

***Arabidopsis* DE-ETIOLATED1 Represses Photomorphogenesis by Positively Regulating Phytochrome-Interacting Factors in the Dark**^{CIW}

Jie Dong,^a Dafang Tang,^a Zhaoxu Gao,^a Renbo Yu,^a Kunlun Li,^a Hang He,^a William Terzaghi,^b Xing Wang Deng,^{a,c} and Haodong Chen^{a,1}

^aPeking-Yale Joint Center for Plant Molecular Genetics and Agro-biotechnology, State Key Laboratory of Protein and Plant Gene Research, Peking-Tsinghua Center for Life Sciences, College of Life Sciences, Peking University, Beijing 100871, China

^bDepartment of Biology, Wilkes University, Wilkes-Barre, Pennsylvania 18766

^cDepartment of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520-8104

ORCID ID: 0000-0001-7228-6341 (H.C.)

***Arabidopsis thaliana* seedlings undergo photomorphogenic development even in darkness when the function of DE-ETIOLATED1 (DET1), a repressor of photomorphogenesis, is disrupted. However, the mechanism by which DET1 represses photomorphogenesis remains unclear. Our results indicate that DET1 directly interacts with a group of transcription factors known as the phytochrome-interacting factors (PIFs). Furthermore, our results suggest that DET1 positively regulates PIF protein levels primarily by stabilizing PIF proteins in the dark. Genetic analysis showed that each *pif* single mutant could enhance the *det1-1* phenotype, and ectopic expression of each PIF in *det1-1* partially suppressed the *det1-1* phenotype, based on hypocotyl elongation and cotyledon opening angles observed in darkness. Genomic analysis also revealed that DET1 may modulate the expression of light-regulated genes to mediate photomorphogenesis partially through PIFs. The observed interaction and regulation between DET1 and PIFs not only reveal how DET1 represses photomorphogenesis, but also suggest a possible mechanism by which two groups of photomorphogenic repressors, CONSTITUTIVE PHOTOMORPHOGENESIS/DET/FUSCA and PIFs, work in concert to repress photomorphogenesis in darkness.**

INTRODUCTION

Light not only provides the energy needed for plant development, but also regulates a number of different processes over the course of the plant life cycle, including seed germination, seedling photomorphogenesis, shade avoidance, photoperiodic responses, and flowering (Chen et al., 2004; Jiao et al., 2007). Following germination, seedlings grown in darkness undergo skotomorphogenic development, which is characterized by long hypocotyls, closed cotyledons, and apical hooks. In contrast, seedlings grown in the light exhibit photomorphogenic development characterized by short hypocotyls and open, expanded cotyledons (Von Arnim and Deng, 1996; Chen et al., 2004).

A group of photomorphogenic repressor genes, *CONSTITUTIVE PHOTOMORPHOGENESIS/DE-ETIOLATED/FUSCA* (*COP/DET/FUS*), were initially isolated by genetic screening (Sullivan et al., 2003). Proteins encoded by these genes can form three different complexes, which have all been shown to play significant roles in the ubiquitin-proteasome system. The first group of complexes contains COP1 and Suppressors of PhyA (SPAs),

which form E3 ubiquitin ligases (Yi and Deng, 2005; Zhu et al., 2008). These COP1-SPA complexes negatively regulate the levels of several photomorphogenesis-promoting proteins, including ELONGATED HYPOCOTYL5 (HY5; Osterlund et al., 2000), HY5 Homolog (Holm et al., 2002), LONG AFTER FAR-RED LIGHT1 (LAF1; Seo et al., 2003), and LONG HYPOCOTYL IN FAR-RED1 (Duek et al., 2004; Jang et al., 2005; Yang et al., 2005). The second complex, the COP9 signalosome (CSN), contains eight subunits and is known to interact with CULLIN-containing E3 ligases and regulate their modification by cutting off Nedd8/RELATED TO UBIQUITIN (RUB) (Schwechheimer et al., 2001; Serino and Deng, 2003; Chen et al., 2006). The third complex, CDD (COP10, DNA DAMAGE BINDING PROTEIN1 [DDB1], DET1), may act as a ubiquitylation-promoting factor to regulate photomorphogenesis (Yanagawa et al., 2004). Mutation of either *DET1* or *COP10* is known to induce photomorphogenic development in the dark (Chory et al., 1989; Wei et al., 1994). The mutants of *DET1* in both *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*) accumulated elevated levels of anthocyanins (Chory et al., 1989; Mustilli et al., 1999).

DET1 is the first identified *COP/DET/FUS* locus, and it encodes a nuclear-localized protein and determines the cell type-specific expression of light-regulated promoters to repress photomorphogenesis in darkness (Chory et al., 1989; Pepper et al., 1994). By in vitro experiments and tests in living plant cells, *DET1* was found to bind the N-terminal tail of histone H2B (Benvenuto et al., 2002). *DET1* was also demonstrated to interact with DDB1 to regulate *Arabidopsis* photomorphogenesis (Schroeder et al., 2002), and these proteins were found to interact with *COP10* to

¹ Address correspondence to chenhaodong@pku.edu.cn.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Haodong Chen (chenhaodong@pku.edu.cn).

^{CIW} Some figures in this article are displayed in color online but in black and white in the print edition.

^{CIW} Online version contains Web-only data.

www.plantcell.org/cgi/doi/10.1105/tpc.114.130666

form the CDD complex in vivo (Yanagawa et al., 2004). Later studies demonstrated that the CDD complex formed an E3 ligase with CULLIN4 (CUL4) and thus mediated light-regulated plant development (H. Chen et al., 2006, 2010). Upon UV stress, DET1 could cooperate with CUL4-DDB1^{DDB2} ligase to maintain genome integrity (Castells et al., 2011).

A second group of photomorphogenic repressors, known as phytochrome-interacting factors (PIFs), was identified later. These repressors are basic helix-loop-helix (bHLH) transcription factors that regulate thousands of genes to promote skotomorphogenesis (Leivar et al., 2009; Leivar and Quail, 2011). PIF3 was the first phytochrome-interacting factor identified by a yeast hybrid screen using phytochrome B (phyB) as the bait (Ni et al., 1998). Mutants lacking PIF3 exhibit short hypocotyls under red light conditions, indicating that it is a negative regulator of photomorphogenesis (Kim et al., 2003; Monte et al., 2004). Similarly, several other bHLH transcription factors have also been found to be involved in light signal regulation. For example, it has been demonstrated that PIF1 protein plays an important role in seed germination, hypocotyl elongation, and chlorophyll accumulation (Huq et al., 2004; Oh et al., 2004; Castillon et al., 2007; Park et al., 2011; Shi et al., 2013). PIF4 and PIF5 are also known negative regulators of photomorphogenesis that mediate hypocotyl elongation in red light (Huq and Quail, 2002; Fujimori et al., 2004). Furthermore, the *pifq* mutant, in which four PIF genes (*PIF1*, *PIF3*, *PIF4*, and *PIF5*) are knocked out, shows constitutive photomorphogenesis in the dark, which indicates that these PIFs suppress plant photomorphogenesis in the dark (Leivar et al., 2008; Leivar and Monte, 2014). Recent studies showed that PIFs directly regulate thousands of genes to repress photomorphogenesis (Oh et al., 2009; Hornitschek et al., 2012; Oh et al., 2012; Zhang et al., 2013).

The regulation of PIF protein stability is critically important for their function in plant development, and PIF3 is the PIF representative that has been the subject of the most in-depth research. PIF3 was proved to specifically interact with the Pfr (active) form of phytochrome induced by light (Ni et al., 1999), and both red and far-red light could induce rapid degradation of PIF3 in a phytochrome-dependent manner (Bauer et al., 2004). In phyA- and phyB-mediated light signaling, PIF3 is rapidly polyubiquitinated and subsequently degraded by the 26S proteasome (Park et al., 2004). Further studies showed that photoactivation of phytochromes induced rapid in vivo phosphorylation of PIF1, PIF3, and PIF5, tagging them for ubiquitination and proteosomal degradation (Al-Sady et al., 2006; Shen et al., 2007, 2008). A recent study made a breakthrough by identifying three LRBs (Light-Response Bric-a-Brack/Tramtrack/Broads) as the E3 ligases that targeted PIF3, which could be recruited to the PIF3-phyB complex after light induction, and promoted concurrent polyubiquitination and degradation of both PIF3 and phyB in vivo (Ni et al., 2014). The phosphorylation and ubiquitination of PIF3 probably occur at phytochrome nuclear bodies whose formation is regulated by HEMERA (Al-Sady et al., 2006; M. Chen et al., 2010; Galvão et al., 2012). Compared with the thorough studies on light-regulated PIF3 degradation, the regulation of PIF3 stability in darkness is quite unclear, although it is known that PIF3's accumulation requires COP1 (Bauer et al., 2004).

Although DET1 was the first photomorphogenic repressor to be identified in *Arabidopsis* (Chory et al., 1989), the mechanism by which DET1 suppresses photomorphogenesis in the dark is relatively unclear compared with other repressors. Genomic analyses have demonstrated that the expression of thousands of genes is altered in *det1-1* mutants in the dark compared with the wild type (Ma et al., 2003). Recent research has shown that DET1 can directly interact with the transcription factors CCA1 and LHY1 to regulate the plant circadian clock (Lau et al., 2011). Consequently, we speculated that DET1 might also interact with key transcription factors in light signal transduction to regulate thousands of downstream genes to repress photomorphogenesis in *Arabidopsis*. Our yeast two-hybrid assays identified interactions between DET1 and PIFs, which were confirmed by several other strategies. Further studies demonstrated that DET1 positively regulates PIF protein levels, primarily through the stabilization of PIF proteins in the dark and thus provides one potential mechanism by which DET1 represses photomorphogenesis in the dark. Furthermore, our results also reveal the existence of a connection between COP/DET/FUS and PIFs, two groups of important photomorphogenic repressors.

RESULTS

DET1 Interacts with PIFs Both in Vitro and in Vivo

Based on our hypothesis that DET1 may repress photomorphogenesis by interacting with transcription factors (Ma et al., 2003; Lau et al., 2011), we tested for interactions between DET1 and four PIF transcription factors (PIF1, PIF3, PIF4, and PIF5) that are known to be key regulators in light signal transduction. Since previous research demonstrated that full-length DET1 can repress transcriptional activity and the DET1 fragment consisting of the 26th to 87th amino acid residues could be used to effectively test interactions in yeast (Lau et al., 2011), we used this DET1 peptide as the bait with which to test possible interactions with PIF proteins. The results of our yeast two-hybrid assays indicated that this DET1 (26 to 87 amino acids) fragment interacted with PIF1, PIF3, PIF4, and PIF5 (Figure 1A), which had been shown to be repressors of photomorphogenesis (Leivar et al., 2008). In addition, our data showed that DET1 could also interact with PIF6 and PIF7 (Supplemental Figure 1), which had been demonstrated to play important roles in seed dormancy and shade avoidance, respectively (Penfield et al., 2010; Li et al., 2012). To further investigate the PIF domains responsible for these interactions, PIF3 was selected as a representative transcription factor. First, we tested interactions between the DET1 (26 to 87 amino acids) fragment and PIF3 fragments lacking one domain. The DET1 fragment showed strong interactions with both the Δ bHLH and Δ APA (active phyA binding region) fragments of PIF3, but almost no interaction with the Δ APB (active phyB binding region) fragment (Figure 1B). These results indicated that the APB domain of PIF3 was essential for the interaction, so we next tested whether the APB domain itself was sufficient for this interaction. However, the DET1 fragment did not interact with PIF3's APB domain alone, which indicates that the interaction between DET1 and PIF3 requires multiple PIF3

domains (Figure 1B). To further confirm the interactions between DET1 and PIFs, we performed *in vitro* and *in vivo* pull-down assays. The glutathione S-transferase (GST)-tagged DET1 (26 to 87 amino acids) fragment was able to pull down both His-tagged PIF1 and His-tagged PIF3 *in vitro* (Figure 1C), and GST-PIF4 and GST-PIF5 could pull down His-DET1 *in vitro* (Figures 1D and 1E), which is consistent with the results obtained using yeast two-hybrid assays. Furthermore, in a semi-*in vivo* pull-down assay, both Myc-tagged PIF1 and Myc-tagged PIF3 from *Arabidopsis* were shown to interact with His-DET1 (Figure 1F). To test for interactions between DET1 and PIFs *in vivo*, coimmunoprecipitation and bimolecular fluorescence complementation (BiFC) assays were performed. Our results showed endogenous DET1 proteins could be pulled down by both Myc-tagged PIF1 and Myc-tagged PIF3 (Figure 1G) and DET1 could interact with PIF1, PIF3, PIF4, and PIF5 in a BiFC assay (Figure 1H).

