Col-0 plants, after being irradiated with light for 4 h, the cotyledons partially turned green (especially the middle region) and the folded apical hook partially opened. The tightly closed cotyledons of Col-0 plants completely opened after being irradiated with light for 12 h, and total chlorophyll contents reached their maximum values after being irradiated with light for 24 h (Fig. 1, B and C). In *det1-1* plants, the greening rate was obviously slower than in Col-0 plants (Fig. 1, B and C). Interestingly, light-induced cotyledon greening was slightly decreased in wild-type Col-0 seedlings after being treated with 10 μM of ABA, and strongly decreased after a high concentration (30 μM) of ABA treatment (Fig. 1, A and C). Compared to Col-0 plants, the *det1-1* mutant exhibited increased sensitivity to ABA treatment (including 1, 10, and 30 μM; Fig. 1, A and C). All these observations suggest that ABA inhibits the light-induced greening of seedlings, and DET1 plays a negative role in this process.

DET1 Physically Interacts with FHY3 In Vitro and In Vivo

To investigate the regulatory mechanism by which DET1 regulates the greening of seedlings, we performed

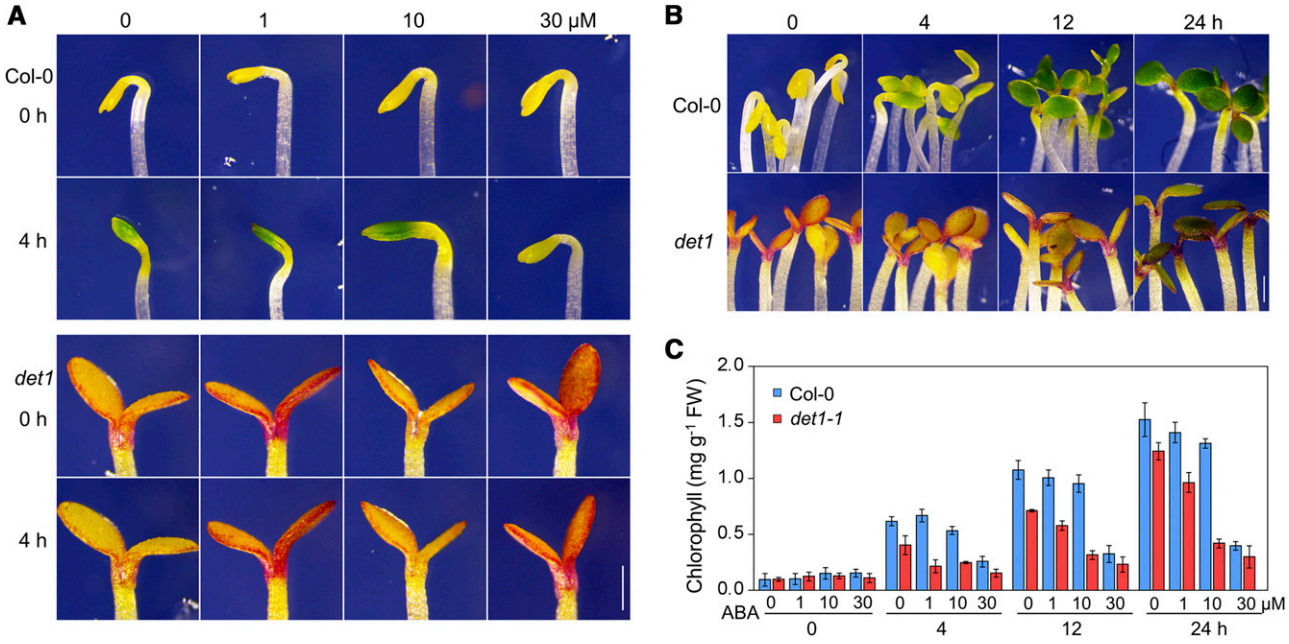

Figure 1. ABA inhibits light-induced greening of etiolated seedlings through DET1. A, Effect of ABA on the greening of etiolated Col-0 and *det1-1* seedlings. Four-day-old etiolated seedlings were treated without or with 1, 10, or 30 μM of ABA for 4 h under light conditions. Scale bar = 1 mm. B, The greening phenotype of 4-d-old etiolated Col-0 and *det1-1* seedlings that were irradiated with light for 0, 4, 12, or 24 h. C, Total chlorophyll contents of the seedlings shown in A and B. Values are means ± SD. FW, Fresh weight.

a yeast two-hybrid screening and found that DET1 interacts with FHY3. As shown in Figure 2, A and B, full-length FHY3 protein weakly interacted with DET1, and the C-terminal region of FHY3 strongly interacted with DET1, indicating that the C-terminal transcriptional activation region of FHY3 is responsible for the physical interaction with DET1 in vitro (Fig. 2, A and B). Given that DET1 forms the CDD complex by interacting with COP10 and DDB1 in planta, we performed a yeast two-hybrid assay and verified that COP10 interacts with the full-length and C-terminal of FHY3 (Supplemental Fig. S1A). These results suggest that DET1 and COP10 physically interact with FHY3 through its C-terminal transcriptional activation domain.

To demonstrate the physical interaction between DET1 and FHY3 in vivo, we performed a bimolecular fluorescence complementation (BiFC) assay in Arabidopsis protoplasts. As shown in Figure 2C, coexpressing DET1-YFP^N (DET1 fused to the N terminus of yellow fluorescent protein [YFP]) and FHY3-YFP^C (FHY3 fused to the C terminus of YFP) produced obvious YFP fluorescence signals in the nucleus, whereas protoplasts harboring DET1-YFP^N/YFP^C or YFP^N/FHY3-YFP^C failed to produce any detectable YFP signal. Further, we performed coimmunoprecipitation (Co-IP) assays using 4-d-old seedlings of *35Sp:MYC-DET1* (in the *det1-1* background) and *35Sp:FLAG-COP10* (in the *cop10-1* background) after being irradiated with light for 30 min (minute). After the total protein immunoprecipitation by anti-MYC (Fig. 2D) or anti-FLAG (Supplemental Fig. S1B) antibodies, specific bands of FHY3 protein were clearly detected in the immunoprecipitated (IP) products of *35Sp:MYC-DET1* and *35Sp:FLAG-COP10* plants, but not in wild-type Col-0 and *fhy3*-11 plants. All these results indicate that DET1 and COP10 physically interact with FHY3 in vitro and in vivo.

FHY3 Acts Downstream of DET1 in Mediating ABA Responses

To investigate the genetic relationship between *DET1* and *FHY3*, we crossed the *fhy3-11* (null allele) mutant with the *det1-1* (weak allele) mutant to generate the *fhy3 det1* double mutant in the Col-0 background. To investigate how DET1 and FHY3 mediate the inhibition of greening by ABA, 4-d-old etiolated Col-0, *fhy3-11*, *det1-1*, and *fhy3 det1* seedlings were treated with different concentrations of ABA after being irradiated with light. Compared to wild-type Col-0 plants, *fhy3-11* plants exhibited faster greening rates and higher chlorophyll contents when treated with a high concentration of ABA (30 μM), while *det1-1* plants exhibited slower greening rates and much lower chlorophyll contents (Fig. 3, A and B). Under ABA treatment conditions, *fhy3 det1* plants showed an intermediate greening response between those of *fhy3-11* and *det1-1* plants, while closer to *fhy3-11*, which indicated that FHY3 and DET1 might antagonistically regulate light-induced greening under ABA treatment conditions.

