The ELF3-PIF7 Interaction Mediates the Circadian Gating of the Shade Response in Arabidopsis

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HIGHLIGHTS

- ELF3 is involved in the inhibition of the shade response at night
- ELF3 interacts with PIF7 and prevents PIF7 from binding DNA
- ELF3 acts upstream of PIF7 in shade-induced growth
- Repressive activity of ELF3 is stronger under SDs than under LDs
The ELF3-PIF7 Interaction Mediates the Circadian Gating of the Shade Response in Arabidopsis

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SUMMARY

Light filtered through dense planting initiates the shade avoidance syndrome (SAS) in plants, which helps them compete against their neighbors. Quantitative trait loci (QTL)-based analysis identified the nighttime-expressed clock component ELF3 as a new player in the SAS, but its detailed mechanism is unclear. Here, we show that the circadian clock gates shade-induced gene expression and hypocotyl elongation at night. ELF3 is involved in nighttime suppression via interaction with and inactivation of PHYTOCHROME-INTERACTING FACTOR 7 (PIF7). Loss of function of ELF3 restores the shade induction, which is largely reduced in the absence of PIF7, indicating that ELF3 acts upstream of PIF7. Finally, we found that the repressive activity of ELF3 on the shade response is stronger under short days than under long days. Our results reveal that the interaction between ELF3 and PIF7 mediates the circadian gating of the SAS, which coordinates the daily control of physiological outputs.

INTRODUCTION

Because of the selective absorption of blue and red wavelengths by chlorophyll, shaded plants perceive reduced ratios of red (R, 660 nm) to far red (FR, 730 nm), which triggers the shade avoidance syndrome (SAS) (Casal, 2012). A classic phenotype of the SAS is manifested as reallocation of energy resources from storage organs to stem-like organs, such as the hypocotyl and petiole, for plants to outgrow their competitors (Ballare and Pierik, 2017). Several microarray and RNA sequencing analyses have revealed transcriptional regulation after shade treatment (Ciolfi et al., 2013; Leivar et al., 2012; Li et al., 2012; Salter et al., 2003; Sellaro et al., 2017; Yang et al., 2018). Interestingly, the transcriptional regulation of PIL1 (a marker gene of the shade response) by a low R/FR is reported to be gated by the circadian clock (Salter et al., 2003). The circadian clock limits the timing of maximum responsiveness to shade light to specific times of the day. However, the mechanism of circadian clock involvement in the shade response is unclear.

PHYTOCHROME-INTERACTING FACTOR 7 (PIF7) is a major regulator of shade-induced gene expression and hypocotyl elongation (Li et al., 2012; Mizuno et al., 2015; Peng et al., 2018). In the shade, PIF7 is dephosphorylated when the Pfr form of phyB is photoconverted to the Pr form, and PIF7 then regulates cell elongation by enhancing the transcription of growth-promoting genes (Li et al., 2012). PIF7 also regulates the shade induction of PIL1; therefore, whether the circadian clock is involved in the gating of PIF7 function is of particular interest.

EARLY FLOWERING 3 (ELF3) was first identified in Arabidopsis as an early-flowering mutant that is insensitive to the photoperiod (Zagotta et al., 1996). ELF4 association with ELF3 directs LUX action in the circadian clock (Herrero and Davis, 2012; Herrero et al., 2012), and this complex is referred to as the evening complex (EC) (Bujdoso and Davis, 2013). ELF3 functions as a transcriptional regulator, repressing clock- and growth-associated transcription factors to regulate the circadian rhythm and hypocotyl elongation (Chow et al., 2012; Dixon et al., 2011; Helfer et al., 2011; Nusinow et al., 2011). Furthermore, natural variation has revealed that the intracellular distribution of ELF3 proteins is associated with specific functions in the circadian clock (Anwer et al., 2014; Anwer and Davis, 2013). The photoreceptor phyB physically interacts with ELF3 in the central oscillator to provide a direct light input to the clock (Kolmos et al., 2011; Oakenfull and Davis, 2017). ELF3 can prevent PIF4 from activating its transcriptional targets in an EC independent manner (Nieto et al., 2015) and also directly regulate PIF4 expression in thermoresponsive growth (Raschke et al., 1998).
et al., 2015). Recently, ELF3 was implicated as a regulator of the shade avoidance response based on quantitative trait loci (QTL) mapping analysis in the Bay x Sha recombinant inbred line population (Coluccio et al., 2011; Jimenez-Gomez et al., 2010). However, the detailed mechanism governing ELF3 involvement in shade avoidance response is unclear.