DET1 Positively Regulates PIF3 Protein Levels during Seedling Development

In order to determine how DET1 regulates seedling development, the phenotypes of *det1-1* mutants and wild-type Columbia ecotype (Col) seedlings were compared in the dark. The phenotypes of Col and *det1-1* were almost indistinguishable after 48 h of growth. However, clear differences began to appear after 60 h of growth and became more obvious after 72 h (Figure 2A). We next examined PIF3 protein levels in Col and *det1-1* seedlings grown in the dark for 2 to 4 d and observed significantly lower levels of PIF3 in *det1-1* than in Col. PIF3 protein was almost undetectable in 4-d-old dark-grown *det1-1* seedlings, which exhibited photomorphogenic phenotypes (Figure 2B). To further confirm that DET1 regulates the accumulation of PIF3 protein, we examined the PIF3 levels in two transgenic lines expressing Myc-DET1 or green fluorescent protein (GFP)-DET1 in the *det1-1* background (Supplemental Figure 2B). The PIF3 levels in these two transgenic lines were much higher than those observed in *det1-1*, and the amounts of PIF3 protein in these plants were consistent with their photomorphogenic phenotypes (Figure 2C; Supplemental Figure 2A). Furthermore, in order to determine whether DET1 positively regulates PIF3 by itself or in the form of a CUL4-CDD complex, we examined PIF3 levels in dark-grown *cul4cs*, *cop10-4*, and *cop10-1* seedlings. PIF3 protein levels in *cul4cs* and *cop10-4* were obviously lower than in Col in the dark, and its level in *cop10-1* was much lower (Figure 2D). The remaining PIF3 levels correlated well with the mutants' photomorphogenic phenotypes (Figure 2D; Supplemental Figures 3A and 3B), which indicated that DET1 positively regulated PIF3 levels, possibly as a component of the CUL4-CDD complex. CSN is a protein complex that can regulate the ubiquitination (RUB conjugation) of CUL4 (Chen et al., 2006), and the PIF3 level in the null mutant *cop9-1* (lack of the CSN complex) was reduced to an undetectable level (Supplemental Figures 3C and 3D). Due to the redundancy of CSN5A and CSN5B, *csn5a* mutants contained normal levels of PIF3 protein and didn't show photomorphogenic phenotypes in the dark (Supplemental Figures 3C and 3D). In contrast, no significant differences were observed between the levels of CUL4 and DET1 proteins in *piif* single and quadruple mutants and Col seedlings (Figure 2E; Supplemental

Figure 4). Taken together, these data demonstrate that while DET1 and other components of the CUL4-CDD complex positively regulate levels of PIF proteins, PIFs have no effect on the levels of DET1 and CUL4 proteins. This result, in turn, suggests that PIFs work downstream of DET1 or the CUL4-CDD complex in light signal transduction.

DET1 Is Necessary for the Stability of PIF Proteins in the Dark

The findings that DET1 interacts with PIFs and that PIF3 protein levels are dramatically lower in *det1-1* mutants (Figures 1 and 2) indicate that DET1 may positively regulate PIF protein levels. To test whether DET1 regulates PIFs at the level of protein or mRNA in the dark, we first examined the levels of *PIF1*, *PIF3*, *PIF4*, and *PIF5* transcripts in *det1-1* and wild-type plants using quantitative RT-PCR (qRT-PCR). The levels of *PIF3* transcripts were significantly lower in *det1-1* than in Col, while the levels of *PIF1*, *PIF4*, and *PIF5* transcripts were similar in Col and *det1-1* (Figure 3A). Previous research has revealed that ethylene can increase the levels of *PIF3* transcripts by stabilizing ETHYLENE INSENSITIVE3 (Zhong et al., 2012). Consequently, we grew *det1-1* and wild-type seedlings on Murashige and Skoog (MS) plates containing 10 μ M 1-aminocyclopropane-1-carboxylic acid (ACC). While the levels of *PIF3* transcripts in *det1-1* seedlings grown on ACC plates were similar to those in Col seedlings grown on MS plates (Figure 3B, top panel), PIF3 protein levels in the *det1-1* mutants treated with ACC were significantly lower than those in the wild type not treated with ACC (Figure 3B, bottom panel). These data thus indicate that there must be a posttranscriptional mechanism that modulates DET1's positive regulation of PIF protein levels. In order to confirm the regulation at the posttranscriptional level, we crossed *35S:PIF1-Myc*, *35S:PIF3-Myc*, *35S:PIF4-Myc*, and *35S:PIF5-Myc* transgenic lines into the *det1-1* mutant and compared the protein levels of these Myc-tagged PIFs in *det1-1* and wild-type backgrounds. The levels of all four Myc-tagged PIF proteins were significantly lower in the *det1-1* than in the wild-type background (Figures 3C and 3D). In order to further determine whether regulation occurs during or after translation, cycloheximide (CHX) was used to block *de novo* protein synthesis in dark-grown seedlings. As shown in Figures 3E to 3L, all four PIF-Myc proteins decayed significantly more rapidly in the *det1-1* than in the wild-type background, indicating that DET1 positively regulates PIF proteins by stabilizing them in the dark.

A Cocktail of Proteasomal Inhibitors Cannot Prevent the Instability of PIFs in *det1-1* Mutants

Since the ubiquitin-proteasome system is the primary pathway for protein degradation, and PIF3 is known to be degraded via this pathway upon exposure to light (Al-Sady et al., 2006), we decided to test whether the instability of PIF proteins in the *det1-1* background was also regulated by this pathway. A cocktail of proteasomal inhibitors (MG132, PS-341, and MLN2238) was used to block proteasomal degradation and then PIF3 protein levels were checked in *det1-1* and wild-type seedlings. Surprisingly, treatment with this cocktail did not increase the levels of

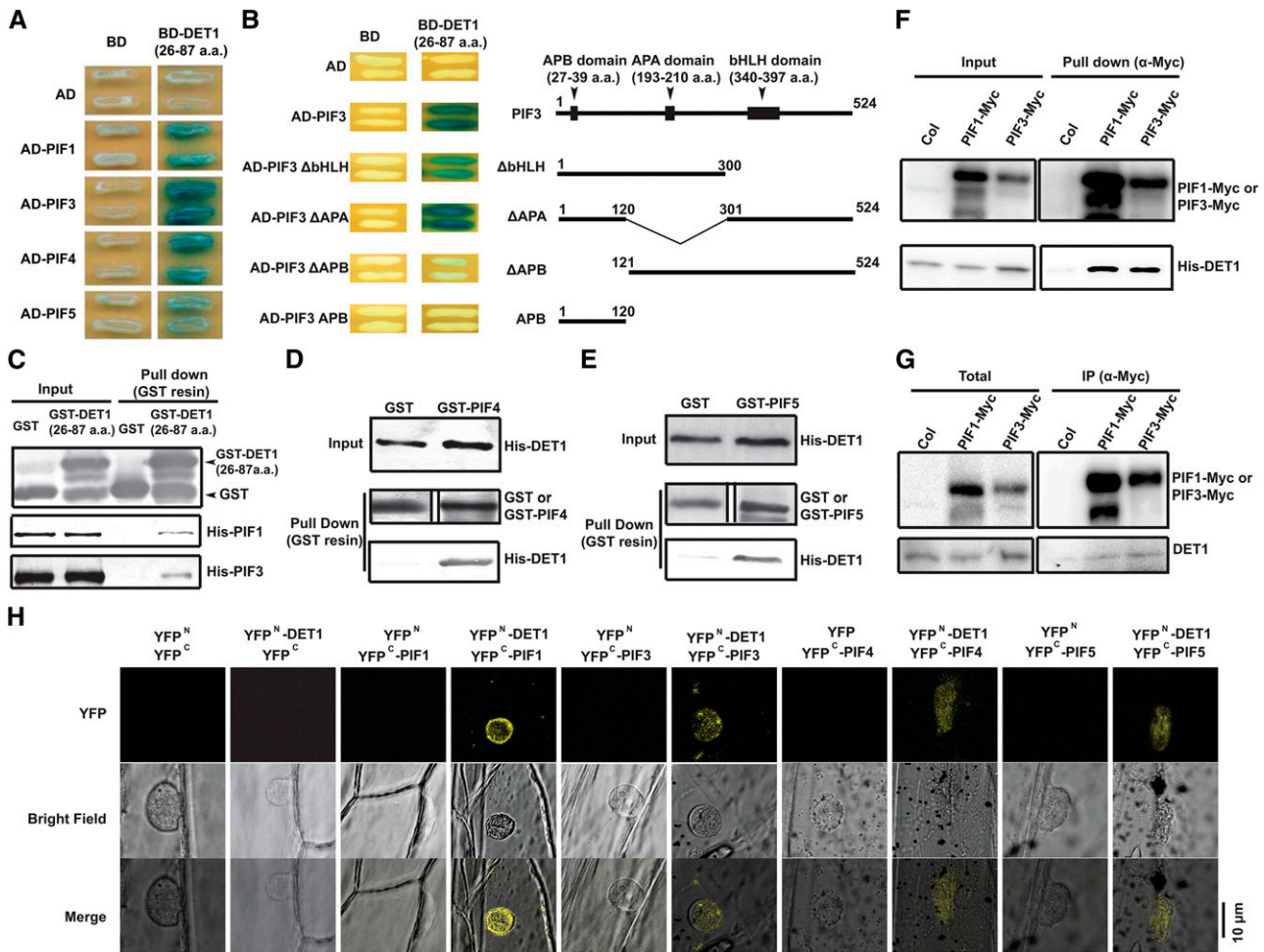


Figure 1. DET1 Interacts with PIFs Both in Vitro and in Vivo.

(A) DET1 interacts with PIF1, PIF3, PIF4, and PIF5 in yeast cells. The DET1 (26 to 87 amino acids) fragment was fused with the binding domain (BD) as bait. Full-length PIF1, PIF3, PIF4, and PIF5 were fused with the activation domain (AD) as prey. Empty vectors were used as negative controls.

(B) DET1 interacts with multiple domains of PIF3 in yeast cells. The DET1 (26 to 87 amino acids) fragment and different fragments of PIF3 were fused with BD and AD, respectively. Empty vectors were used as negative controls. The schematic structures of full-length and various fragments of PIF3 are shown on the right. Δ bHLH, PIF3 protein without bHLH domain; Δ APA, PIF3 protein without APA domain; Δ APB, PIF3 protein without APB domain; APB, PIF3 protein with only APB domain.

(C) GST-tagged DET1 (26 to 87 amino acids) fragment can pull down His-tagged PIFs in vitro. His-tagged PIF1 and PIF3 were incubated with immobilized GST or GST-tagged DET1 (26 to 87 amino acids) fragments and then the precipitated fractions were analyzed with GST and His antibodies.

(D) and **(E)** GST-PIF4 and GST-PIF5 can pull down His-DET1 in vitro. Recombinant His-DET1 was incubated with either PIF4 and PIF5 fused to GST and then the precipitated fractions were analyzed with GST and His antibodies.

(F) *Arabidopsis* Myc-tagged PIFs can pull down recombinant His-DET1 in semi-in vivo pull-down assays. Total plant proteins were extracted from 4-d-old dark-grown seedlings of Col, *35S::PIF1-Myc* (abbreviated as PIF1-Myc), and *35S::PIF3-Myc* (abbreviated as PIF3-Myc). Recombinant His-DET1 was purified from *Escherichia coli* and added to total plant protein extracts. The precipitates and total extracts were analyzed with Myc and His antibodies.

(G) Myc-tagged PIFs associate with DET1 in vivo. Total proteins were extracted from 4-d-old dark-grown seedlings of Col, *35S::PIF1-Myc*, and *35S::PIF3-Myc*. Anti-Myc antibody was used for immunoprecipitation, and the precipitates and total extracts were further analyzed with Myc and DET1 antibodies.