To further verify this notion, we measured the expression of the well-studied ABA rapid-response marker

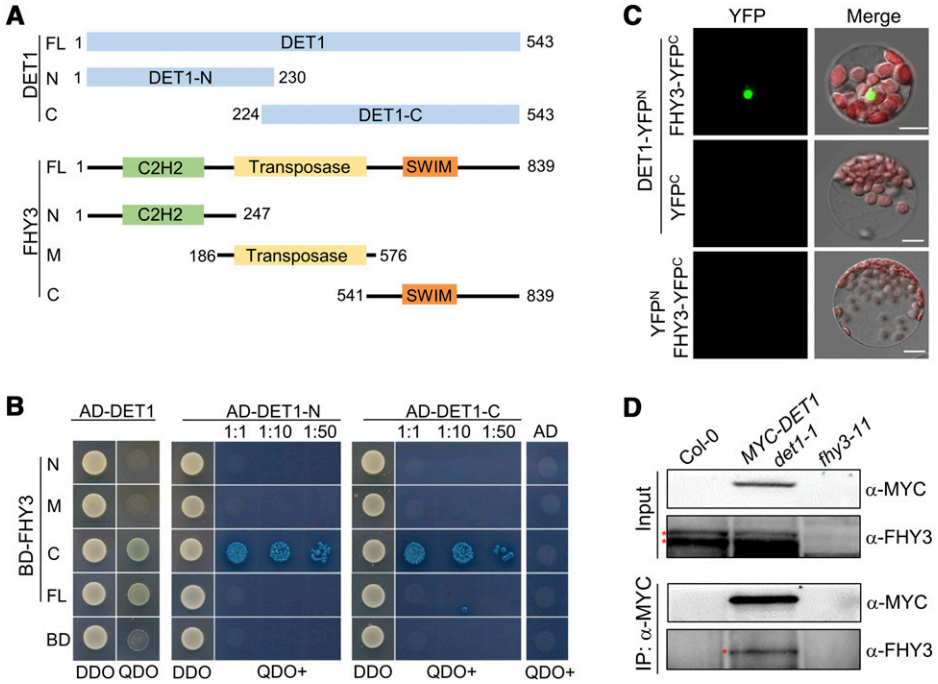

Figure 2. DET1 physically interacts with FHY3 in vitro and in vivo. A and B, Yeast two-hybrid assays showing DET1 interacts with FHY3 in vitro. The positions of various fragments that used in the yeast two-hybrid assays are shown in A. The various fragments of DET1 and FHY3 were fused to DNA binding domain (BD) or activation domain (AD) of GAL4, respectively. 1:10 and 1:50 indicate the dilutions of yeast cells that were spotted on plates for X-α-gal assays. DDO, Yeast synthetic medium without Trp/Leu; QDO, yeast synthetic medium without Trp/Leu/His/Ade, but with 20 μg mL^{-1} of X-α-gal and 125 ng mL^{-1} of AbA; QDO+, QDO medium-plus with 40 μg mL^{-1} of X-α-gal and 250 ng mL^{-1} of AbA. C, BiFC assays showing DET1 interacts with FHY3 in Arabidopsis protoplasts. YFP^N and YFP^C indicate the N- or C-terminal parts of YFP, respectively. Scale bars = 5 μm. D, Co-IP assays showing DET1 interacts with FHY3 in Arabidopsis. Four-day-old various etiolated seedlings were irradiated with light for 30 min and then used to perform Co-IP assays. The *fhy3-11* was used as a negative control showing the positions of endogenous FHY3 protein (indicates by asterisk) that detected by anti-FHY3 polyclonal antibodies. Anti-MYC monoclonal antibodies were used to perform the IP and detect the abundance of the MYC-DET1 fusion protein.

genes *ABI1*, *ABI3*, *ABI4*, and *ABI5* in seedlings treated without (mock) or with ABA, under constant darkness (DD) or after plants were irradiated with light (DD-L). Without ABA treatment, reverse transcription quantitative PCR (RT-qPCR) analyses showed that their expression levels were not significantly altered in Col-0, *det1-1*, *fhy3-11*, and *fhy3 det1* plants under DD and DD-L conditions (Fig. 3C; Supplemental Fig. A). After ABA treatment, the expression levels of *ABI1*, *ABI3*, *ABI4*, and *ABI5* were obviously increased in Col-0 and *det1-1* plants, but only slightly increased in *fhy3-11* and *fhy3 det1* plants (Fig. 3C; Supplemental Fig. S2A). Interestingly, we noticed that the induction of *ABI1*, *ABI3*, *ABI4*, and *ABI5* expression after ABA treatment was much higher in the wild-type Col-0 plants under DD, compared with their expression levels under DD-L, which indicated that ABA-induced expression of these *ABI* genes was significantly repressed by light (Fig. 3C; Supplemental Fig. S2A).

Further, ABA inhibited seed germination and response curves of Col-0, *fhy3-11*, *det1-1*, and *fhy3 det1* plants were observed under both DD and long-day (LD) conditions. Compared to wild-type Col-0 plants, *det1-1* plants had obviously increased sensitivity to ABA treatment; seed germination and postgerminative growth of *det1-1* plants were dramatically inhibited and arrested by a very low concentration of ABA (0.1 μM), while *fhy3-11* plants exhibited reduced sensitivity to ABA treatment under both DD and LD conditions (Fig. 3, D and E; Supplemental Fig. S2, B and C). More importantly, although *fhy3 det1* plants exhibited intermediate ABA responses between those of *fhy3* and *det1* plants, the inhibited seed germination and arrested post-germinative growth of *det1-1* plants were nearly completely rescued by disruption of *FHY3* (Fig. 3, D and E). ABA inhibited seed germination and response curves of Nossen-0 (No-0), *fhy3-4* (No-0 ecotype), *MYC-DET1 No-0*, and *MYC-DET1 fhy3-4* plants were further examined. Consistently, under ABA treatment, *MYC-DET1 fhy3-4* plants exhibited a very similar (but not additive) seed germination ratio to that of *fhy3-4* plants, although a higher percent of germination ratio than No-0 plants was observed in *MYC-DET1 No-0* plants (Supplemental Fig. S2, D and E). In addition, with ABA treatment, the root elongation of *det1-1* plants was completely inhibited, while *fhy3-4*, *fhy3-4 det1-1*, and *MYC-DET1 fhy3-4* plants exhibited much longer root lengths than wild-type plants (Supplemental Fig. S2,