Here, we found that the time-specific inhibition of shade-induced gene expression and hypocotyl elongation appeared around CT16-CT20. ELF3 is responsible for this inhibition via interactions with PIF7 and represses PIF7’s DNA-binding activity, antagonizing PIF7-induced gene expression and hypocotyl elongation. Our findings reveal that ELF3 affects the shade avoidance response with respect to circadian rhythm traits.

RESULTS

Inhibition of the Shade Avoidance Response Occurs during the Night

To determine whether shade-induced gene expression is gated by the circadian clock, wild-type seedlings were grown under short days (SDs) for 5 days and then transferred to continuous white light, under which the internal circadian clock of the plants was still functional. We considered these conditions to be the circadian conditions. Then, the seedlings were kept under white light or treated with shade light for 1 h every 4 h (Figure 1A), at which point the transcript levels of IAA29 and PIL1 were measured. As shown in Figure 1B, shade treatment induced the expression of these two genes from 4 to 8 h after transfer to constant conditions, whereas shade induction was inhibited from 16 to 20 h after transfer to constant conditions (Figure 1B, circadian conditions).

To assess whether shade-induced hypocotyl elongation is also gated by the circadian clock, we measured the new growth of hypocotyls during the first 2 h when SD-grown wild-type seedlings were treated with shade light at different times of the day using a seedling phenotyping platform (DynaPlant) (Figure 1C). We observed that the shade-induced growth response at 4 h was nearly identical to that at 8 h after transfer to constant conditions. However, shade-induced elongation was inhibited from 16 to 20 h after transfer to constant conditions (Figure 1C). Loss of function of PIF7 abolished the shade-induced hypocotyl elongation. Combined with shade-induced gene expression data, these data suggested that the inhibition of the shade avoidance response mainly occurs at night. As a master regulator for shade-induced hypocotyl elongation, PIF7 functions continuously.

Because IAA29 and PIL1 are targets of PIF7 and because PIF7 is a master regulator of shade-induced hypocotyl elongation, we were interested in whether the translational or transcript levels of PIF7 are gated by the circadian clock. However, shade light consistently dephosphorylates PIF7 regardless of the time (from 4 to 28 h after transfer to constant conditions, circadian conditions) (Figures S1A and S1B), indicating that the phosphorylation of PIF7 is regulated by light, but not the circadian clock. The phosphorylation and dephosphorylation of PIF7 consistently switched slowly following light conditions when the seedlings were kept under SD (light/dark cycle) conditions (diurnal conditions) (Figure S1C). Although the transcript level of PIF7 is lower at ZT12-ZT21 than that at ZT3-ZT6, the degree of oscillation is less than that of PIF4 (Figure S1D, diurnal conditions). Therefore, in addition to the weak transcriptional rhythm of PIF7, it is possible that a circadian component might be involved in the inhibition of shade induction at night.