(H) DET1 interacts with PIFs in vivo. YFP^N-DET1 and YFP^C-PIF1, -PIF3, -PIF4, and -PIF5 were transiently transformed into onion epidermal cells to test their interactions using the BIFC assay. After 24 h incubation in the dark, YFP signal was detected by confocal microscopy. Bright field was used to indicate the localization of nuclei. Empty vectors were used as negative controls.

endogenous PIF3 or ectopic PIF-Myc proteins in the *det1-1* background (Figures 4A to 4D), contrasting with the previously published result that treatment with proteasomal inhibitors inhibited red light-induced PIF3 degradation (Al-Sady et al., 2006; Supplemental Figures 5A and 5B). Similarly, treating *cop1-4*

mutants with this cocktail in the dark did not increase the level of PIF3 protein (Supplemental Figures 5C and 5D). Overall, if this cocktail of proteasomal inhibitors worked efficiently to inhibit the proteasome system in the *det1-1* mutant, these data indicate that the instability and degradation of PIF3 in *det1-1* in the dark

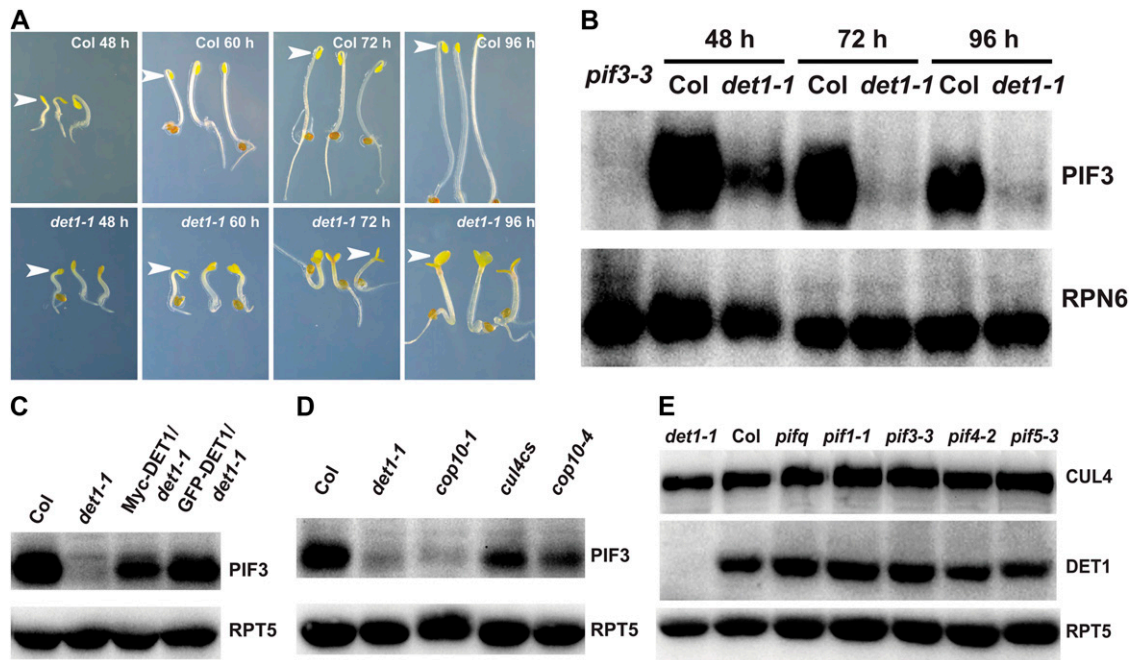


Figure 2. DET1 Positively Regulates PIF3 Protein Levels.

(A) Seedlings of Col and *det1-1* mutants grown in the dark for 48, 60, 72, and 96 h. Arrowheads indicate the cotyledons of seedlings. Col, wild type of Columbia ecotype.

(B) PIF3 protein levels in Col and *det1-1* mutants. Total proteins were extracted from seedlings grown in the dark for indicated times (48, 72, and 96 h) and then analyzed by immunoblots using anti-PIF3 and anti-RPN6 antibodies. RPN6 was used as a control.

(C) PIF3 protein levels in Col, *det1-1*, GFP-DET1/*det1-1*, and Myc-DET1/*det1-1* seedlings. Total proteins were extracted from 4-d-old dark-grown seedlings and then analyzed by immunoblots using antibodies against PIF3 and RPT5.

(D) PIF3 protein levels in Col, *det1-1*, *cul4cs*, *cop10-1*, and *cop10-4* seedlings. Total proteins were extracted either from 4-d-old dark-grown seedlings and then analyzed by immunoblots using antibodies against PIF3 and RPT5.

(E) DET1 and CUL4 protein levels in Col and various *pif* mutants. Total proteins were extracted from 4-d-old dark-grown seedlings and then analyzed by immunoblots using CUL4, DET1, and RPT5 antibodies.

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

is possibly not driven by the ubiquitin-proteasome system, which may be distinct from the light-induced proteasomal PIF3 degradation.

Mutation of Each PIF Gene Results in Enhancement of the *det1-1* Mutant Phenotype

To further clarify the relationship between DET1 and PIFs in photomorphogenic repression in the dark, we examined genetic interactions between *det1-1* and *pif* mutants by crossing *det1-1* with each *pif* single mutant. Interestingly, the dark-grown double mutants *pif1-1 det1-1*, *pif3-3 det1-1*, *pif4-2 det1-1*, and *pif5-3 det1-1* exhibited more exaggerated photomorphogenic phenotypes than either parental single mutant in terms of both hypocotyl lengths and cotyledon opening angles (Figures 5A to 5D). Moreover, all of these double mutants also exhibited shorter hypocotyls than *det1-1* mutants under red light except for *pif5-3 det1-1* (Supplemental Figure 6). Although the single *pif* mutants showed no obvious phenotypic changes compared with the wild type in the dark, further removal of each PIF in the *det1-1* background resulted in an enhanced phenotype. Together with the biochemical data that DET1 positively regulates PIFs abundance,

these genetic analyses support the notion that DET1 works upstream of PIFs to repress photomorphogenesis in the dark.

Overexpression of Each PIF Can Partially Suppress the *det1-1* Mutant Phenotype in Seedling Development

Given that DET1 is known to positively regulate PIF protein levels and that each *pif* single mutant has been shown to enhance the *det1-1* mutant phenotype in the dark, it seems likely that PIFs may work downstream of DET1 to repress photomorphogenesis. To verify this hypothesis, the phenotypes of 35S:PIF1-Myc/*det1-1* (PIF1-Myc/*det1-1*), 35S:PIF3-Myc/*det1-1* (PIF3-Myc/*det1-1*), 35S:PIF4-Myc/*det1-1* (PIF4-Myc/*det1-1*), and 35S:PIF5-Myc/*det1-1* (PIF5-Myc/*det1-1*) seedlings were compared with that of *det1-1*. The hypocotyl lengths of PIF1-Myc/*det1-1* and PIF3-Myc/*det1-1* were comparable to those of *det1-1*, whereas those of PIF4-Myc/*det1-1* and PIF5-Myc/*det1-1* were significantly longer (Figures 6A and 6B). At the same time, both the cotyledon opening percentages and opening angles of PIF1-Myc/*det1-1*, PIF3-Myc/*det1-1*, and PIF4-Myc/*det1-1* were much lower than their *det1-1* counterparts. The opening percentages and angles of PIF5-Myc/*det1-1*, however, were comparable

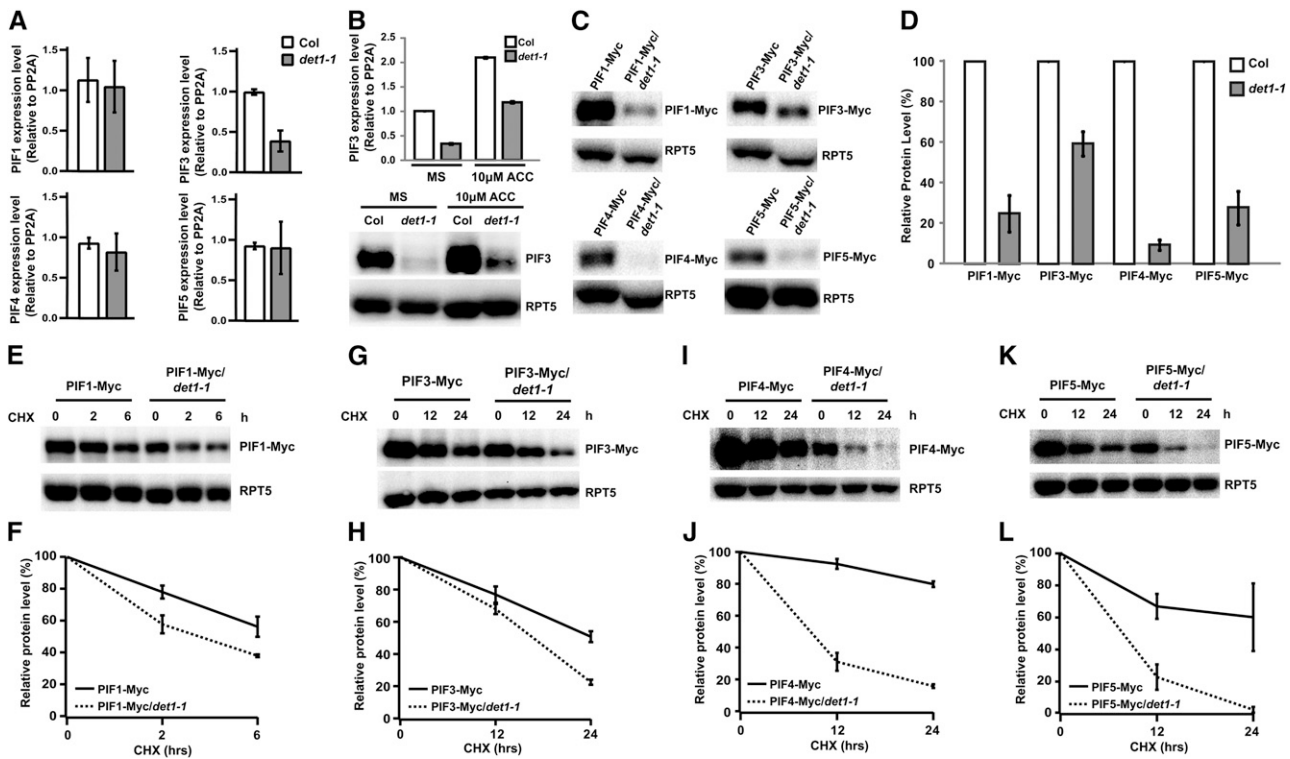


Figure 3. DET1 Stabilizes PIF Proteins in the Dark.

(A) *PIF1*, *PIF3*, *PIF4*, and *PIF5* transcript levels in Col and *det1-1* mutants. Total RNAs were extracted from 4-d-old dark-grown seedlings. The levels of different PIF transcripts were quantified by qRT-PCR. The expression of *PP2A* was used as an internal control. Data are shown as mean \pm SD, $n = 3$. (B) Increasing PIF3 transcripts in *det1-1* to wild-type level failed to rescue PIF3 proteins to normal levels. Top panel: Total RNA was extracted from 4-d-old seedlings grown on MS medium in darkness with or without ACC and then PIF3 transcripts were quantified by qRT-PCR. The expression of *PP2A* was used as an internal control. Data are shown as mean \pm SD, $n = 3$. Bottom panel: Total proteins were extracted from seedlings treated the same as in the top panel and then were subjected to immunoblot analysis with PIF3 and RPT5 antibodies. RPT5 was used as a control. (C) and (D) Comparison of PIF1-Myc, PIF3-Myc, PIF4-Myc, and PIF5-Myc protein levels in Col and *det1-1* backgrounds. *35S:PIF1-Myc*, *35S:PIF3-Myc*, *35S:PIF4-Myc*, and *35S:PIF5-Myc* were respectively crossed into *det1-1* and designated PIF1-Myc/*det1-1*, PIF3-Myc/*det1-1*, PIF4-Myc/*det1-1*, and PIF5-Myc/*det1-1*, respectively. Total proteins were extracted from 4-d-old dark-grown seedlings and then analyzed by immunoblot (C) and quantified using Image J software (D). RPN6 or RPT5 was used as a control. Quantitative data are shown as mean \pm SE, $n = 3$. (E) to (L) PIF-Myc protein stability in Col and *det1-1* backgrounds. Total proteins were extracted from 4-d-old dark-grown seedlings treated with 100 μ M CHX for the indicated times and then analyzed by immunoblot (E), (G), (I), and (K) and quantified using image J software (F), (H), (J), and (L). PIF-Myc protein levels were normalized to RPT5, and the value of starting point was set to 100. Quantitative data are shown as mean \pm SE, $n = 3$.

to those of *det1-1* seedlings (Figures 6C to 6E). Moreover, the hypocotyls of PIF3-Myc/*det1-1* and PIF4-Myc/*det1-1* seedlings were significantly longer than those of *det1-1* seedlings under red light (Supplemental Figure 7). During the dark to light transition, significantly less anthocyanin accumulated and greening percentages were higher in PIF1-Myc/*det1-1*, PIF3-Myc/*det1-1*, PIF4-Myc/*det1-1*, and PIF5-Myc/*det1-1* seedlings than in *det1-1* seedlings (Supplemental Figure 8). Together, these data support the conclusion that DET1 represses photomorphogenesis partially via PIFs.