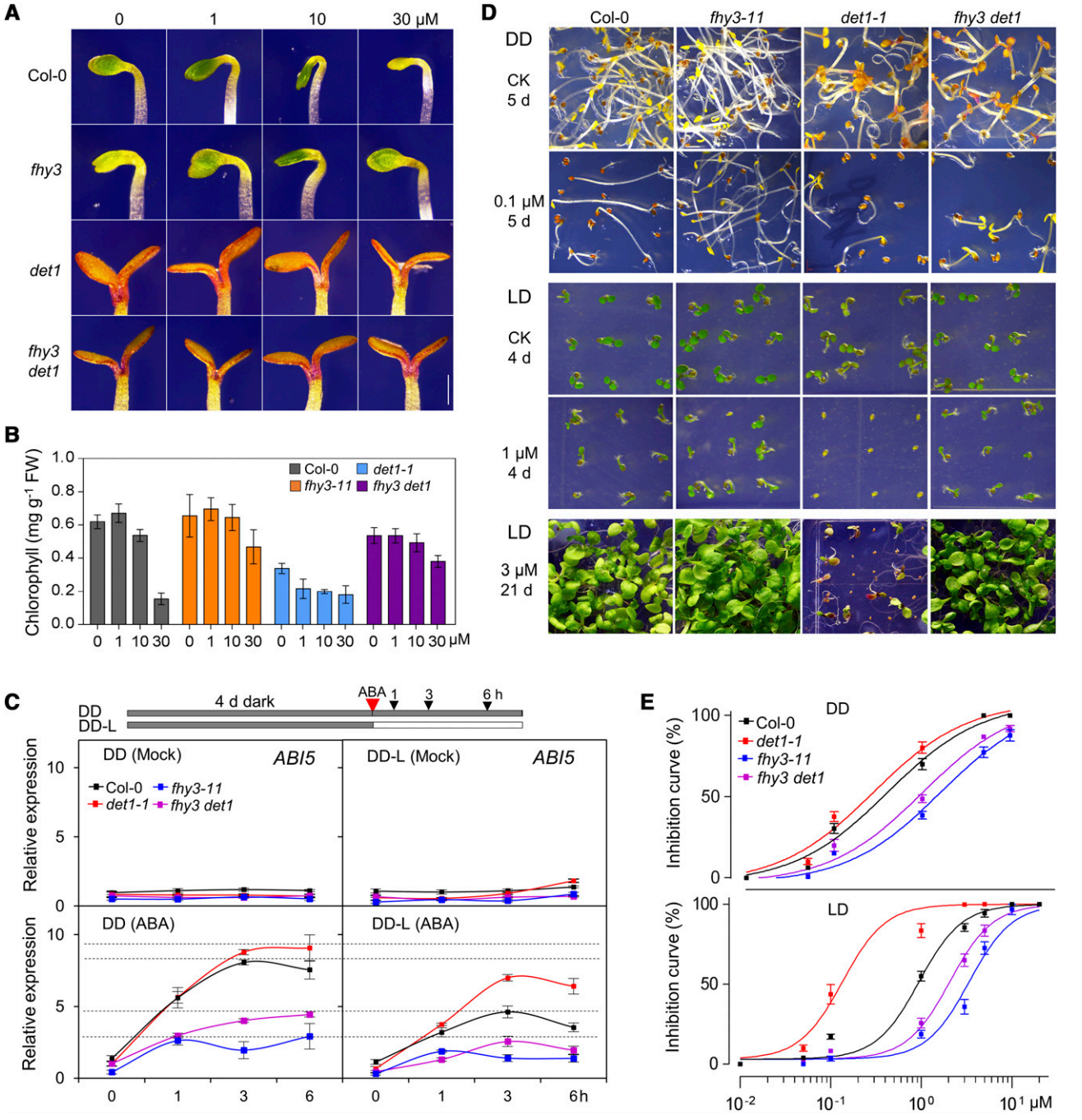

Figure 3. FHY3 acts downstream of DET1 in mediating ABA responses. A and B, The greening phenotype (A) and chlorophyll contents (B) of 4-d-old various etiolated seedlings treated with 0, 1, 10, or 30 μM of ABA for 4 h under light conditions. FW, Fresh weight. Scale bar = 1 mm. Values are means ± SD. C, RT-qPCR analyses showing the relative expression of *ABI5* in various seedlings. Four-day-old various etiolated seedlings were treated without (Mock) or with 30 μM of ABA for the indicated times under DD or after being transferred to light conditions (DD-L). A typical experiment out of three repeated experiments is shown, and means are values ± SD of three technical replicates are presented. D, Phenotypes of various plants germinated and continuously grown on GM without or with 0.1 μM of ABA under DD for 5 d, with 1 μM of ABA under LD for 4 d, or with 3 μM of ABA under LD for 21 d. E, The ABA inhibition response curves of seed germination for various plants under DD (upper) or LD (lower) conditions. ABA inhibition curves were calculated based on the inhibition effect of ABA on germination at the time point that ~50% wild-type seeds germinated under 1 μM of ABA treatment (at 60 h after imbibition under LD; at 72 h after imbibition under DD, respectively) using the EC50 shift. Values are means ± SD.

F and G). We also noticed that, without ABA treatment, the *fhy3-4* and *fhy3-4 det1-1* plants exhibited longer root lengths than wild-type plants, while the *det1-1* mutant exhibited shorter root lengths than wild-type plants. All these results indicate that FHY3 acts downstream of DET1-mediated ABA responses, including gene expression, seed germination, greening of etiolated seedlings, and postgerminative growth.

DET1 Inhibits FHY3 Activation of its Direct Target *ABI5* under Light and ABA Treatment Conditions

To reveal the molecular mechanisms by which DET1 regulates ABA responses through FHY3, we first checked the tissue-specific expression pattern of *FHY3* and *DET1* using *FHY3p*:*GUS* and *DET1p*:*GUS* reporter lines. GUS staining assays showed that both *FHY3* and *DET1* were strongly expressed in cotyledons and the radicle (Supplemental Fig. S3A). Further, RT-qPCR and immunoblot assays showed that *FHY3* and *DET1* shared a similar expression pattern under diurnal and ABA treatment conditions (Supplemental Fig. S3, B–E). Immunoblot assays showed that the abundance of FHY3 protein was not significantly altered in *det1-1* plants, while the abundance of MYC-DET1 was slightly increased in *MYC-DET1 fhy3-4* plants (Supplemental Fig. S4, A and B).

To investigate the regulatory mechanisms of the DET1 and FHY3 interaction during the dark-to-light transition, we performed Co-IP assays in etiolated *MYC-DET1 No-0* seedlings. As shown in Figure 4A, after protein immunoprecipitated by anti-MYC monoclonal antibodies, specific FHY3 protein bands were clearly detected in the IP product in *MYC-DET1 No-0* seedlings under light conditions (DD-L) but not DD conditions (Fig. 4A), suggesting that the protein interaction between DET1 and FHY3 is induced by light. A Co-IP assay further revealed that ABA treatment significantly enhanced the protein interaction between DET1 and FHY3 under light conditions (Fig. 4B). Meanwhile, the ABA- and FHY3-induced high expression of *ABI5* could be obviously reduced by light (Fig. 3C), implying that light promotes the DET1 interaction with FHY3 and