ELF3 Is Involved in Shade-Induced Hypocotyl Elongation

The circadian gating of the shade-induced increase in PIL1 and IAA29 transcript levels and hypocotyl elongation led us to investigate which circadian-related components are involved in this mechanism. We measured the hypocotyl lengths of SD-grown seedlings treated with end-of-day (EOD) shade in Col-0, toc1-2 (a mutant of TOC1 [Timing of CAB expression 1]), prr5-11 (a mutant of PRR5 [PSEUDO-RESPONSE REGULATOR 5]), cca1-1 lhy (a double mutant of CCA1 [Circadian Clock Associated 1] and LHY [Late elongated hypocotyl]), elf3-1, elf3-7, and elf3-8. Shade-induced hypocotyl elongation was manifested in Col-0, toc1-2, and prr5-11 but was diminished in cca1-1 lhy and the three elf3 mutants (Figures 2A–2C, diurnal conditions). Although TOC1 has been reported to interact with PIF3 to act as a gate of growth during predawn conditions (Soy et al., 2016) and interact with PIF4 to regulate the circadian gating of thermoresponsive growth (Zhu et al., 2016), toc1-2 displays a reduced but significant shade response. ELF3 and CCA1/LHY appear to be more important than TOC1 in the SAS. CCA1 has been reported to regulate hypocotyl elongation by modulating the transcription of DWARF4 (Zheng et al., 2018) and ELF3 (Lu et al., 2012; Reed et al., 2000). The maximal expression of ELF3 occurs around CT12-CT20 under SDs (Herrero et al., 2012; Liu et al., 2015).
We measured the effects of shade on the transcriptional and translational levels of ELF3. As shown in Figures S2A and S2B (circadian conditions), there was no significant effect of shade treatment on the expression of ELF3 from 12 to 24 h after transfer to constant conditions. To measure the effects of shade on the translational level of ELF3, we obtained ELF3 antibodies (Ding et al., 2018). A western blot was performed to determine the specificity of the ELF3 antibodies, and the results are shown in Figure S2C. There was also no significant effect of shade treatment on the translational level of ELF3 at 16 or 20 h after transfer to constant conditions (Figure S2D).

We also measured the shade-induced hypocotyl elongation of elf3 mutants that were grown under continuous white light conditions and then transferred to shade conditions (Figures 2D and 2E). An enhanced shade response confirmed that ELF3 is a negative regulator of shade-induced hypocotyl elongation. Under dark conditions, elf3 mutants look like wild-type plants (Figure 2F). We then checked the new growth
of hypocotyls during the first 2 h when SD-grown elf3-1 seedlings were treated with shade light at different times of the day. The results showed that the shade responses are almost the same at any time of day in elf3-1 (Figure 2G), indicating that ELF3 is involved in the inhibition of the shade response at night.

We took advantage of published RNA sequencing data of elf3-1 and checked the effect of ELF3 on the expression level of PIF7 targets (IAA29, PIL1, IAA19, YUCCA8, YUCCA9, IAA2, GH3.3, ATHB4, and...
HAT2) at different times under SDs (Ezer et al., 2017). The expression of all these genes dramatically increased at ZT12–ZT22 in elf3-1 (Figure S3A, diurnal conditions). The transcript levels of YUCCA8, IAA29, and PIL1 were confirmed by qRT-PCR (Figure S3B, diurnal conditions). We also obtained an ELF3ox overexpression line (Liu et al., 2001), which was confirmed by qRT-PCR (Figure S3C) and western blotting (Figure S3D). We then detected that the transcript levels of IAA29 and PIL1 were reduced in the ELF3ox line after shade treatment (Figure S3E), which suggests that ELF3 negatively moderates these genes’ transcript levels.

**ELF3 Physically Interacts with PIF7**

It has been known that the circadian clock plays a pivotal role in the control of hypocotyl elongation by regulating the rhythmic expression of PIF4 and PIF5 (Nusinow et al., 2011). Therefore, we measured the transcript level of PIF7 in SD-grown Col-0 and elf3 at CT9–CT21. Compared with the expression of PIF4, the expression of PIF7 is not significantly altered in elf3 (Figure S4, diurnal conditions), indicating that there are other mechanisms of ELF3 in addition to the transcriptional regulation of PIF7.

Recently, PIF7 was identified as a protein that is associated with ELF3 by affinity purification and mass spectrometry (AP-MS) (Huang et al., 2016). We examined the protein interaction by a luciferase complementation imaging (LCI) assay, which revealed that ELF3-cLUC could interact with nLUC-PIF7 when transiently expressed in Nicotiana benthamiana leaf cells (Figure 3A). A bimolecular fluorescence complementation assay also revealed that the interaction occurred in the nucleus (Figure 3B). In an in vitro pull-down experiment, ELF3 extracted from Col-0 seedlings directly bound with glutathione S-transferase (GST) fusion proteins of PIF7 (Figure 3C), and PIF7-Flash extracted from both white light-grown and shade-treated PIF7ox (35S:PIF7-Flash) seedlings (Li et al., 2012), could bind with the GST-fused ELF3 (Figure 3D). Coimmunoprecipitation experiments further confirmed that ELF3 precipitated with PIF7 (Figure 3E). These data indicate that ELF3 can physically interact with PIF7 both in vitro and in vivo.