DET1 Regulates Light-Directed Transcriptomic Changes Partially through PIFs during Seedling De-Etiolation

DET1 and PIFs have both been demonstrated to regulate large numbers of light-mediated genes during *Arabidopsis* seedling

development via microarray-based expression profiling analyses (Ma et al., 2003; Leivar et al., 2009). However, their regulation profiles have not been compared directly. If DET1 represses photomorphogenesis through PIFs, many genes regulated by light via DET1 should in turn be regulated by PIFs. To test this hypothesis, we used mRNA deep-sequencing analysis to examine and compare changes in seedling transcriptomes regulated by DET1, PIFs, and light (Supplemental Data Set 1). We performed transcriptomic analyses of wild-type, *det1-1*, and *pifq* seedlings grown in the dark and of wild-type seedlings exposed to white light for 6 h. Compared with dark-grown Col (Col_D), we identified 3050 differentially expressed genes (statistically significant 2-fold changes [SSTF]) in seedlings exposed to white light for 6 h (Col_DL6 h) that are hereafter referred to as light-regulated genes (Supplemental Data Set 2). We then compared the expression profiles of *det1-1* (*det1-1_D*) and wild-type

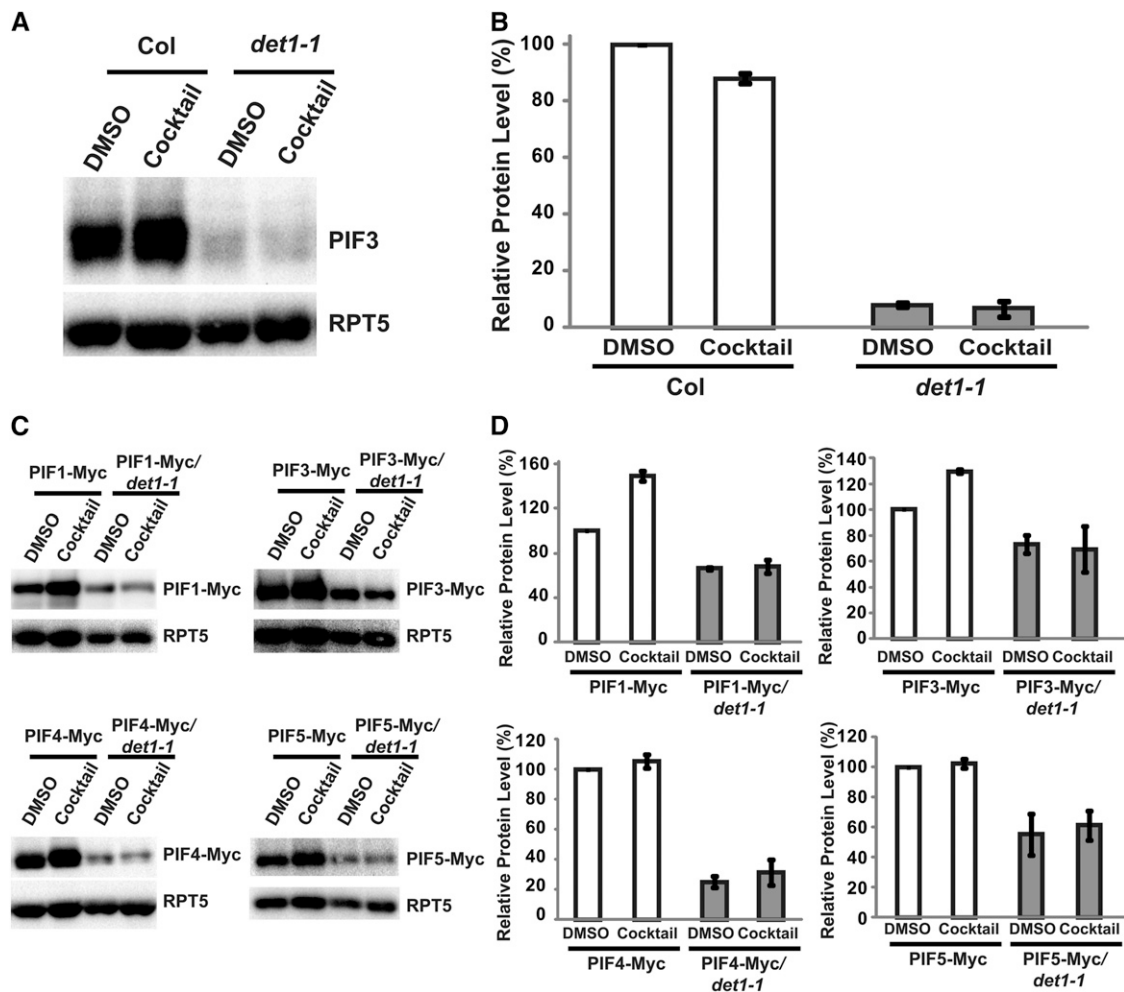


Figure 4. A Cocktail of Proteasomal Inhibitors Cannot Prevent the Instability of PIF Proteins in the *det1-1* Background in the Dark.

(A) and **(B)** The instability of endogenous PIF3 in *det1-1* cannot be inhibited by a cocktail of proteasomal inhibitors. Four-day-old dark-grown seedlings were treated with DMSO or 50 μ M of the cocktail of proteasomal inhibitors (MG132, PS-341, and MLN2238) for 4 h in the dark. Seedlings treated with DMSO were used as negative controls. Total proteins were then extracted and analyzed by immunoblots using PIF3 and RPT5 antibodies **(A)**, and quantitative data are shown as mean \pm SE, $n = 3$ **(B)**.

(C) and **(D)** The instability of all the four ectopic PIF-Myc proteins in *det1-1* cannot be inhibited by the cocktail of proteasomal inhibitors. The experimental conditions are the same as in **(A)**. Myc and RPT5 antibodies were used for the immunoblots analysis **(C)**, and quantitative data are shown as mean \pm SE, $n = 3$ **(D)**.

dark-grown seedlings and identified 3740 SSTF genes, referred to as *det1*-regulated genes (Supplemental Data Set 2). Similarly, 3775 SSTF genes were identified in dark-grown *pifq* seedlings (*pifq*_D), referred to as *pifq*-regulated genes (Supplemental Data Set 2). A heat map of light-, *det1*-, and *pifq*-regulated genes showed very similar transcriptomic changes in the three data sets (Figure 7A). We then analyzed the overlapping light-, *pifq*-, and *det1*-regulated genes by Venn diagrams to find out the key genes regulated by light through DET1 and PIFs. Among the 1684 genes regulated by both light and *det1-1*, 940 genes (55.8%) were regulated by *pifq*, which supports our hypothesis that DET1 represses photomorphogenesis partially through PIFs (Figure 7B). This result indicates

that DET1 may repress photomorphogenesis through multiple transcription factors in the dark, but PIFs seem to play major roles among them. The 940 genes coregulated by light, *det1-1*, and *pifq* were considered to be the key genes regulating photomorphogenesis (Figure 7B; Supplemental Data Set 3). Among those 940 genes, 575 were upregulated together, 296 were downregulated together, and 69 showed other regulation patterns (Figure 7C; Supplemental Data Set 3). The cluster analysis and the heat map revealed that these coregulated genes showed highly similar expression patterns (Figure 7D). Therefore, our data support the conclusion that DET1 affects the expression of light-regulated genes to repress photomorphogenesis, and this is partially

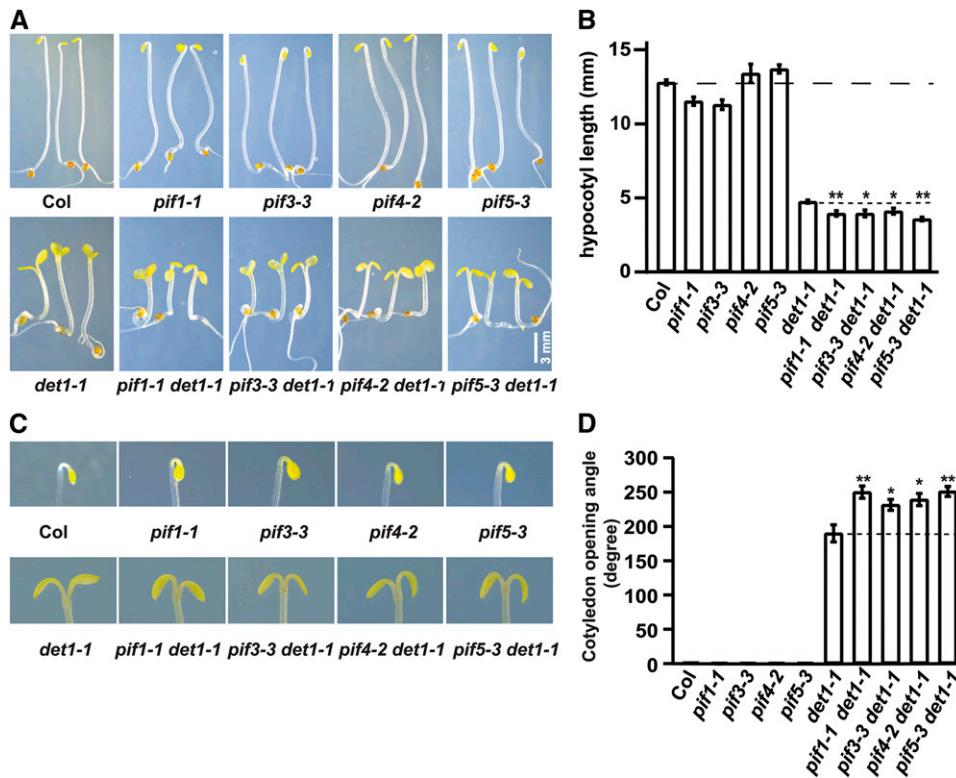


Figure 5. Each Single *PIF* Mutation Can Enhance the *DET1* Mutant Phenotype in the Dark.

(A) and (B) Hypocotyl lengths of 4-d-old dark-grown seedlings. *pif1-1*, *pif3-3*, *pif4-2*, and *pif5-3* were crossed into *det1-1*, respectively. Then, the double mutants and parental lines were grown in the dark for phenotype comparison.

(C) and (D) Cotyledon opening angles of 4-d-old dark-grown seedlings. The genotypes are the same as in (A).

In (B) and (D), data are shown as mean \pm SE, which were analyzed based on more than 20 seedlings. Statistical significance was determined using Student's *t* tests between double mutants and *det1-1*. **P* < 0.01; ***P* < 0.001.

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

mediated through the interaction with and stabilization of PIFs.

DET1 Regulates Photosynthesis, Cell Wall Organization, and Auxin-Responsive Genes through PIFs to Repress Photomorphogenesis

To identify the biological processes in which the genes coregulated by light, *det1*, and *pifq* were involved, we performed gene ontology analysis and functional clustering using the functional annotation of DAVID (Huang et al., 2009). The genes that were coregulated by light, *det1*, and *pifq* were enriched in photosynthesis, light stimulus, pigment biosynthesis, cell redox homeostasis, glucan metabolic process, and protein complex assembly (Figure 8A, left panel). We then divided these coregulated genes into two classes, coregulated and codownregulated genes, to further identify which biological processes were coregulated by light, *det1*, and *pifq*. The coregulated genes were enriched in photosynthesis, light stimulus, pigment biosynthesis, cell redox homeostasis, glucan metabolic process, protein complex assembly, as well as defense against bacteria

and tetraterpenoid biosynthesis (Figure 8A, middle panel). The codownregulated genes were enriched in far-red light stimulus, cell wall organization, response to organic substance, and response to auxin stimulus (Figure 8A, right panel). Among these categories, photosynthesis, cell wall loosening, and response to auxin stimulus are known to be important biological processes affecting plant development. Quantitative PCR assays further confirmed that the representative genes involved in photosynthesis, chlorophyll biosynthesis, cell wall loosening, and response to auxin stimulus indeed showed the same changes in expression in *det1* and *pifq* (Figures 8B and 8C), which indicates that *DET1* may regulate these genes through PIFs to repress photomorphogenesis.

DISCUSSION

DET1 Directly Interacts with the PIF Group of Transcription Factors and Positively Modulates Their Protein Stability

DET1 was the first photomorphogenic suppressor identified and has been shown to affect the expression of many light-regulated

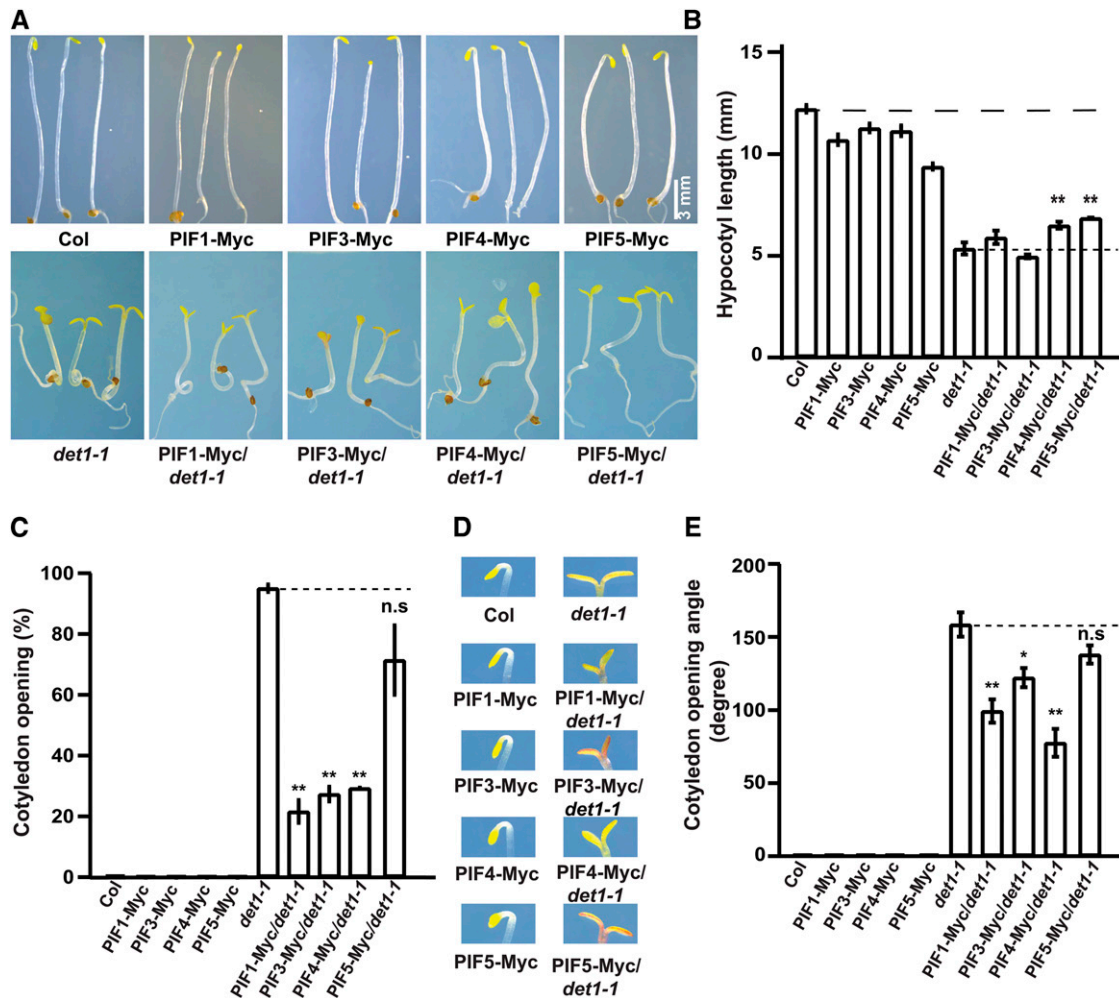


Figure 6. Ectopic Expression of PIFs Can Partially Suppress the Phenotype of the DET1 Mutant in the Dark.