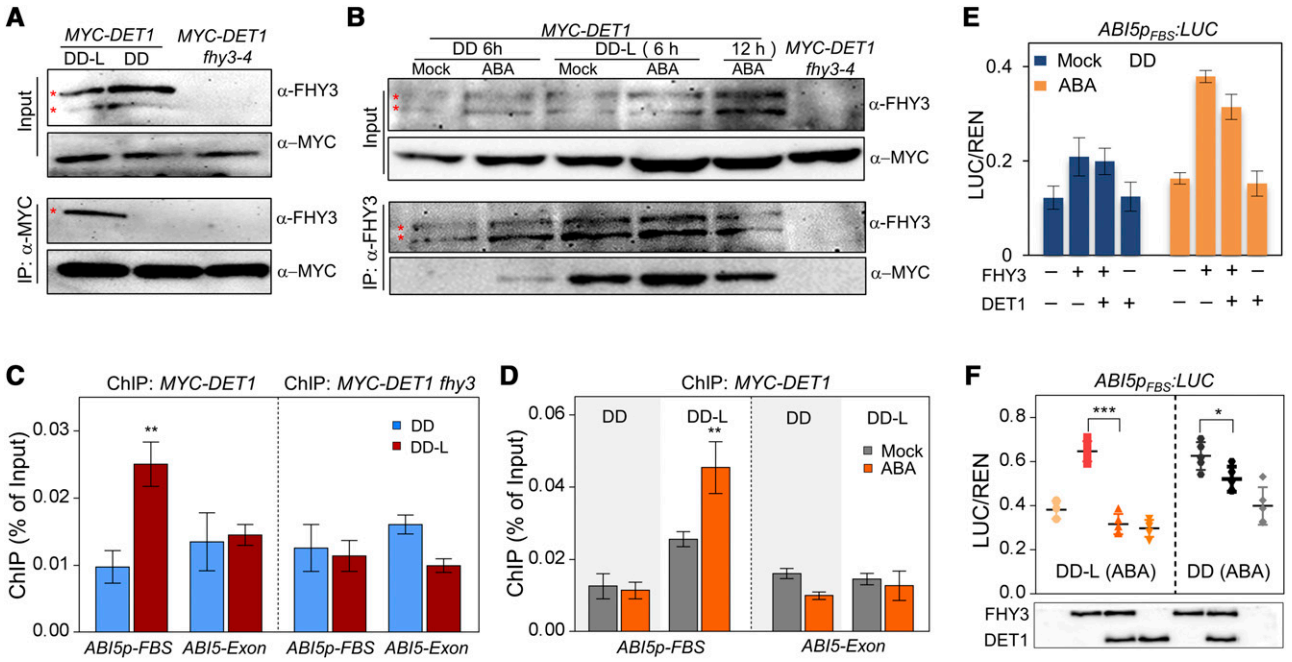

Figure 4. DET1 inhibit the transcriptional activation of *ABI5* by FHY3. A and B, Co-IP analyses showing the protein interaction between DET1 and FHY3 dependent on light (A) and enhanced by ABA (B). In A, 4-d-old etiolated *MYC-DET1* seedlings (DD) being irradiated with light (DD-L) for 1 h were used to perform Co-IP assays. In B, 4-d-old etiolated *MYC-DET1* seedlings treated without (Mock) or with ABA (30 μM) for the indicated times under DD or DD-L conditions were used to perform Co-IP assays. In A and B, *MYC-DET1 fhy3* was used as a negative control to indicate the positions (asterisk) of FHY3 protein. C and D, ChIP-qPCR assays showing the association of DET1 with the *ABI5* promoter is dependent on FHY3 (C) and enhanced by ABA under light (D). In C, 4-d-old etiolated seedlings (DD) after being irradiated with light (DD-L) for 3 h were used to perform ChIP assays. In D, 4-d-old etiolated seedlings were treated without (Mock) or with ABA (30 μM) for 3 h under DD or DD-L conditions, and then used to perform a ChIP assay. E and F, Transient expression assays showing DET1 represses the transcriptional activation of *ABI5* by FHY3. In E, the transformed Arabidopsis protoplasts were first incubated without (Mock) or with 0.3 μM of ABA under darkness for 16 h (DD), then irradiated with light (DD-L) or not for 3 h and used to measure the expression of *LUC* reporter. In F, the abundances of FHY3-3FLAG and DET1-3FLAG were detected by anti-FLAG antibodies. Data are means of five biological replicates, and error bars represent SD. In C, D, and F, asterisks indicate the statistical significance by Student's *t* test (**P* <0.05, ***P* <0.01, and ****P* <0.001).

subsequently may inhibit the FHY3-mediated transcriptional activation of its direct targets such as *ABI5*.

To reveal the molecular mechanism by which DET1 affects the FHY3-mediated transcriptional activation of its downstream targets, we first tested whether DET1 associated with the promoter region of FHY3 targets such as *ABI5* by interacting with FHY3. To this end, 4-d-old etiolated *MYC-DET1 No-0* and *MYC-DET1 fhy3-4* seedlings without or with light treatment were used to perform the chromatin immunoprecipitation quantitative PCR (ChIP-qPCR) assays. As shown in Figure 4C, the fragments containing the FHY3/FAR1 binding site (FBS) cis-elements of the *ABI5* promoter were specifically enriched in the ChIP products by the MYC-DET1 protein in *MYC-DET1 No-0* seedlings after being irradiated with light, but not in *MYC-DET1 fhy3-4* seedlings (Fig. 4C). ChIP-qPCR assays in ABA-treated *MYC-DET1 No-0* seedlings showed that ABA treatment enhanced the association of DET1 with the *ABI5* promoter (Fig. 4D). Meanwhile, ChIP-qPCR assays showed that FHY3 directly bound to the promoter region of *ABI5* in both *FHY3p*:*FHY3-YFP* and *FHY3p*:*FHY3-YFP det1-1* seedlings (Supplemental Fig. S4C), which suggested that the DNA binding activity of FHY3 was not directly affected by DET1. All these results suggest that the association of DET1 with the promoter region of *ABI5* is dependent on FHY3 under light and ABA treatment conditions.

To confirm whether DET1 directly represses the transcriptional activation of *ABI5* by FHY3, we performed transient expression assays in yeast cells and Arabidopsis protoplasts. In yeast cells, expression of FHY3 significantly increased the transcription of $ABI5p_{FBS}$:*LacZ*, and coexpression of DET1 and FHY3 repressed the transcriptional activation of $ABI5p_{FBS}$:*LacZ* by FHY3 (Supplemental Fig. S4D). In Arabidopsis protoplasts, compared with expression of FHY3 alone, coexpression of DET1 and FHY3 repressed the activation of $ABI5p_{FBS}$:*LUC*, especially under light and ABA treatment conditions (Fig. 4, E and F; Supplemental Fig. S4E), which is consistent with the specific interaction between DET1 and FHY3 in light (Fig. 4, A and B). All these results suggest that DET1 interacts with FHY3 and subsequently associates to the promoter regions of FHY3 direct targets (such as *ABI5*) under light or ABA treatment conditions, and then inhibits their expression.

DET1 Mediates H3K27ac and H3K4me3 Modification during the Dark-to-Light Transition and ABA Response

To investigate whether DET1 represses the FHY3-mediated transcriptional activation by influencing histone modifications at the FHY3 target locus, we first measured the global abundances of H3K27ac (acetylation of histone H3lys27) and H3K4me3 (trimethylation of histone H3lys4), two well-studied transcriptional activation-related histone modifications (Santos-Rosa et al., 2002; Katoh et al., 2018), in Col-0, *det1-1*, *fhy3-11*, and *fhy3 det1* seedlings (Fig. 5A; Supplemental Fig. S5, A and B). In darkness, compared to wild-type Col-0 plants, the global abundances of H3K27ac and H3K4me3 were reduced in *det1-1* and *fhy3 det1* plants, but were not significantly altered in *fhy3-11* plants (Fig. 5A; Supplemental Fig. S5B). Upon irradiating seedlings with light, compared to seedlings grown in darkness, the global abundances of H3K27ac and H3K4me3 were decreased slightly in Col-0 plants, did not significantly change in *fhy3-11* plants, and were obviously increased in *det1-1* and *fhy3 det1* plants (Fig. 5A; Supplemental Fig. S5B). After ABA treatment, compared to untreated plants, the global abundances of H3K27ac and H3K4me3 were significantly increased in *det1-1* plants, but not in Col-0 plants and *fhy3-11* plants (Fig. 5A). The specific enrichments of H3ac, H3K27ac, and H3K4me3 modifications in the *ABI5* promoter and exon regions were further detected in etiolated Col-0 and *det1-1* seedlings using ChIP-qPCR. In Col-0 plants, the enrichments of H3ac, H3K27ac, and H3K4me3 in the promoter region of *ABI5* were repressed by light and induced by ABA treatment (Fig. 5B; Supplemental Fig. S5C). Compared to wild-type Col-0 plants, the enrichments of H3K27ac and H3K4me3 in the promoter and exon regions of *ABI5* were obviously induced in *det1-1* plants under both light and ABA treatment conditions (Fig. 5B), consistent with its positive role in the decrease of H3K27ac and H3K4me3 modifications after light or ABA treatment (Fig. 5A). Interestingly, these results revealed that DET1 promoted the accumulation of H3K27ac and H3K4me3 in darkness, while it promoted a decrease of H3K27ac and H3K4me3 after light or ABA treatment.

The contrasting regulatory roles of DET1 in dark and light suggest that DET1 may specifically interact with HDAs or other histone modification regulators after light or ABA treatment, thus affecting H3K27ac or other modifications. To test this, we performed RT-qPCR assays with Trichostatin A (TSA) treatment, an inhibitor of HDAs (Finnin et al., 1999). In wild-type Col-0 and *fhy3-11* plants, TSA treatment obviously increased the induction of *ABI5* in response to ABA treatment (Fig. 5C). Compared to wild-type Col-0 plants, TSA treatment only slightly increased the induction of *ABI5* in response to ABA treatment in *det1-1* and *fhy3 det1* plants (Fig. 5C), which suggested that DET1 might inhibit the transcriptional activation of *ABI5* through regulating the enrichments of H3K27ac, H3K4me3, or other modifications.