**ELF3 Suppresses the Shade-Induced Expression of IAA29 and PIL1 by Preventing PIF7 from Binding DNA**

To explore the biological implications of this interaction, we generated PIF7oxELF3ox Arabidopsis lines, in which both the PIF7 and ELF3 genes were expressed under control of the 35S promoter. We then examined PIF7 and ELF3 protein levels in double-overexpression lines. As shown in Figure 4A, overexpression of ELF3 does not affect the protein level of PIF7 in continuous white light-grown seedlings. A chromatin immunoprecipitation-PCR assay that used primers that anneal to the G-box PIF7 recognition motifs and coding regions in IAA29 and PIL1 (Figures 4B and S5) was performed to detect the DNA-binding ability of PIF7 in both PIF7ox and PIF7oxELF3ox plants after shade treatment. The G-box regions were less enriched in the chromatin fractions from PIF7ox ELF3ox plants compared with PIF7ox plants (Figures 4B and S5). Moreover, no enrichment was detected with primers in the coding regions of IAA29 and PIL1. These results demonstrate that ELF3 suppresses PIF7 activity by a sequestration mechanism.

Then we would like to investigate whether loss of function of ELF3 could restore the downstream gene expression of PIF7 at night. The circadian clock regulates hypocotyl elongation by affecting cell elongation-related gene expression, and the expression of these genes is also modulated by light (Figure S3A). To exclude the effects of the circadian clock on the expression of these genes, we transferred the seedlings from diurnal conditions to continuous white light (Figures 1A and 4C, circadian conditions). Under these conditions, the expression of downstream genes such as IAA29 and PIL1 is strongly inhibited by white light (Figure 4C). As we expected, elf3 mutant restores the shade-induced expression of PIL1 and IAA29 from 16 to 20 h after transfer to constant conditions (Figure 4C, circadian conditions). These results suggest that ELF3 negatively regulates shade-induced gene expression by suppressing the DNA-binding activity of PIF7 during night in SD-grown seedlings.

**ELF3 Acts Upstream of PIF7 in Shade-Induced Growth**

To explore the genetic interactions between ELF3 and PIF7, we generated an elf3-1pi7-1 double mutant. The rescuing of the shade-induced expression of IAA29 and PIL1 at 16 h after transfer to constant conditions (Figure 5A, circadian conditions) and 20 h after transfer to constant conditions (Figure 5A, circadian conditions) by the loss of function of ELF3 was abolished again by a pif7 mutation, confirming that ELF3 acts upstream of PIF7 in shade-induced gene expression. We further tested the shade-induced expression of IAA29 and YUCCA8 in continuous white light-grown elf3-1 and elf3-1pif7-1 mutants. Similar
expression patterns were found, which are shown in Figure 5B with Figure 5A, which suggests that the suppression of ELF3 on PIF7 activity also occurs under continuous white light conditions. Previous studies have shown that elf3 mutants are taller than phyB mutants in diurnal light/dark cycles but shorter than phyB mutants in cRL (Nieto et al., 2015), indicating that ELF3 plays a dominant role in the diurnal light/dark cycle, rather than in cRL. We investigated the hypocotyl length of seedlings grown under SD conditions or SDs with EOD shade treatments. The hypocotyl length of elf3-1pif7-1 double mutant is between that of elf3-1 and pif7-1 (Figure 5C), and the hypocotyl length of PIF7oxELF3ox under SD conditions or EOD shade treatment (Figure S6B), which might result from redundant functions of PIF4 and PIF5 in circadian-regulated hypocotyl elongation (Hornitschek et al., 2009; Nieto et al., 2015; Nusinow et al., 2011). We further measured the hypocotyl length of seedlings that were grown under continuous white light (WLc) and then either kept in continuous white light or transferred to continuous shade conditions (Shadec), in which PIF7 is a dominant player. Under these conditions, the hypocotyl length of the elf3-1pif7-1 double mutant is more similar to that of pif7-1, which is consistent with the shade-induced gene expression results (Figure 5D), indicating the dominant action of PIF7. Together, these results suggested that ELF3 acts upstream of PIF7 in shade-induced gene expression and growth.
Repression of ELF3 in the Shade Response Is Stronger under SDs than under LDs

We found that ELF3 represses PIF7 binding activity at night. Therefore, we were interested in whether the effects of ELF3 are decreased under LD conditions with night-shade treatment compared with SDs with night-shade treatment (Figure 6A). The hypocotyl response to night shade was measured as the percent increase in hypocotyl length. As expected, the percent hypocotyl increase was 57.4% under SDs but 139.6% under LDs (Figures 6B and 6C). Although the shade duration is longer under SDs than under LDs, the stronger repression of ELF3 resulted in reduced shade response under SDs. These results suggest that PIF7 plays a major role in both conditions, whereas ELF3 is more important to night-shade treatment under SDs than under LDs.