(A) and (B) Hypocotyl lengths of 4-d-old dark-grown seedlings. *35S:PIF1-Myc*, *35S:PIF3-Myc*, *35S:PIF4-Myc*, and *35S:PIF5-Myc* were crossed into *det1-1* and then the double mutants and parental lines were grown in the dark for phenotype comparison.

(C) Cotyledon opening (%) of 3-d-old dark-grown seedlings. The genotypes are the same as in (A).

(D) and (E) Cotyledon opening angles of 4-d-old dark-grown seedlings. The genotypes are the same as in (A).

In (B), (C), and (E), data are shown as mean \pm SE, which were analyzed based on more than 20 seedlings. Statistical significance was determined using Student's *t* test between crossed plants and *det1-1*. n.s., $P > 0.01$; * $P < 0.01$; ** $P < 0.001$.

genes (Chory et al., 1989; Chory and Peto, 1990; Mayer et al., 1996). Although the DET1 protein was shown to localize in the nucleus in vivo, it was not shown to bind DNA (Pepper et al., 1994). Thus, DET1 may regulate the expression of light-regulated genes by interacting with other transcription factors involved in light signaling.

Previous research has shown that DET1 can repress transcription. Specifically, it has been shown to physically interact with two MYB transcription factors, CCA1 and LHY, to repress the expression of targeted genes in the plant circadian clock (Lau et al., 2011). Recent research has also demonstrated that DET1 can inhibit the ubiquitination of LHY by the E3 ubiquitin ligase SINAT5 and thus plays a role in regulating *Arabidopsis* flowering (Song and Carré, 2005; Park et al., 2010).

However, the mechanism by which DET1 regulates the expression of genes involved in photomorphogenesis remains unclear.

In this study, we demonstrated that DET1 could physically interact with PIF proteins both in vitro and in vivo (Figure 1; Supplemental Figure 1) and that DET1 and other components of the CUL4-CDD complex could positively regulate PIF protein levels (Figure 2). Further examination also revealed that this positive regulation was accomplished at both the transcriptional and posttranscriptional levels (Figure 3). While DET1 was shown to positively regulate PIF3 at the transcriptional level, it was not observed to influence the transcription of PIF1, PIF4, and PIF5 (Figures 3A and 3B). In contrast, DET1 positively regulated all four PIFs at the protein level via posttranslational regulation

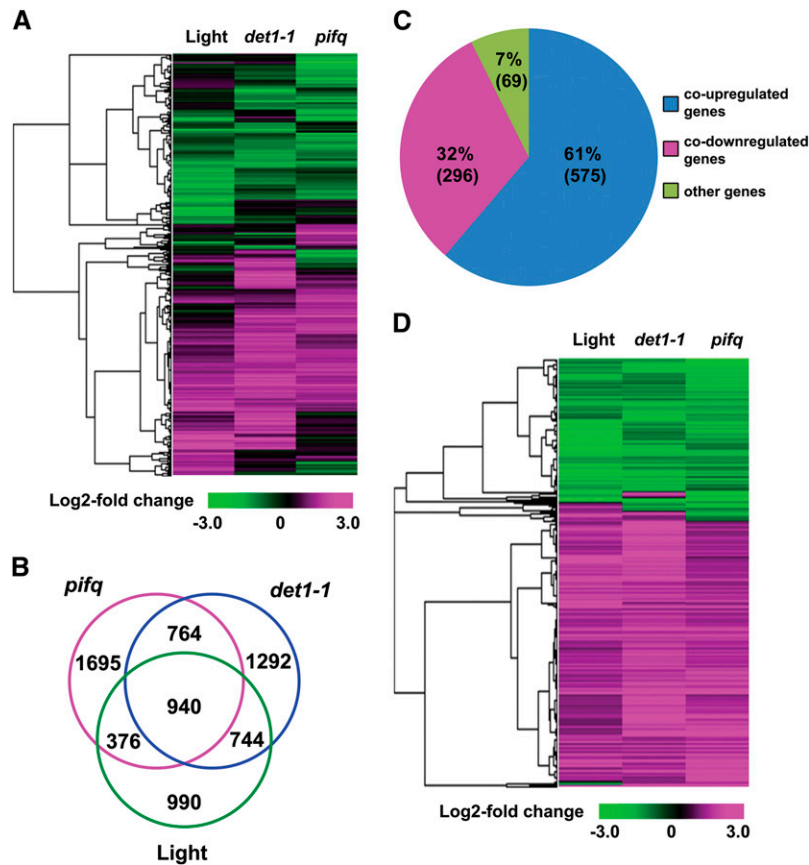


Figure 7. DET1 Mediates the Light-Regulated Transcriptomic Changes in Seedlings Partially through PIFs.

- (A) Heat map of light-, *det1-1*-, and *pifq*-regulated genes. Genes that were differentially expressed in at least one condition were analyzed. The color scale represents the log₂ of fold change.
- (B) Venn diagram showing the number of overlapping light-, *det1-1*-, and *pifq*-regulated genes.
- (C) Percentages of coregulated genes by light, *det1-1*, and *pifq*: couperegulated, codownregulated, or in other patterns. The numbers in parentheses refer to the numbers of genes.
- (D) Heat map of genes coregulated by light, *det1-1*, and *pifq*. Genes that were differentially expressed in all three conditions were analyzed. The color scale represents the log₂ of fold change.

(Figures 3C to 3L). Since proteasomal inhibitors have been shown to inhibit red light-induced PIF3 degradation via the ubiquitin-proteasomal pathway (Supplemental Figure 5A), we set out to determine whether the instability of PIFs in *det1-1* was also mediated through the ubiquitin-proteasomal pathway. To our surprise, however, treatment with a cocktail of proteasomal inhibitors (MG132, PS-341, and MLN2238) was not observed to increase PIF protein levels in the *det1-1* background (Figure 4). This, in turn, suggested that DET1 possibly stabilized targeted proteins through alternative pathways.

To date, DET1 has been shown to regulate transcription factors through at least three different pathways. First, DET1 can interact with and thus repress the transcriptional activity of transcription factors (Lau et al., 2011). Second, DET1 can stabilize target proteins by inhibiting their ubiquitination by E3 ligases (Park et al., 2010). Finally, this study indicates that DET1 possibly stabilizes target proteins through pathways other than

the regular ubiquitin-proteasomal pathway. However, the details of the mechanism by which DET1 positively regulates PIF protein levels remain unclear. Overall, this study both increases our knowledge regarding the role of DET1 in repressing photomorphogenesis and reveals a possible mechanism through which DET1 interacts with and regulates transcription factors.

COP/DET/FUS Proteins Act through PIFs in Mediating Genome Expression and Thus Repression of Photomorphogenesis

COP/DET/FUS is a group of photomorphogenic repressors that were initially identified through genetic screening and were later found to form three complexes involved in the ubiquitin-proteasomal pathway (Sullivan et al., 2003). It was further shown that these complexes are connected through the formation or regulation of CUL4-based E3 ligases (H. Chen et al., 2006, 2010;

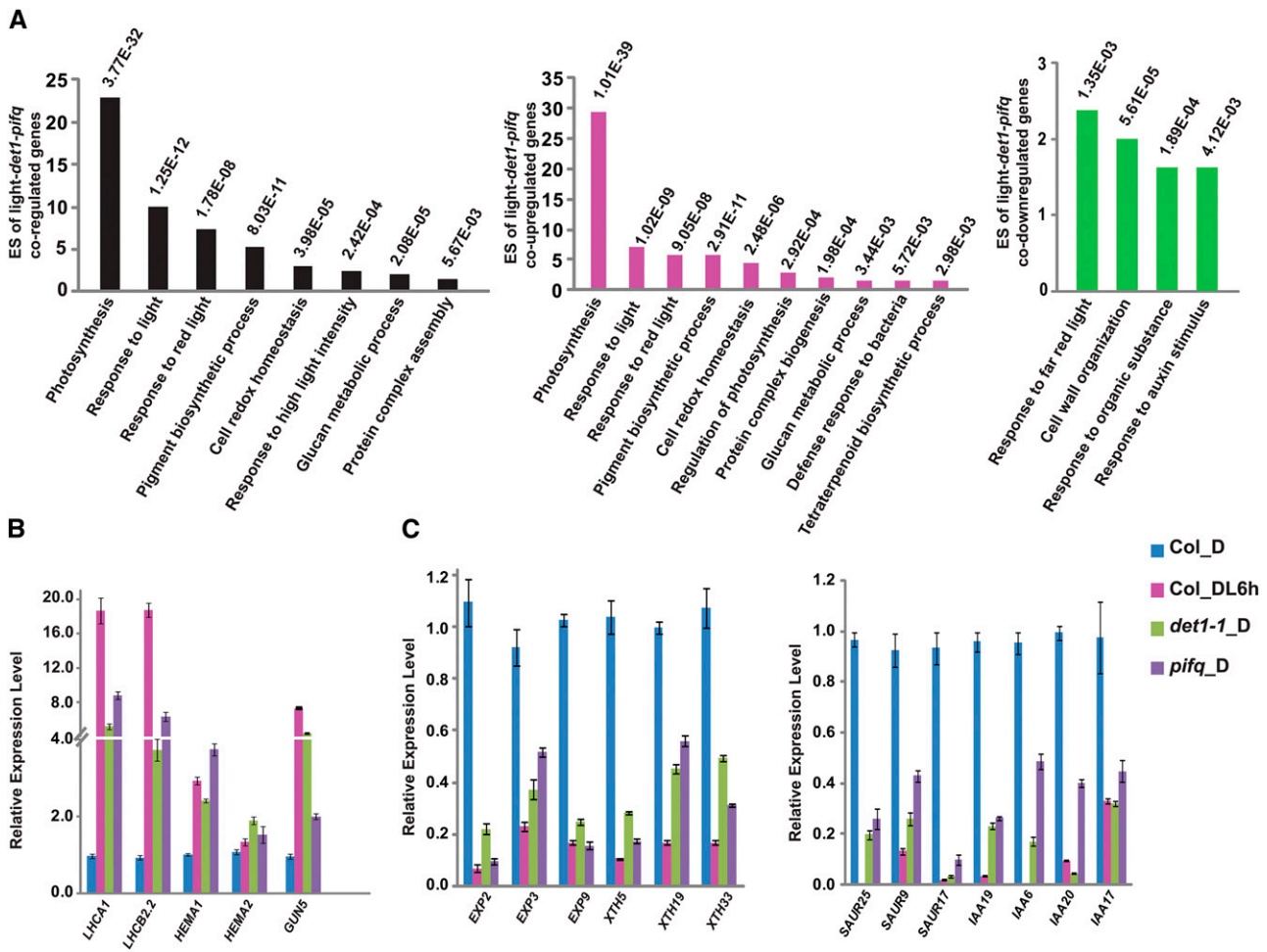


Figure 8. Functional Categories of Light, *det1*, and *pifq*.

(A) The enrichment scores (ES) of light-*det1*-*pifq* coregulated, co-upregulated, and co-downregulated genes.

(B) and (C) qRT-PCR analysis of several major genes involved in photosynthesis and chlorophyll biosynthesis (B), cell wall loosening and response to auxin stimulus (C). Seedlings dark grown for 90 h were either kept in darkness (D) or transferred to white light for 6 h (DL6 h) before RNA extraction. The expression of *PP2A* was used as an internal control. Data are shown as mean \pm SD, $n = 3$.