DET1 Recruits HDA6 To Inhibit the Transcriptional Activity of FHY3

To investigate whether HDAs were involved in the DET1-mediated inhibition of FHY3, we first tested the protein interaction between DET1 and HDA6, an HDA member that has been reported to function in light, ABA, and stress responses (Earley et al., 2006; Tanaka

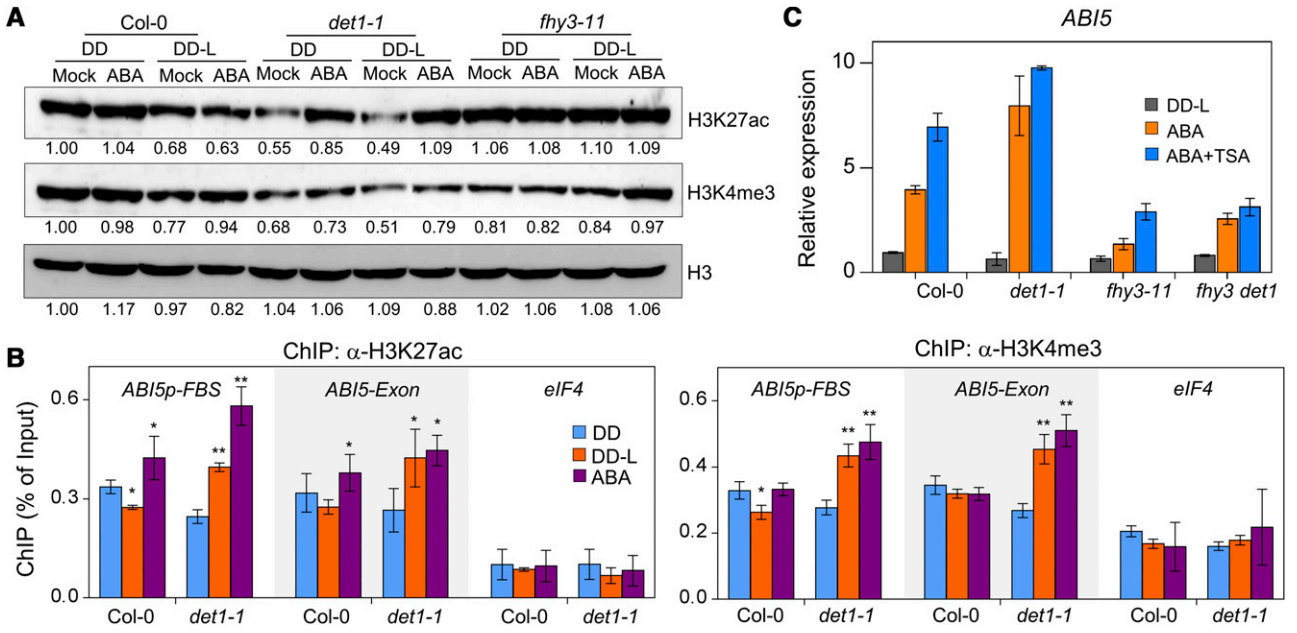

Figure 5. DET1 mediates histone modification during the dark-to-light transition and ABA response. A, Immunoblot assays showing the dynamic changes of H3K27ac and H3K4me3 abundance during dark (DD) to light (L) transition and ABA treatment. Four-day-old various etiolated seedlings were treated without (Mock) or with ABA (30 μM) under DD or DD-L for 3 h, and then used to perform immunoblot assays. The abundance of histone H3 was used as a loading control. B, ChIP-qPCR assays showing the association of H3K27ac and H3K4me3 at the promoter and exon regions of *ABI5*. The exon region of *eIF4* (*eIF4A1*) was used as a negative control for ChIP-qPCR. Asterisks indicate the statistical significance by Student's *t* test (*$P<0.05$ and **$P<0.01$). C, RT-qPCR analysis showing the effect of ABA and TSA treatment on the expression of *ABI5*. Four-day-old etiolated seedlings were treated with ABA (30 μM) or ABA+TSA (10 μM) for 3 h under light (DD-L), and then used to perform RT-qPCR assays.

et al., 2008; Tessadori et al., 2009; Chen et al., 2010). As shown in Figure 6A, after being immunoprecipitated by anti-MYC antibodies, specific HDA6-HA protein bands were detected in the samples that coexpressed DET1-MYC and HDA6-HA, which suggested that DET1 interacted with HDA6 in plants (Fig. 6A). To test whether DET1 recruits HDA6 to associate with FHY3, we performed Co-IP assays in the protoplasts of Col-0 and *det1-1* plants. After immunoprecipitation by anti-FHY3 antibodies, DET1-MYC and HDA6-FLAG proteins were detected in the protoplasts that coexpressed DET1-MYC and HDA6-FLAG, indicating that FHY3 associated with DET1 and HDA6 in plants (Fig. 6B, left). In *det1-1* protoplasts, after being immunoprecipitated with anti-FHY3 antibodies, weak HDA6-FLAG bands could be detected. When coexpressing DET1-MYC and HDA6-FLAG in *det1-1* protoplasts, the detected abundance of HDA6-FLAG was significantly increased after being immunoprecipitated with anti-FHY3 antibodies, compared with that in *det1-1* protoplasts expressing HDA6-FLAG alone (Fig. 6B), which suggested that DET1 recruited HDA6 to associate with FHY3 in Arabidopsis.

To further investigate whether DET1 could interact with HDA6 to coordinately regulate ABA responses, we examined ABA responses in *hda6* plants (an *HDA6-RNAi* transgenic line in the Wassilewskija ecotype [Ws]; Wu et al., 2008). With a low concentration of ABA (1 μM) treatment, germination of *hda6* seeds was completely inhibited (Fig. 6, C and D), similar to *det1-1* plants (Fig. 3, D and E). Compared with the Ws control plants, the global abundances of H3K27ac and H3K4me3 were significantly increased when *hda6* seedlings were transferred from dark to light (Fig. 6E; Supplemental Fig. S5B). ChIP-qPCR assays further verified that the specific enrichments of H3K27ac and H3K4me3 modifications in the promoter region of *ABI5* were significantly higher in *hda6* plants, compared with Ws control plants (Fig. 6F). Consistent with these results, the fragments containing the FBS cis-elements in the *ABI5* promoter were specifically enriched in the DNA products immunoprecipitated by anti-FLAG of *HDA6-FLAG* seedlings under DD-L conditions (Fig. 6G). Meanwhile, the expression level of *ABI5* was slightly higher in *hda6* plants in light (mock), and significantly higher than Ws plants when seedlings were treated with ABA (Fig. 6H). More importantly, when HDA6 was coexpressed with FHY3, the expression level of *ABI5p$_{FBS}$:LUC* was slightly repressed, and the repression effect was enhanced when both HDA6 and DET1 were coexpressed with FHY3, suggesting that HDA6 and DET1 have additive roles in this repression process (Fig. 6I). Taken together, our results revealed that DET1 recruits HDA6 to FHY3 target loci and subsequently represses their transcription