DISCUSSION

In the current study, we demonstrated that ELF3 negatively regulates shade-induced gene expression and hypocotyl elongation by suppressing the DNA-binding activity of PIF7 during ZT16-ZT20 both in SD-grown seedlings and in continuous white light-grown seedlings. Our data provide evidence that a core clock
Figure 5. ELF3 Acts Upstream of PIF7 in Shade-Induced Growth

(A) Relative expression of IAA29 and PIL1 in Col-0, elf3-1, elf3-1pif7-1 and pif7-1 seedlings at 20 h after transfer to constant conditions. The top panel represents the light treatment for the detection of shade-induced gene expression. Wild-type seedlings were grown under SD conditions for 5 days and transferred to continuous white light, after which the shade treatment was started or the seedlings were kept under continuous white light for 1 h. The white, black, and gray colors represent white light, darkness, and shade, respectively. The bottom panels represent the expression of IAA29 and PIL1. The error bars indicate the SEMs of three independent experiments. The asterisk indicates statistically significant differences between mean values according to Student’s t test (*p < 0.05, **p < 0.01, ***p < 0.001).

(B) Relative expression of IAA29 and PIL1 in Col-0, elf3-1, elf3-1pif7-1, and pif7-1. The seedlings were grown under continuous white light (WLC) or transferred to continuous shade (Shadec) for 1 h. The error bars indicate the SEMs of three independent experiments. The asterisk indicates statistically significant differences between mean values according to Student’s t test (*p < 0.05, **p < 0.01, ***p < 0.001).

(C) Hypocotyl phenotypes of Col-0, elf3-1, elf3-1pif7-1, and pif7-1 seedlings under SDs or end-of-day (EOD) shade treatment. The top panel represents the light treatment for the hypocotyl measurements of seedlings grown under SDs and EOD shade conditions. The seedlings were grown for 3 days under SD and either kept under SDs or treated for 2 h with shade at the end of each day for 4 days. The white, black, and gray colors represent white light, darkness, and shade, respectively. The bottom panels represent the phenotypes of hypocotyl length. The data are presented as the means with SEMs; more than 20 seedlings were measured. The bars marked with different letters denote significant differences (p < 0.05), calculated by Student’s t test. Scale bar, 2 mm.

(D) Hypocotyl length of Col-0, elf3-1, elf3-1pif7-1, and pif7-1 seedlings grown under WLC or Shadec conditions. The seedlings were germinated and grown for 5 days under continuous white light and either kept under white light or
component directly connects to a transcription-centered signaling hub involved in various environmental sensory systems.

Similar to that which occurs with thermoresponsive growth (Zhu et al., 2016) and circadian-regulated growth, the circadian component is also involved in the inhibition of shade-induced growth at night (Figure 1). However, the detailed molecular mechanism is not the same. TOC1 has been reported to interact with PIF3 and repress PIF3 activity by preventing PIF3 from binding DNA (Soy et al., 2016). Moreover, TOC1 binds to PIF4 and suppresses thermomorphogenesis by directly repressing PIF4 activity without affecting its DNA-binding activity (Zhu et al., 2016). In our study, toc1-2 exhibited a reduced but significant shade response (Figures 2B and 2C). ELF3 is more important than TOC1 in the SAS. ELF3 can interact with PIF4 to inhibit the DNA-binding activity of PIF4 (Nieto et al., 2015). Here, it appears that a similar mechanism of ELF3 occurs with respect to PIF4 and PIF7. However, the PIF7 protein stability was not significantly affected by the overexpression of ELF3, whereas the protein stability of PIF4 and ELF3 influences each other. Although the PIF proteins belong to the same subfamily 15 of the Arabidopsis bHLH superfamily and similarly bind the Pfr form of phyB (Leivar and Quail, 2011), their regulatory mechanisms seem to be different in different physiological settings, indicative of additional layers of complexity for functional diversity. Owing to the importance of PIF7 in SAS, the biological significance of the PIF7-ELF3 interaction could be more relevant to shade response.