Huang et al., 2013). PIFs are another group of photomorphogenic repressors that were initially identified through yeast two-hybrid screens using phytochrome (Ni et al., 1998; Leivar et al., 2008). However, little is known about how these two groups of repressors work together to regulate photomorphogenesis. One possible connection was discovered in a recent study that revealed that PIF1 could enhance COP1's E3 ligase activity to repress photomorphogenesis in the dark (Xu et al., 2014). While previous research has demonstrated that *cop1-4* mutants accumulated less PIF3 protein than the wild type (Bauer et al., 2004), it is unclear whether this regulation is accomplished via a direct or indirect interaction. It is also unclear whether this regulation occurs at the mRNA or protein level.

In this study, we found that DET1 positively regulated PIFs possibly in the form of the CUL4-CDD complex and that this

regulation occurred at both the transcriptional and post-translational levels (Figures 2 to 4). Genetic analyses demonstrated that PIFs might work downstream of DET1 to repress photomorphogenesis (Figures 5 and 6; Supplemental Figures 6 to 8). Previous research has shown that COP1-SPA complexes work as E3 ligases to degrade factors promoting photomorphogenesis, such as HY5, in the dark (Osterlund et al., 2000; Zhu et al., 2008). As one of the key transcription factors involved in light signaling, HY5 can directly bind to and regulate a large number of genes involved in photomorphogenesis (Lee et al., 2007). Previous work has illustrated that HY5 can also accumulate in *det1* mutants in the dark and that the pattern of this accumulation resembles that observed among *cop1* mutants (Osterlund et al., 2000). Likewise, PIF3 protein levels have been shown to decrease dramatically when the function of

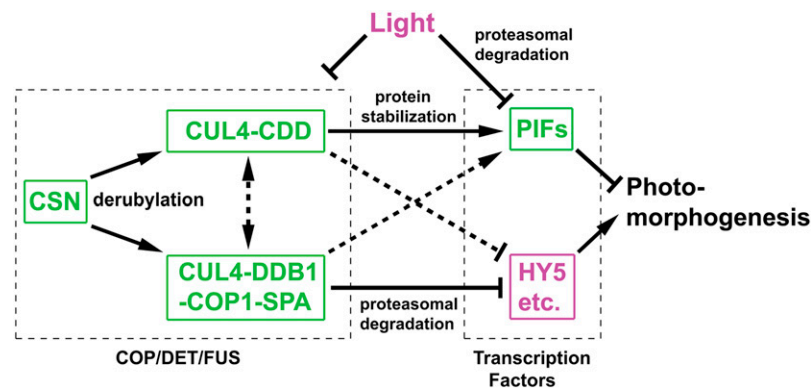


Figure 9. Model of How COP/DET/FUS and PIFs Work in Concert to Repress Plant Photomorphogenesis in the Dark.

There are three main COP/DET/FUS complexes in plants: COP1-SPA, CDD, and CSN. COP1-SPA and CDD complexes may further form CUL4-based E3 ligases in the dark. CSN can regulate the derubylation of CUL4 and thus positively regulates the activity of CUL4-CDD and CUL4-DDB1-COP1-SPA complexes. CUL4-DDB1-COP1-SPA complexes target and degrade photomorphogenesis-promoting factors such as HY5 (Osterlund et al., 2000; H. Chen et al., 2006, 2010). In this study, we determined that DET1 can directly interact with and stabilize PIFs to repress photomorphogenesis, possibly in the form of CUL4-CDD. Previous data also showed that DET1 negatively regulates HY5 (Osterlund et al., 2000), and COP1 positively regulates PIF3 (Bauer et al., 2004), but the mechanisms are unclear. It has been proved that light can inactivate COP1-SPA protein complexes via direct interactions between photoreceptors and COP1 or SPA1, and nucleocytoplasmic movement of COP1 is involved in the inactivation process (Torii et al., 1998; Wang et al., 2001; Liu, et al., 2011). Since the functions of COP/DET/FUS proteins in repressing photomorphogenesis are dependent on each other (Yanagawa et al., 2004; H. Chen et al., 2010; Lau and Deng, 2012), all of their repression of photomorphogenesis can be disrupted by light, although it is not clear whether light can directly inactivate other COP/DET/FUS proteins besides COP1-SPA complexes. Together, COP/DET/FUS may function in concert to stabilize PIFs and degrade HY5 to repress photomorphogenesis, and this repression can be removed by light. Magenta, photomorphogenesis promoting factors; green, photomorphogenesis repressors; solid lines, direct interactions and regulations; dashed lines, mechanisms are unclear.

either COP1 or DET1 is disrupted (Bauer et al., 2004; this study).

The genome expression profiles of the viable mutants of COP1 and DET1 (*cop1-6* and *det1-1*) in darkness have been shown to mimic those of the light-regulated genomic expression profiles (Ma et al., 2003). It has also been demonstrated that most gene expression changes elicited by the absence of the PIFs in dark-grown *pifq* seedlings are normally induced by prolonged light in wild-type seedlings (Leivar et al., 2009). In this study, we further compared the profiles of light-, *det1*-, and *pifq*-regulated genes directly using mRNA sequencing data. Among the light-regulated genes mediated by DET1, most were regulated by PIFs, which are primarily involved in several pathways affecting plant development, such as photosynthesis, cell wall organization, and auxin response (Figures 7 and 8). These data support a conclusion that COP/DET/FUS may partially act through PIFs in mediating the whole-genome expression and thus repressing photomorphogenesis.

Based on the results of previous research and this study, we propose a possible model of how COP/DET/FUS work together with transcription factors to regulate photomorphogenesis (Figure 9). COP/DET/FUS may form several different complexes capable of stabilizing PIFs and degrading HY5 to repress photomorphogenesis in the dark, and this repression can be removed by light. The mechanism by which COP/DET/FUS stabilizes PIFs remains to be determined.

Shared and Distinct Functions of PIFs

It has been shown that the various PIFs possess both shared and distinct functions and regulation (Jeong and Choi, 2013). By comparing various *pif* mutants with the wild type, it has been revealed that in the dark, all four PIFs promote hypocotyl elongation. In contrast, under red light, hypocotyl elongation is mainly promoted by PIF3 and PIF4, while only weakly by PIF5 and not at all by PIF1. Furthermore, in the dark, cotyledon opening is mainly regulated by PIF1 and PIF3 but also slightly by PIF4 and PIF5 (Shin et al., 2009). This assertion has been further supported by the results of this study. Specifically, while DET1 was shown to stabilize all the PIF proteins (PIF1, PIF3, PIF4, and PIF5), it was only found to enhance the transcription of PIF3 (Figure 3). In the *det1-1* background, removal of each PIF can further inhibit the hypocotyl lengths and enhance the cotyledon opening angles in the dark (Figure 5). Ectopic expression of PIF4 and PIF5 were shown to enhance *det1-1* hypocotyl elongation in the dark (Figures 6A and 6B), while PIF1, PIF3, and PIF4 were found to be the major suppressors of cotyledon opening in the dark (Figures 6C to 6E). In contrast, PIF3 and PIF4 were both found to be significant positive regulators of hypocotyl elongation under red light (Supplemental Figure 7). Finally, all four PIFs were shown to suppress anthocyanin accumulation during the dark to light transition (Supplemental Figure 8). Overall, while the results of this study have shed more light on both the functions shared by all PIFs and those that are specific to individual PIFs, much more research remains to be done to determine the role of each PIF in the regulation of photomorphogenesis.

METHODS

Plant Materials and Growth Conditions

The wild-type *Arabidopsis thaliana* plants used in this study were of the Columbia-0 ecotype. The *35S:PIF1-Myc*, *35S:PIF4-Myc*, and *35S:PIF5-Myc* transgenic lines were provided by Peter Quail. The processes by which the other mutants and transgenic lines were created have been previously described as follows: *det1-1* (Chory et al., 1989); *pif1-1*, *pif3-3*, *pif4-2*, *pif5-3*, and *pif1-1 pif3-3 pif4-2 pif5-2(pifq)* (Leivar et al., 2008); *35S:PIF3-Myc* (Kim et al., 2003); GFP-DET1/*det1-1* and Myc-DET1/*det1-1* (Schroeder et al., 2002); *cop10-4* (Wei et al., 1994); and *cul4cs* (Chen et al., 2006).

Seeds were first vernalized for 3 d at 4°C in the dark after having been surface-sterilized with 15% NaClO for 8 min. Seedlings were then exposed to white light for 4 h and finally transferred to darkness unless otherwise specified. For biochemical analyses, full-strength MS plates (4.4 g/L MS powder, 10 g/L sucrose, and 8 g/L agar, pH 5.8) were used. For phenotypic observations, half-strength MS plates (2.2 g/L MS powder, 3 g/L sucrose, 0.5 g/L MES, and 8 g/L agar, pH 5.8) were used. For MG132 or CHX treatments, 4-d-old dark-grown seedlings were treated with 100 μM MG132, 100 μM CHX, or DMSO in liquid full-strength MS medium.

To calculate phenotypes including hypocotyl length, cotyledon opening, anthocyanin accumulation, and greening percentage, more than 20 seedlings were measured. Hypocotyl length and cotyledon opening angles were calculated using Image J software (Kretsch, 2010). Unless specifically mentioned, all statistical analyses were performed between double mutants and *det1-1* single mutants.

Yeast Two-Hybrid Assays

Yeast two-hybrid assays were performed according to the instructions provided with the Matchmaker LexA two-hybrid system (Clontech). BD-DET1 (26 to 87 amino acids) has been described previously (Lau et al., 2011). AD-PIF constructs were obtained by inserting PIF1, PIF3, PIF4, and PIF5 cDNAs into the *EcoRI* and *XhoI* sites of the pB42AD plasmid (Clontech). Corresponding pairs of plasmids were transformed into yeast strain EGY48, which contained a reporter plasmid (p8op-lacZ). Yeast transformants were then plated on minimal SD/-His-Trp-Ura agar plates for 4 d at 30°C. Finally, well-grown colonies were plated onto minimal SD/Gal/Raf/-His-Trp-Ura agar plates with X-Gal for our interaction tests.

In Vitro GST Pull-Down Assays

The bacterial expression constructs for GST-DET1 (26 to 87 amino acids) and His-DET1 were described previously (Lau et al., 2011). The expression constructs for His-PIF1 and His-PIF3 were generated by cloning the corresponding cDNAs into the *EcoRI* and *SalI* sites of vector pET28a (Novagen). The expression constructs for GST-PIF4 and GST-PIF5 were generated by cloning the corresponding cDNAs into the *EcoRI* and *XhoI* sites of vector pGEX-4T-1 (Amersham).

Two micrograms of GST or GST fusion proteins were mixed with 2 μg of His-tagged protein in 1 mL GST binding buffer (20 mM Tris-HCl, pH 7.5, 250 mM NaCl, and 0.1% Nonidet P-40), and the mixture was rotated at 4°C for 4 h. The GST resin was washed with GST binding buffer three times prior to being added to the mixture, which was then kept rotating at 4°C for another 2 h. After washing five times with GST binding buffer, the GST resin was boiled with 1× SDS loading buffer in order to elute the binding proteins, which, in turn, were analyzed using immunoblots.

Semi-in Vivo Pull-Down Assays

Four-day-old dark-grown Col, *35S:PIF1-Myc*, and *35S:PIF3-Myc* seedlings were collected and ground into powder in liquid nitrogen. Four

hundred microliters of GST binding buffer containing 1 mM phenylmethylsulfonyl fluoride and 1× cocktail was added to the powder. The mixture was then centrifuged twice at 14,000 rpm for 10 min at 4°C. The protein concentrations of different samples were examined and adjusted until they were equal. GST binding buffer was then added to the protein extract in the amount necessary to maintain a stable pH value. Five micrograms of His-DET1 recombinant protein was added to the mixture, which was then rotated at 4°C for 5 h. Anti-Myc affinity gel was then added to the mixture, which, in turn, was rotated for another hour. After centrifugation (500g, 3 min, 4°C) and washing three times, the pellet fraction was boiled with 1× SDS loading buffer and analyzed by immunoblot.

Coimmunoprecipitation Assays

Four-day-old dark-grown Col, *35S:PIF1-Myc*, and *35S:PIF3-Myc* seedlings were collected and ground into powder in liquid nitrogen. Four hundred microliters of IP buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM MgCl₂, 1 mM EDTA, 10 mM NaF, 2 mM Na₃VO₄, 25 mM glycerol, phosphate, 10% glycerol, and 0.1% Nonidet P-40) containing 1 mM phenylmethylsulfonyl fluoride, 1× cocktail, and 20 μM MG132 were then added to the powder. The mixture was then centrifuged twice at 16,000g for 10 min at 4°C. Anti-Myc affinity gel was then added to the mixture, which was then rotated at 4°C for 6 h. After centrifugation (500g, 3 min, 4°C) and washing three times, the pellet fraction was boiled in 1× SDS loading buffer and analyzed by immunoblots.