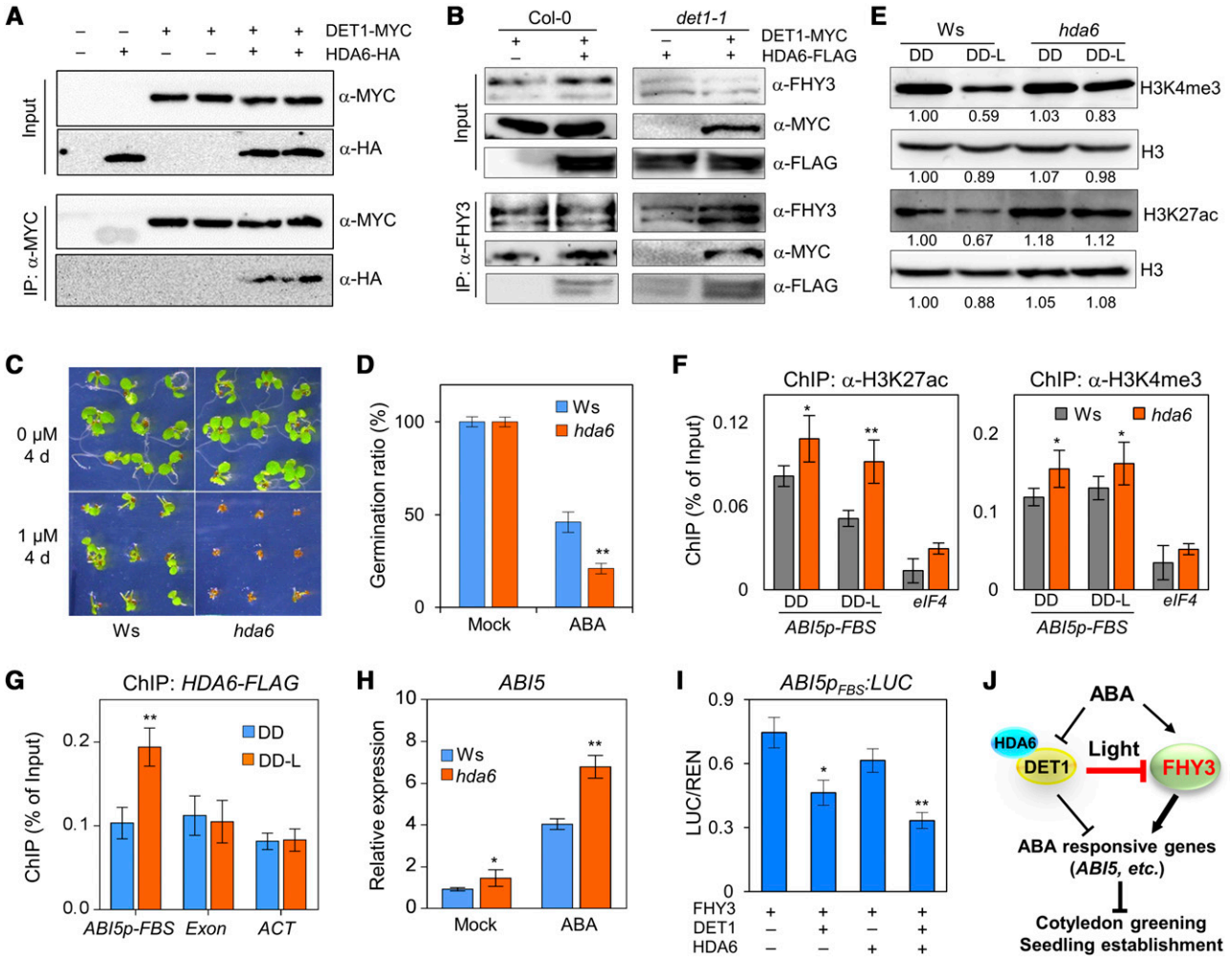

Figure 6. DET1 recruits HDA6 to inhibit the transcriptional activity of FHY3. A, Co-IP assays in *N. benthamiana* leaves showing DET1 interacts with HDA6. B, Co-IP assays in protoplasts of wild type (Col-0) and *det1-1* showing FHY3 interacts with HDA6 via DET1. C and D, Seed germination phenotypes (C) and ratio (D) of wild-type (Ws) and *hda6* plants under 1-μM ABA treatment conditions. Germination ratios were determined from three biological replicates of ~40 seeds each. E, Immunoblot assays showing the dynamic changes of H3K4me3 and H3K27ac abundance in Ws and *hda6* plants during the dark-to-light transition. The abundance of histone H3 was used as a loading control. F, ChIP-qPCR assays showing the effect of HDA6 on the association of H3K4me3 and H3K27ac at *ABI5* promoter during the dark-to-light transition. The exon of *eIF4* was used as a negative control for ChIP assay. G, ChIP-qPCR assays showing HDA6 associates with the *ABI5* promoter after etiolated seedlings irradiated with light (DD-L). The promoter of *ACT* was used as a negative control. H, RT-qPCR analysis showing the effect of HDA6 on the expression of *ABI5*. Four-day-old etiolated seedlings were treated with 30 μM of ABA for 3 h under light and then used to perform RT-qPCR assays. I, Transient expression assays in Arabidopsis protoplasts showing HDA6 and DET1 repress the transcriptional activation of *ABI5* by FHY3. Data are means of five biological replicates, and error bars represent SD. In F to I, asterisks indicate the statistical significance by Student's *t* test (*P <0.05 and **P <0.01). J, Working model showing DET1-HDA6 and FHY3 coordinately regulate the effects of light and ABA in the greening of seedling during the dark-to-light transition.

by reducing the abundance of H3K4me3 and H3K27ac modifications.

DISCUSSION

FHY3 and ABI5 Act Downstream of DET1 in Mediating ABA Responses in Light

As a key repressor of photomorphogenesis and light signal transduction, mutation of *DET1* in plants causes a de-etiolated phenotype in darkness, while *det1-1* plants exhibit a delayed greening rate after being irradiated with light (Fig. 1). Consistent with this, the *det1-1* plants failed to survive after being transferred to light from long-term (such as 7 d) growth in continuous darkness. Besides a delayed greening rate, disruption of *DET1* causes very low seed germination, inhibited elongation of primary root, and severely arrested early seedling establishment phenotypes under ABA treatment conditions (Fig. 3, A, B, D, and E; Supplemental

Fig. S2). At the transcription level, DET1 repressed the induction of multiple *ABI* genes (*ABI1*, *ABI3*, *ABI4*, and *ABI5*), especially *ABI5* in the seedlings (Fig. 3C; Supplemental Fig. S2A). Considering the important regulatory roles of these genes (such as *ABI5*) in ABA responses (Tang et al., 2013; Guan et al., 2014; Hu et al., 2019), the high induction levels of *ABI5* or other response genes in *det1-1* plants could be one of the major reasons for enhanced ABA response in *det1* plants (Figs. 1 and 3; Supplemental Fig. S2). Meanwhile, mutation of *ABI5* completely rescues the ABA hypersensitivity in *det1-1* plants (Fernando and Schroeder, 2015), which further indicates that *ABI5* acts downstream of DET1 in ABA responses. More importantly, *ABI5* is one of the direct targets of FHY3, which directly physically interacts with DET1, and disruption of *FHY3* in *det1-1* plants completely recovers its enhanced ABA responses (Figs. 2 and 3). Additionally, *MYC-DET1 fhy3* plants exhibited a similar ABA sensitivity to *fhy3* plants, not an additive phenotype (Supplemental Fig. S2, D–G). These results indicate that FHY3 is a major downstream factor of DET1 in mediating ABA response by activating the transcription of *ABI5*. Therefore, the cascade of DET1-FHY3-*ABI5* plays an essential role in ABA response during the dark-to-light transition.

We noticed that the interaction between DET1 and FHY3 only occurred after the seedlings were irradiated with light and enhanced by ABA treatment, which indicated that this protein interaction was induced by light. Interestingly, Co-IP assays showed that DET1 only interacted with the upper-shifted band of FHY3 in plants, suggesting that only modified FHY3 protein interacted with DET1. Phosphatase treatment experiments showed that the upper band contained the phosphorylated form of FHY3 (Supplemental Fig. S6), while the upper band of FHY3 is very stable, not rapidly and significantly changed during the dark-to-light transition or ABA treatment (Fig. 4, A and B; Supplemental Fig. S6). These results indicate that the light-induced interaction between FHY3 and DET1 is not dependent on the phosphorylation of FHY3. In addition, other FHY3 or DET1 interacting proteins related to light signals, such as PIFs or COP1, may interfere with the interaction between FHY3 and DET1. As reported, FHY3 can interact with PIF1 and PIF3 in the dark, and DET1 can interact with PIF1/3/4/5 and COP1 in the dark (Lau and Deng, 2012; Tang et al., 2012; Dong et al., 2014). These FHY3 or DET1 interacting proteins may act as competitors to prevent the protein interaction between DET1 and FHY3 in darkness by an unknown mechanism, while upon being irradiated with light, the competition may decrease and thus allow the interaction between FHY3 and DET1.

In this study, we showed that DET1 repressed the FHY3-mediated transcriptional activation of *ABI5* under light and ABA treatment conditions (Figs. 3 and 4). In addition, a recent study has revealed that the induction of *ABI5* is mediated by PIF4 in darkness and ABA treatment conditions (Qi et al., 2020). Therefore, in darkness, ABA- or stress-induced high expression of *ABI5* could be mediated by both FHY3 and PIF4, while FHY3 is the major transcription factor responsible for the induction of *ABI5* upon irradiating etiolated seedlings with light. After the dark-to-light transition, ABA or abiotic stress induced high expression of *ABI5* could be further repressed by DET1 through interacting with FHY3, thus, to promote the greening of etiolated seedlings and increase their adaptation to light. Other transcriptional regulators may also be involved in the transcriptional regulation of *ABI5* or other ABA response genes during the dark-to-light transition that need to be further explored.

Although we observed that ABA treatment obviously inhibited the greening of etiolated seedlings, the detailed molecular regulatory mechanisms are still largely unknown. Indeed, the transcriptional levels of *PROTOCHLOROPHYLLIDE OXIDOREDUCTASEs* (*PORs*), encoding the enzymes that catalyze the conversation from Pchlide to chlorophyllide, were significantly repressed by ABA and dramatically decreased in *det1* seedlings (Kusnetsov et al., 1998; Nassrallah et al., 2018), consistent with the delayed greening phenotype (Figs. 1 and 3, A and B). Considering *HEMB1*, which encodes 5-aminolevulinic acid dehydratase and is a direct target of FHY3, its transcriptional level was not significantly altered in *det1-1* plants (Nassrallah et al., 2018), but was obviously reduced in the *fhy3* mutant (Tang et al., 2012), which suggested that DET1-FHY3 might also affect the greening of seedlings through directly affecting the expression of chlorophyll-biosynthesis–related genes, such as *PROTOCHLOROPHYLLIDE OXIDOREDUCTASEs* and *HEMB1*. In addition to ABA signaling, reactive oxygen species caused by excess accumulation of Pchlide after irradiation by light probably also influence the greening of seedlings during the dark-to-light transition.

DET1 and HDA6 Repress FHY3-Mediated Transcriptional Activation of *ABI5*

DET1 is an evolutionarily conserved component in plants and animals (Pick et al., 2007; Dubin et al., 2011; Lau and Deng, 2012). Previous studies have revealed that DET1 not only affects the histone modification of Histone H2 by controlling the monoubiquitination of Histone 2B, it also affects histone modification of Histone H3, including H3K4me3, H3K27me3, and H3K36ac by an unknown mechanism (Kang et al., 2015; Nassrallah et al., 2018). In this study, we noticed that the global abundances of H3K27ac and H3K4me3 in *det1-1* plants were significantly decreased in darkness, and increased after light or ABA treatment (Fig. 5A; Supplemental Fig. S5, A and B). These results suggest that DET1 plays contrasting regulatory roles in dark and light: In the dark, it promotes the accumulation of H3K27ac and H3K4me3, but after light and ABA treatment, it promotes the decrease of H3K27ac and H3K4me3 (Fig. 5A; Supplemental Fig. S5, A and B). These contrasting regulatory roles of DET1 under dark and light suggest that DET1 may specifically interact

with light-regulated histone modification regulators. Subsequently, we showed that DET1 physically interacted with HDA6 (Fig. 6, A and B), an HDA responsible for the deacetylation of H3K27 and affected the trimethylation of H3K4 (Probst et al., 2004; Earley et al., 2006; Tanaka et al., 2008; Tessadori et al., 2009; Chen et al., 2010). Meanwhile, HDA6 plays a negative role in ABA responses (Chen et al., 2010), similar to the physiological role of DET1 in ABA response (Figs. 1 and 3). Disruption of *HDA6* produced similar changes of H3K27ac and H3K4me3 as those observed in *det1-1* plants (Figs. 5A and 6E). More importantly, DET1 recruits HDA6 to the *ABI5* promoter thus coordinately inhibiting the activation of *ABI5* by regulating the enrichment of H3K27ac and H3K4me3 modifications (Fig. 6, G–I–I). All these physiological, biochemical, and molecular evidence support the idea that HDA6 functions together with DET1 to coordinately inhibit the transcriptional activity of FHY3.

In this study, we showed that DET1 repressed the transcription of *ABI5* by recruiting HDA6 to the promoter region of *ABI5* (Fig. 6). Interestingly, we noticed that DET1 could not bind to the exon region of *ABI5*, but the specific enrichments of H3K27ac and H3K4me3 at the *ABI5* exon were significantly increased in *det1-1* plants (Fig. 5B). These results suggest that HDA6 or other histone modification regulators that interact with DET1 might also affect the enrichment of H3K27ac and H3K4me3 at the *ABI5* promoter and exon regions. Meanwhile, DET1 might influence the stability or function of FHY3 in other mechanisms, although the abundance of FHY3 was not obviously altered in the *det1-1* plants (Supplemental Fig. S4A), but this possibility cannot be excluded. Indeed, DET1 may repress the transcription of *ABI5* by directly inhibiting the transcription activity of FHY3, because DET1 strongly interacts with the C-terminal region of FHY3 (Fig. 2, A and B) that is responsible for the transcriptional regulation (Lin et al., 2007). Moreover, we noticed that in the transient expression assays, expressed DET1 alone could only slightly reduce the transcription of the *LUC* reporter driven by the *ABI5* promoter (Fig. 4, E and F; Supplemental Fig. S4D), which suggested that DET1 might repress the expression of *ABI5* in FHY3-independent manners. Consistently, DET1 has been reported to interact with other transcription factors such as PIF4 that also mediate the transcriptional activation of *ABI5* (Dong et al., 2014; Qi et al., 2020). Therefore, DET1 affects the transcription of *ABI5* and other aspects of ABA responses through multiple regulatory levels.

Finally, we showed that during the dark-to-light transition, light induced the interaction between DET1 and FHY3, then DET1 associated to the promoters of FHY3 direct targets (such as *ABI5*), and subsequently recruited HDA6 to the target locus to reduce the enrichments of H3K27ac and H3K4me3 modifications, thus repressing the transcription of *ABI5* and other ABA response genes (Fig. 6J). Therefore, our study revealed the molecular mechanisms by which light and ABA coordinately regulate the greening of etiolated seedlings and possibly other physiological processes.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The Arabidopsis (*Arabidopsis thaliana*) *fhy3-11* (Salk_002711), *det1-1* (Chory et al., 1989), *35Sp:MYC-DET1 det1-1* (Saijo et al., 2003), *35Sp:FLAG-COP10 cop10-1* (Yanagawa et al., 2004), and *FHY3p:GUS* (Lin and Wang, 2004) lines were in the Col-0 ecotype. *hda6* (cs24039) is an *HDA6-RNAi* transgenic line in the Ws ecotype (Wu et al., 2008). *fhy3-4* (Wang and Deng, 2002) and *FHY3p:FHY3-YFP fhy3-4* plants (Lin et al., 2007) were in the No-0 ecotype. *fhy3-4 det1-1*, *MYC-DET1 fhy3-4*, *MYC-DET1 No-0*, and *FHY3p:FHY3-YFP det1-1* plants were generated by genetic crosses. Arabidopsis seeds sown on germination medium (GM) with 1% (w/v) Suc and 0.8% (w/v) agar were incubated at 4°C overnight and transferred to different growth conditions as indicated at 22°C. The light intensity under LD and DD-L conditions was ~100 μmol m^{-2} s^{-1}.

Plasmid Construction and Generation of Transgenic Arabidopsis Plants

To generate *pCAMBIA1305-35Sp:DET1-3MYC* and *pCAMBIA1305-35Sp:HDA6-3HA*, the coding regions of *DET1* and *HDA6* were PCR-amplified from complementary DNA (cDNA) of Col-0 plants and inserted into the *Kpn*I- and *Sal*I-digested *pCAMBIA1305-3MYC* and *pCAMBIA1305-3HA*, respectively. To generate *pPZP211-35Sp:HDA6-3FLAG* and *pPZP211-35Sp:FHY3-3FLAG*, the fragments containing the *HDA6* and *FHY3* coding regions were inserted into the *Kpn*I- and *Sal*I-digested *pPZP211-3FLAG* (Ma et al., 2016), respectively. To generate *DET1p:GUS*, the fragments containing the promoter region of *DET1* (1785 bp) were inserted into the *Sac*I- and *Sal*I-digested *pPZP211-GUS*. The *pPZP211-DET1p:GUS* and *pPZP211-35Sp:HDA6-3FLAG* vectors were transformed into Col-0 plants. After transformation, more than 30 independent T1 generation transgenic lines were screened and transplanted in soil. Two representative lines were identified by GUS staining, RT-qPCR, or immunoblot analysis, and further used for functional assays.

ABA Treatments and Greening Assays

Arabidopsis seeds were first incubated at 4°C for 72 h and then transferred to sugar-free GM to perform ABA treatments and greening assays. For ABA-induced inhibition of seed germination, Arabidopsis seeds were transferred to medium with 0, 0.01, 0.05, 0.1, 1, 3, 5, 10, or 20 μM of ABA under LD conditions, or with 0, 0.05, 0.1, 1, 5, or 10 μM of ABA under DD conditions. The seed germination is characterized by radicle protrusion. Germination ratios were measured from three biological replicates of ~80 seeds each and ABA inhibition curves were calculated based on the inhibition effect of ABA on germination at the time point that ~50% of wild-type seed germinated under 1 μM of ABA treatment using the EC50 shift in the program Prism 7.0 (https://www.graphpad.com/scientific-software/prism/).