Immunoblot Analyses

Briefly, protein samples were separated by SDS-PAGE and then proteins were transferred to a polyvinylidene fluoride film. After blocking with 5% milk, the film was incubated with primary antibody overnight at 4°C, then washed three times with PBST for 10 min and incubated with secondary antibody for 1 h at room temperature. After washing three times with PBST for 10 min, the film was illuminated using a Bio-Rad illumination detection device.

For protein quantification, Image J software was used and targeted proteins were normalized to the loading control, RPT5. For in vivo degradation assays, the relative protein levels at the start were set to 100. Unless specifically mentioned, all statistical data were obtained from three replicates.

BiFC Assay

The full-length cDNAs of PIF1, PIF3, PIF4, PIF5, and DET1 were amplified and cloned into the *SacI* and *SpeI* sites of pSY735 (C terminus of yellow fluorescent protein [YFP^c]) and the *SpeI* and *BamHI* sites of pSY736 (YFPⁿ) vectors (Bracha-Drori et al., 2004), resulting in plasmids YFP^c-PIF1, YFP^c-PIF3, YFP^c-PIF4, YFP^c-PIF5, and YFPⁿ-DET1. The plasmids were extracted and concentrated to 2 μg/μL. Then, the in vivo interaction was assayed by particle-mediated transformation using onion epidermal cells (Von Arnim, 2007). After 24 h of incubation, YFP signal was detected using a Zeiss LSM 710 confocal microscope.

RNA Extractions and qRT-PCR

Total RNA was extracted from 4-d-old dark-grown seedlings using the RNeasy Plant Mini Kit (Qiagen). One microgram of total RNA was used to synthesize cDNA using ReverTra Ace qPCR RT Master Mix (TOYOBO). Gene-specific primers listed in Supplemental Table 1 were used for qRT-PCR assays using SYBR Premix Ex Taq (Takara) in an ABI 7500 fast real-time instrument. The relative expression levels were normalized to internal control *PP2A*. Materials for qRT-PCR assays were collected from three biological replicates, and three technical replicates were performed in each experiment.

Transcriptomic Analysis

Total RNAs were extracted from the seedlings of 4-d-old dark-grown Col, *det1-1*, and *pifq*, and Col that had been exposed to white light for 6 h. The

RNAs were then sequenced using an Illumina HiSeq2000 following standard protocol.

The RNA-Seq data were initially processed by removing the adapter sequences and low-quality reads, which resulted in high-quality 36-bp single-end reads. Then, these reads were mapped to the *Arabidopsis* TAIR10 genome using TopHat (Trapnell et al., 2012), in which default TopHat parameters were used and two mismatches were allowed. The sequence alignment files generated by TopHat were then used as inputs for Cufflinks (Trapnell et al., 2012), which assembled the mapping files into transcripts. Cufflinks assembled transcripts independently of the existing gene annotations and calculated the transcript abundance by estimating FPKM values (reads per kilobase of exon model per million mapped reads). After that, the significantly differentially expressed genes were identified by the program Cuffdiff (Trapnell et al., 2012), in which the criteria were set as more than 2-fold change and the *q*-value < 0.05. For illustrating gene expression patterns, the clustering applications Cluster3.0 and Treeview from the Eisen Lab were used (Eisen et al., 1998). For classification of differentially expressed genes, the DAVID functional annotation clustering tool (Huang et al., 2009) was used to cluster genes for expression patterns.

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative database under the following accession numbers: *DET1* (AT4g10180), *PIF1* (AT2g20180), *PIF3* (AT1g09530), *PIF4* (AT2g43010), *PIF5* (AT3g59060), *LHCA1* (AT3g54890), *LHCB2.2* (AT2g05070), *HEMA1* (AT1g58290), *HEMA2* (AT1g09940), *GUN5* (AT5g13630), *EXP2* (AT5g05290), *EXP3* (AT2g37640), *EXP9* (AT5g02260), *XTH5* (AT5g13870), *XTH19* (AT4g30290), *XTH33* (AT1g10550), *SAUR25* (AT4g13790), *SAUR9* (AT4g36110), *SAUR17* (AT4g09530), *IAA6* (AT1g52830), *IAA20* (AT2g46990), *IAA17* (AT1g04250), *IAA19* (AT3g15540), and *PP2A* (AT1g13320). The original expression profiling data have been deposited in the Gene Expression Omnibus database under accession number GSE60835.

Supplemental Data

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

Supplemental Figure 1. DET1 Interacts with PIF6 and PIF7 in Yeast.

Supplemental Figure 2. Phenotypic Complementation of *det1-1* by Ectopic Expression of Myc-DET1 and GFP-DET1.

Supplemental Figure 3. Phenotypes and PIF3 Protein Levels of Mutants Defective in CUL4-CDD or CSN Components.

Supplemental Figure 4. *pifq* but not Single *pif* Mutant Seedlings Show Similar Photomorphogenic Phenotypes to *det1-1* in the Dark.

Supplemental Figure 5. A Cocktail of Proteasomal Inhibitors Can Inhibit Light-Induced but Not *cop1-4*-Mediated PIF3 Degradation.

Supplemental Figure 6. Several *PIF* Single Mutations Can Enhance the Phenotype of DET1 Mutants under Red Light.

Supplemental Figure 7. Ectopic Expression of PIFs Can Partially Suppress the Short Hypocotyls of DET1 Mutant under Red Light.

Supplemental Figure 8. Ectopic Expression of PIFs Can Suppress Anthocyanin Accumulation in DET1 Mutants during the Dark-to-Light Transition.

Supplemental Table 1. List of Primers Used for qRT-PCR Analyses.

Supplemental Data Set 1. Summary of the mRNA Sequencing Data Mapping Results.

Supplemental Data Set 2. List of Light-, *det1*-, or *pifq*-Regulated Genes.

Supplemental Data Set 3. List of Coregulated, Coupregulated, and Codownregulated Genes by Light, *det1*, and *pifq*.

ACKNOWLEDGMENTS

We thank Peter Quail for providing the *35S:PIF1-Myc*, *35S:PIF4-Myc*, and *35S:PIF5-Myc* transgenic seeds, Xinhao Ouyang for assistance with experiments, Yu Zhang and Xi Huang for suggestions, and Abigail Coplin for article editing. This work was supported by grants to H.C. from the National Natural Science Foundation of China (31271294), the National Program on Key Basic Research Project of China (973 Program: 2011CB100101), the National High Technology Research and Development Program of China (863 Program: 2012AA10A304), the Ministry of Agriculture of China (948 Program: 2011-G2B), and State Key Laboratory of Protein and Plant Gene Research; and by grants to X.W.D. from the National Natural Science Foundation of China (31330048, U1031001), the National Program on Key Basic Research Project of China (973 Program: 2012CB910900), Peking-Tsinghua Center for Life Sciences, and State Key Laboratory of Protein and Plant Gene Research.

AUTHOR CONTRIBUTIONS

J.D. and H.C. designed the research. J.D., D.T., R.Y., and K.L. performed the experiments. Z.G. and H.H. conducted the bioinformatics analysis. J.D., X.W.D., and H.C. analyzed the data. J.D., W.T., X.W.D., and H.C. wrote the article.

Received July 31, 2014; revised August 28, 2014; accepted September 4, 2014; published September 23, 2014.

REFERENCES

- Al-Sady, B., Ni, W., Kircher, S., Schäfer, E., and Quail, P.H.** (2006). Photoactivated phytochrome induces rapid PIF3 phosphorylation prior to proteasome-mediated degradation. *Mol. Cell* **23**: 439–446.
- Bauer, D., Viczián, A., Kircher, S., Nobis, T., Nitschke, R., Kunkel, T., Panigrahi, K.C., Adám, E., Fejes, E., Schäfer, E., and Nagy, F.** (2004). Constitutive photomorphogenesis 1 and multiple photoreceptors control degradation of phytochrome interacting factor 3, a transcription factor required for light signaling in Arabidopsis. *Plant Cell* **16**: 1433–1445.
- Benvenuto, G., Formiggini, F., Laflamme, P., Malakhov, M., and Bowler, C.** (2002). The photomorphogenesis regulator DET1 binds the amino-terminal tail of histone H2B in a nucleosome context. *Curr. Biol.* **12**: 1529–1534.
- Bracha-Drori, K., Shichrur, K., Katz, A., Oliva, M., Angelovici, R., Yalovsky, S., and Ohad, N.** (2004). Detection of protein-protein interactions in plants using bimolecular fluorescence complementation. *Plant J.* **40**: 419–427.
- Castells, E., Molinier, J., Benvenuto, G., Bourbousse, C., Zabulon, G., Zalc, A., Cazzaniga, S., Genschik, P., Barneche, F., and Bowler, C.** (2011). The conserved factor DE-ETIOLATED 1 cooperates with CUL4-DDB1DDB2 to maintain genome integrity upon UV stress. *EMBO J.* **30**: 1162–1172.
- Castillon, A., Shen, H., and Huq, E.** (2007). Phytochrome Interacting Factors: central players in phytochrome-mediated light signaling networks. *Trends Plant Sci.* **12**: 514–521.