Four-d–old etiolated seedlings were irradiated with light or treated with different concentrations of ABA for the indicated times and then used to perform RT-qPCR, ChIP-qPCR, Co-IP, and immunoblot assays. To measure the total chlorophyll contents, various seedlings were incubated in 95% (v/v) ethanol for 2 d in darkness and then the absorbance at 665 and 649 nm was measured, and the contents of chlorophyll were calculated using the equation $(6.63A_{665} + 18.08A_{649})$/g fresh weight (Tian et al., 2020).

Yeast Assays

To generate the full-length or various fragments of *DET1* or *FHY3* in *pGADT7* and *pGBKT7* plasmids, the fragments containing full-length, N-terminal (1–741 bp), transposase (555–1,728 bp), and C-terminal (1,620–2,517 bp) of FHY3 were inserted into the *BamH*I- and *Sal*I-digested *pGBKT7*, and full-length (1–1,629 bp), N-terminal (1–690 bp), and C-terminal (669–1,629 bp) fragments of *DET1* were inserted into the *BamH*I- and *Sal*I-digested *pGADT7*. To perform yeast two-hybrid assays, various *pGADT7*- and *pGBKT7*-fusion plasmids were cotransformed into strain Y2H Gold following the procedure described in the Yeast Protocols Handbook (Clontech). DDO indicates yeast

synthetic medium without Trp/Leu, QDO indicates yeast synthetic medium without Trp/Leu/His/Ade, but with 20 μg mL^{-1} of X-α-gal and 125 ng mL^{-1} of Aureobasidin A (AbA). QDO+ indicates QDO medium with 40 μg mL^{-1} of X-α-gal and 250 ng mL^{-1} of AbA.

To generate the *pJG-FHY3* plasmid, the fragment containing the full-length *FHY3* coding region was inserted into the *BamH*I- and *Sal*I-digested *pJG4-5* vector. The fragment containing FBS cis-elements in the promoter of *ABI5* was repeated three times and inserted into the *Kpn*I- and *Xho*I-digested *pLacZi-2μ* (Lin et al., 2007) to generate the *ABI5p$_{FBS}$:LacZ*. To perform transient expression assays in yeast cells, *pGAD-DET1* and *pJG-FHY3* plasmids were cotransformed with the *ABI5p$_{FBS}$:LacZ* reporter into yeast strain EGY48. After transformation, all cultures were grown on the appropriate yeast synthetic medium. The liquid assay was conducted as described in the Yeast Protocols Handbook (Clontech).

BiFC Assay

Three-week-old wild-type Col-0 plants grown under short-day conditions were used to prepare protoplasts and perform the BiFC assay. The full-length coding regions of *DET1* and *FHY3* were individually subcloned into the *pTOPO* vector and further recombined into the *pSITE-BIFC-nEYFP* (YFP^{n}) or *pSITE-BIFC-cEYFP* (YFP^{c}) vectors (Martin et al., 2009). The combinations of DET1-YFP^{N} and FHY3-YFP^{C}, DET1-YFP^{N}, and YFP^{C}, YFP^{N} and FHY3-YFP^{C} were cotransformed into Arabidopsis protoplasts. After 16 h of incubation under dim light, the fluorescent signals of YFP were detected by laser scanning confocal microscopy (Zeiss).

Immunoblot and Co-IP Assays

For immunoblot assays, total proteins from various seedlings or protoplasts were extracted in extraction buffer containing 0.5% (w/v) SDS. For Co-IP assays, total proteins were extracted from seedlings, protoplasts, or *Nicotiana benthamiana* leaves using the IP buffer and further immunoprecipitated using the specific primary antibodies as indicated. The detailed procedures for immunoblot and Co-IP assays have been described in Li et al. (2011). The primary antibodies anti-FHY3 (catalog no. PHY1892A; PhytoAB), anti-MYC (catalog no. 2278S; Cell Signaling Technology), anti-FLAG (catalog no. A8592; Sigma-Aldrich), anti-HA (catalog no. 11583816001; Roche), anti-Actin (catalog no. CW0264M; CWBIO), anti-H3K4me3 (catalog no. 07-473; Millipore), anti-H3K4ac (catalog no. ab176799, Abcam), anti-H3K14ac (catalog no. ab52946; Abcam), anti-H3K27ac (catalog no. ab4729; Abcam), anti-H3ac (K9/K14/K18/K23/K2; catalog no. ab47915; Abcam), and anti-H3 (catalog no. AF0009; Beyotime) were used to perform IP or immunoblot assays. The intensities of each band were quantified using the program ImageJ (https://imagej.nih.gov/ij/). All the immunoblot and Co-IP assays were repeated at least three times.

RT-qPCR and ChIP-qPCR

Total RNA was extracted from various seedlings with an RNA Extraction Kit (Omega), and first-strand cDNA was synthesized with cDNA Synthesis SuperMix (Transgene). qPCR assays were further performed using UltraSYBR Mixture (CWBIO) using a LightCycler 96 PCR machine (Roche). The expression levels of various genes were normalized to the expression level of the internal control gene *UBQ1*.

Four-day-old etiolated seedlings after light or ABA treatments were used to perform ChIP assays. The primary antibodies anti-MYC, anti-FLAG, anti-GFP, anti-H3K4me3, and anti-H3K27ac were used in the ChIP assays. The detailed procedure for ChIP assays has been described in Ma et al. (2016). The exon region of *ABI5* and the promoter region of *ACTIN12* (*ACT*) were used as the negative control for ChIP assays with DET1 and HDA6. The exon region of *eIF4A1* (*eIF4*) and *ACT* was used as the negative control for ChIP with H3K27ac, H3K4me3, and H3ac. All the RT-qPCR and ChIP-qPCR assays were repeated at least three times. Primers for RT-qPCR and ChIP-qPCR are listed in Supplemental Table S1.

Transient Transcription Dual-Luciferase Assay

To generate the *pGreen-0800-ABI5p$_{FBS}$:LUC* plasmid, the fragment containing the FBS cis-elements in the *ABI5* promoter region was repeated three times and inserted into the *Sal*I- and *Kpn*I-digested *pGreen-0800-35Smini:LUC* vector (Chen et al., 2019). The plasmids *35Sp:FHY3-3FLAG*, *35Sp:DET1-3FLAG*, and *35Sp: HDA6-3FLAG* were coexpressed with *pGreen-0800-ABI5p$_{FBS}$:LUC* as indicated in the dual-luciferase assays in Arabidopsis protoplasts. The detailed procedure for dual-luciferase assays has been described in Li et al. (2011). For LUC/REN, the expression values of the *LUC* reporter were normalized to the expression values of *REN* luciferase driven by the *35S* promoter. Data are means of five biological replicates, and error bars represent SD.

Statistical Analysis

To determine statistical significance, we employed Student's *t* test. *$P < 0.05$ was considered to indicate statistical significance, and **$P < 0.01$ or ***$P < 0.001$ were considered extremely significant.

Accession Numbers

The Arabidopsis Genome Initiative (https://www.arabidopsis.org) locus identifiers for the genes mentioned in this study are as follows: *DET1* (At4g10180), *FHY3* (At3g22170), *HDA6* (At5g63110), *ABI5* (At2g36270), *COP10* (At3g13550), *ACT* (At3g46520), *UBQ1* (At3g52590), and *eIF4A1* (AT3g13920).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. FHY3 physically interacts with COP10 in vitro and in vivo.

Supplemental Figure S2. FHY3 acts downstream of DET1 in mediating ABA responses.

Supplemental Figure S3. Expression pattern of FHY3 and DET1.

Supplemental Figure S4. Effect of DET1 on the protein abundance and DNA binding activity of FHY3.

Supplemental Figure S5. Influence of DET1 on histone modifications of histone H3 during dark-to-light transition and ABA responses.

Supplemental Figure S6. Phosphatase treatment assays of FHY3 protein in plants.

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

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