- Chen, H., Huang, X., Gusmaroli, G., Terzaghi, W., Lau, O.S., Yanagawa, Y., Zhang, Y., Li, J., Lee, J.H., Zhu, D., and Deng, X.W. (2010). Arabidopsis CULLIN4-damaged DNA binding protein 1 interacts with CONSTITUTIVELY PHOTOMORPHOGENIC1-SUPPRESSOR OF PHYA complexes to regulate photomorphogenesis and flowering time. *Plant Cell* **22**: 108–123.
- Chen, H., Shen, Y., Tang, X., Yu, L., Wang, J., Guo, L., Zhang, Y., Zhang, H., Feng, S., Strickland, E., Zheng, N., and Deng, X.W. (2006). Arabidopsis CULLIN4 forms an E3 ubiquitin ligase with RBX1 and the CDD complex in mediating light control of development. *Plant Cell* **18**: 1991–2004.
- Chen, M., Chory, J., and Fankhauser, C. (2004). Light signal transduction in higher plants. *Annu. Rev. Genet.* **38**: 87–117.
- Chen, M., Galvão, R.M., Li, M., Burger, B., Bugea, J., Bolado, J., and Chory, J. (2010). Arabidopsis HEMERA/pTAC12 initiates photomorphogenesis by phytochromes. *Cell* **141**: 1230–1240.
- Chory, J., and Peto, C.A. (1990). Mutations in the DET1 gene affect cell-type-specific expression of light-regulated genes and chloroplast development in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **87**: 8776–8780.
- Chory, J., Peto, C., Feinbaum, R., Pratt, L., and Ausubel, F. (1989). *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. *Cell* **58**: 991–999.
- Duek, P.D., Elmer, M.V., van Oosten, V.R., and Fankhauser, C. (2004). The degradation of HFR1, a putative bHLH class transcription factor involved in light signaling, is regulated by phosphorylation and requires COP1. *Curr. Biol.* **14**: 2296–2301.
- Eisen, M.B., Spellman, P.T., Brown, P.O., and Botstein, D. (1998). Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA* **95**: 14863–14868.
- Fujimori, T., Yamashino, T., Kato, T., and Mizuno, T. (2004). Circadian-controlled basic/helix-loop-helix factor, PIL6, implicated in light-signal transduction in *Arabidopsis thaliana*. *Plant Cell Physiol.* **45**: 1078–1086.
- Galvão, R.M., Li, M., Kothadia, S.M., Haskel, J.D., Decker, P.V., Van Buskirk, E.K., and Chen, M. (2012). Photoactivated phytochromes interact with HEMERA and promote its accumulation to establish photomorphogenesis in Arabidopsis. *Genes Dev.* **26**: 1851–1863.
- Holm, M., Ma, L.G., Qu, L.J., and Deng, X.W. (2002). Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in Arabidopsis. *Genes Dev.* **16**: 1247–1259.
- Hornitschek, P., Kohnen, M.V., Lorrain, S., Rougemont, J., Ljung, K., López-Vidriero, I., Franco-Zorrilla, J.M., Solano, R., Trevisan, M., Pradervand, S., Xenarios, I., and Fankhauser, C. (2012). Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. *Plant J.* **71**: 699–711.
- Huang, W., Sherman, B.T., and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* **4**: 44–57.
- Huang, X., Ouyang, X., Yang, P., Lau, O.S., Chen, L., Wei, N., and Deng, X.W. (2013). Conversion from CUL4-based COP1-SPA E3 apparatus to UVR8-COP1-SPA complexes underlies a distinct biochemical function of COP1 under UV-B. *Proc. Natl. Acad. Sci. USA* **110**: 16669–16674.
- Huq, E., and Quail, P.H. (2002). PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in Arabidopsis. *EMBO J.* **21**: 2441–2450.
- Huq, E., Al-Sady, B., Hudson, M., Kim, C., Apel, K., and Quail, P.H. (2004). Phytochrome-interacting factor 1 is a critical bHLH regulator of chlorophyll biosynthesis. *Science* **305**: 1937–1941.
- Jang, I.C., Yang, J.Y., Seo, H.S., and Chua, N.H. (2005). HFR1 is targeted by COP1 E3 ligase for post-translational proteolysis during phytochrome A signaling. *Genes Dev.* **19**: 593–602.
- Jeong, J., and Choi, G. (2013). Phytochrome-interacting factors have both shared and distinct biological roles. *Mol. Cells* **35**: 371–380.
- Jiao, Y., Lau, O.S., and Deng, X.W. (2007). Light-regulated transcriptional networks in higher plants. *Nat. Rev. Genet.* **8**: 217–230.
- Kim, J., Yi, H., Choi, G., Shin, B., Song, P.S., and Choi, G. (2003). Functional characterization of phytochrome interacting factor 3 in phytochrome-mediated light signal transduction. *Plant Cell* **15**: 2399–2407.
- Kretsch, T. (2010). Phenotypic characterization of photomorphogenic responses during plant development. *Methods Mol. Biol.* **655**: 189–202.
- Lau, O.S., and Deng, X.W. (2012). The photomorphogenic repressors COP1 and DET1: 20 years later. *Trends Plant Sci.* **17**: 584–593.
- Lau, O.S., Huang, X., Charron, J.B., Lee, J.H., Li, G., and Deng, X.W. (2011). Interaction of Arabidopsis DET1 with CCA1 and LHY in mediating transcriptional repression in the plant circadian clock. *Mol. Cell* **43**: 703–712.
- Lee, J., He, K., Stolz, V., Lee, H., Figueroa, P., Gao, Y., Tongprasit, W., Zhao, H., Lee, I., and Deng, X.W. (2007). Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. *Plant Cell* **19**: 731–749.
- Leivar, P., and Quail, P.H. (2011). PIFs: pivotal components in a cellular signaling hub. *Trends Plant Sci.* **16**: 19–28.
- Leivar, P., and Monte, E. (2014). PIFs: systems integrators in plant development. *Plant Cell* **26**: 56–78.
- Leivar, P., Tepperman, J.M., Monte, E., Calderon, R.H., Liu, T.L., and Quail, P.H. (2009). Definition of early transcriptional circuitry involved in light-induced reversal of PIF-imposed repression of photomorphogenesis in young Arabidopsis seedlings. *Plant Cell* **21**: 3535–3553.
- Leivar, P., Monte, E., Oka, Y., Liu, T., Carle, C., Castillon, A., Huq, E., and Quail, P.H. (2008). Multiple phytochrome-interacting bHLH transcription factors repress premature seedling photomorphogenesis in darkness. *Curr. Biol.* **18**: 1815–1823.
- Li, L., et al. (2012). Linking photoreceptor excitation to changes in plant architecture. *Genes Dev.* **26**: 785–790.
- Liu, B., Zuo, Z., Liu, H., Liu, X., and Lin, C. (2011). Arabidopsis cryptochrome 1 interacts with SPA1 to suppress COP1 activity in response to blue light. *Genes Dev.* **25**: 1029–1034.
- Ma, L., Zhao, H., and Deng, X.W. (2003). Analysis of the mutational effects of the COP/DET/FUS loci on genome expression profiles reveals their overlapping yet not identical roles in regulating Arabidopsis seedling development. *Development* **130**: 969–981.
- Mayer, R., Raventos, D., and Chua, N.H. (1996). *det1*, *cop1*, and *cop9* mutations cause inappropriate expression of several gene sets. *Plant Cell* **8**: 1951–1959.
- Monte, E., Tepperman, J.M., Al-Sady, B., Kaczorowski, K.A., Alonso, J.M., Ecker, J.R., Li, X., Zhang, Y., and Quail, P.H. (2004). The phytochrome-interacting transcription factor, PIF3, acts early, selectively, and positively in light-induced chloroplast development. *Proc. Natl. Acad. Sci. USA* **101**: 16091–16098.
- Mustilli, A.C., Fenzi, F., Ciliento, R., Alfano, F., and Bowler, C. (1999). Phenotype of the tomato high pigment-2 mutant is caused by a mutation in the tomato homolog of DEETIOLATED1. *Plant Cell* **11**: 145–157.
- Ni, M., Tepperman, J.M., and Quail, P.H. (1998). PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein. *Cell* **95**: 657–667.
- Ni, M., Tepperman, J.M., and Quail, P.H. (1999). Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. *Nature* **400**: 781–784.
- Ni, W., Xu, S.L., Tepperman, J.M., Stanley, D.J., Maltby, D.A., Gross, J.D., Burlingame, A.L., Wang, Z.Y., and Quail, P.H. (2014).

- A mutually assured destruction mechanism attenuates light signaling in Arabidopsis. *Science* **344**: 1160–1164.
- Oh, E., Zhu, J.Y., and Wang, Z.Y.** (2012). Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. *Nat. Cell Biol.* **14**: 802–809.
- Oh, E., Kang, H., Yamaguchi, S., Park, J., Lee, D., Kamiya, Y., and Choi, G.** (2009). Genome-wide analysis of genes targeted by PHYTOCHROME INTERACTING FACTOR 3-LIKE5 during seed germination in Arabidopsis. *Plant Cell* **21**: 403–419.
- Oh, E., Kim, J., Park, E., Kim, J.I., Kang, C., and Choi, G.** (2004). PIL5, a phytochrome-interacting basic helix-loop-helix protein, is a key negative regulator of seed germination in *Arabidopsis thaliana*. *Plant Cell* **16**: 3045–3058.
- Osterlund, M.T., Hardtke, C.S., Wei, N., and Deng, X.W.** (2000). Targeted destabilization of HY5 during light-regulated development of Arabidopsis. *Nature* **405**: 462–466.
- Park, B.S., Eo, H.J., Jang, I.C., Kang, H.G., Song, J.T., and Seo, H.S.** (2010). Ubiquitination of LHY by SINAT5 regulates flowering time and is inhibited by DET1. *Biochem. Biophys. Res. Commun.* **398**: 242–246.
- Park, E., Kim, J., Lee, Y., Shin, J., Oh, E., Chung, W.I., Liu, J.R., and Choi, G.** (2004). Degradation of phytochrome interacting factor 3 in phytochrome-mediated light signaling. *Plant Cell Physiol.* **45**: 968–975.
- Park, J., Lee, N., Kim, W., Lim, S., and Choi, G.** (2011). ABI3 and PIL5 collaboratively activate the expression of SOMNUS by directly binding to its promoter in imbibed Arabidopsis seeds. *Plant Cell* **23**: 1404–1415.
- Penfield, S., Josse, E.M., and Halliday, K.J.** (2010). A role for an alternative splice variant of PIF6 in the control of Arabidopsis primary seed dormancy. *Plant Mol. Biol.* **73**: 89–95.
- Pepper, A., Delaney, T., Washburn, T., Poole, D., and Chory, J.** (1994). DET1, a negative regulator of light-mediated development and gene expression in Arabidopsis, encodes a novel nuclear-localized protein. *Cell* **78**: 109–116.
- Schroeder, D.F., Gahrtz, M., Maxwell, B.B., Cook, R.K., Kan, J.M., Alonso, J.M., Ecker, J.R., and Chory, J.** (2002). De-etiolated 1 and damaged DNA binding protein 1 interact to regulate Arabidopsis photomorphogenesis. *Curr. Biol.* **12**: 1462–1472.
- Schwechheimer, C., Serino, G., Callis, J., Crosby, W.L., Lyapina, S., Deshaies, R.J., Gray, W.M., Estelle, M., and Deng, X.W.** (2001). Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCFTIR1 in mediating auxin response. *Science* **292**: 1379–1382.
- Seo, H.S., Yang, J.Y., Ishikawa, M., Bolle, C., Ballesteros, M.L., and Chua, N.H.** (2003). LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. *Nature* **423**: 995–999.
- Serino, G., and Deng, X.W.** (2003). The COP9 signalosome: regulating plant development through the control of proteolysis. *Annu. Rev. Plant Biol.* **54**: 165–182.
- Shen, H., Zhu, L., Castillon, A., Majee, M., Downie, B., and Huq, E.** (2008). Light-induced phosphorylation and degradation of the negative regulator PHYTOCHROME-INTERACTING FACTOR1 from Arabidopsis depend upon its direct physical interactions with photo-activated phytochromes. *Plant Cell* **20**: 1586–1602.
- Shen, Y., Khanna, R., Carle, C.M., and Quail, P.H.** (2007). Phytochrome induces rapid PIF5 phosphorylation and degradation in response to red-light activation. *Plant Physiol.* **145**: 1043–1051.
- Shi, H., Zhong, S., Mo, X., Liu, N., Nezames, C.D., and Deng, X.W.** (2013). HFR1 sequesters PIF1 to govern the transcriptional network underlying light-initiated seed germination in Arabidopsis. *Plant Cell* **25**: 3770–3784.
- Shin, J., Kim, K., Kang, H., Zulfugarov, I.S., Bae, G., Lee, C.H., Lee, D., and Choi, G.** (2009). Phytochromes promote seedling light responses by inhibiting four negatively-acting phytochrome-interacting factors. *Proc. Natl. Acad. Sci. USA* **106**: 7660–7665.
- Song, H.R., and Carré, I.A.** (2005). DET1 regulates the proteasomal degradation of LHY, a component of the Arabidopsis circadian clock. *Plant Mol. Biol.* **57**: 761–771.
- Sullivan, J.A., Shirasu, K., and Deng, X.W.** (2003). The diverse roles of ubiquitin and the 26S proteasome in the life of plants. *Nat. Rev. Genet.* **4**: 948–958.
- Torii, K.U., McNellis, T.W., and Deng, X.W.** (1998). Functional dissection of Arabidopsis COP1 reveals specific roles of its three structural modules in light control of seedling development. *EMBO J.* **17**: 5577–5587.
- Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., and Pachter, L.** (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat. Protoc.* **7**: 562–578.
- Von Arnim, A.** (2007). Subcellular localization of GUS- and GFP-tagged proteins in onion epidermal cells. *Cold Spring Harb. Protoc.* **2007**: pdb prot4689.
- Von Arnim, A., and Deng, X.W.** (1996). Light control of seedling development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**: 215–243.
- Xu, X., Paik, I., Zhu, L., Bu, Q., Huang, X., Deng, X.W., and Huq, E.** (2014). PHYTOCHROME INTERACTING FACTOR1 enhances the E3 ligase activity of CONSTITUTIVE PHOTOMORPHOGENIC1 to synergistically repress photomorphogenesis in Arabidopsis. *Plant Cell* **26**: 1992–2006.
- Wang, H., Ma, L.G., Li, J.M., Zhao, H.Y., and Deng, X.W.** (2001). Direct interaction of Arabidopsis cryptochromes with COP1 in light control development. *Science* **294**: 154–158.
- Wei, N., Kwok, S.F., von Arnim, A.G., Lee, A., McNellis, T.W., Piekos, B., and Deng, X.W.** (1994). Arabidopsis COP8, COP10, and COP11 genes are involved in repression of photomorphogenic development in darkness. *Plant Cell* **6**: 629–643.
- Yanagawa, Y., Sullivan, J.A., Komatsu, S., Gusmaroli, G., Suzuki, G., Yin, J., Ishibashi, T., Saijo, Y., Rubio, V., Kimura, S., Wang, J., and Deng, X.W.** (2004). Arabidopsis COP10 forms a complex with DDB1 and DET1 in vivo and enhances the activity of ubiquitin conjugating enzymes. *Genes Dev.* **18**: 2172–2181.
- Yang, J., Lin, R., Sullivan, J., Hoecker, U., Liu, B., Xu, L., Deng, X.W., and Wang, H.** (2005). Light regulates COP1-mediated degradation of HFR1, a transcription factor essential for light signaling in Arabidopsis. *Plant Cell* **17**: 804–821.
- Yi, C., and Deng, X.W.** (2005). COP1 - from plant photomorphogenesis to mammalian tumorigenesis. *Trends Cell Biol.* **15**: 618–625.
- Zhang, Y., Mayba, O., Pfeiffer, A., Shi, H., Tepperman, J.M., Speed, T.P., and Quail, P.H.** (2013). A quartet of PIF bHLH factors provides a transcriptionally centered signaling hub that regulates seedling morphogenesis through differential expression-patterning of shared target genes in Arabidopsis. *PLoS Genet.* **9**: e1003244.
- Zhong, S., Shi, H., Xue, C., Wang, L., Xi, Y., Li, J., Quail, P.H., Deng, X.W., and Guo, H.** (2012). A molecular framework of light-controlled phytohormone action in Arabidopsis. *Curr. Biol.* **22**: 1530–1535.
- Zhu, D., Maier, A., Lee, J.H., Laubinger, S., Saijo, Y., Wang, H., Qu, L.J., Hoecker, U., and Deng, X.W.** (2008). Biochemical characterization of Arabidopsis complexes containing CONSTITUTIVELY PHOTOMORPHOGENIC1 and SUPPRESSOR OF PHVA proteins in light control of plant development. *Plant Cell* **20**: 2307–2323.