The circadian gating of shade-induced hypocotyl elongation has been previously published (Sellaro et al., 2012). In that study, the authors showed that afternoon shade events promote hypocotyl growth, whereas morning shade is ineffective. In their experiments, the seedlings returned to white light after a morning shade treatment, and darkness followed the afternoon shade treatment. The final phenotype was measured after 3 days. In the current study, we should emphasize that we did not check how the long-
term phenotypes were affected by the timing of daily shade events. We investigated the short-term shade-induced gene expression and hypocotyl elongation and compared them between the daytime and nighttime (Figure 1). The window we focused on is the early stage of the shade response. Consistent with short-term changes in gene expression and hypocotyl elongation, there are related reports in which different shade starting points during the day result in the same growth rate pattern along short time scales via real-time monitoring methods (Cole et al., 2011; Gommers et al., 2017).

Our molecular and genetic tests present a compelling mechanistic model; however, it remains unclear why plants have evolved a mechanism for inhibiting shade responses at a time when shade signals are rare. The different shade responses under SDs and LDs are probably related to the various types of growth during the different seasons. However, thus far, more work needs to be done to understand the importance of the inhibition of ELF3 activity on PIF7 under shade.

Limitations of the Study
In this study, we revealed that, by repressing the activity of PIF7, ELF3 affects the expression of shade-induced genes (PIL1) involved in circadian rhythm traits. The regulatory circuit has both negative and positive gated points depending on the time of day a shade pulse has already occurred (Salter et al., 2003). However, the transcriptional regulation of PIF7 by PIL1 is still unknown. Therefore, ELF3 regulation of PIF7 target expression followed by ELF3-PIF7 direct protein interaction regulation creates a feedforward mechanism that would be interesting to investigate further. Additional studies are required to elucidate whether other circadian components such as CCA1/LHY are also involved in the circadian gating of the shade response.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2019.11.029.

ACKNOWLEDGMENTS
We thank Dr. Xingwang Deng (Peking University) for sharing the seeds of ELF3ox, elf3-1, elf3-7, and elf3-8 and Dr. Hongtao Liu (Shanghai Institutes for Biological Sciences-Institute of Plant Physiology and Ecology) for sharing the seeds of toc1-2, prr5-11, and cca1-1 lhy. This research was supported by the National Key R&D Program of China (2017YFA0503800).

AUTHOR CONTRIBUTIONS
Y.J., C.Y., S.H., F.X., Y.X., and C.L. performed the experiments. L.L. conceived the project and wrote the paper.

DECLARATION OF INTERESTS
The authors declare that they have no competing or financial interests.

Received: June 2, 2019
Revised: June 4, 2019
Accepted: November 14, 2019
Published: December 20, 2019

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Supplemental Information

The ELF3-PIF7 Interaction Mediates the Circadian Gating of the Shade Response in Arabidopsis

Yupei Jiang, Chuanwei Yang, Sha Huang, Famin Xie, Yitian Xu, Chang Liu, and Lin Li
Transparent Methods

Plant material and growth conditions. All Arabidopsis thaliana plants used in this study were of the Columbia-0 ecotype. The following mutants were used in this study and have been described previously: pif7-1, PIF7-Flash (PIF7ox), toc1-2, prr5-11, cca1-1 lhy, elf3-1, elf3-7 and elf3-8. The ELF3ox overexpression line was previously published (Liu et al., 2001). Plants overexpressing ELF3 were obtained by in planta transformation of Col-0 with a pBI121 vector (Clontech, CA) containing the 35S promoter and ELF3 cDNA. ELF3ox overexpression was confirmed by immunoblots of total protein with anti-ELF3 antibodies.

For the phenotypic analysis, seeds were germinated on plates that contained 1/2-strength Murashige and Skoog (MS) medium (Duchefa Biochemie, Netherlands) with 1% agar (Sangon, China) but without sucrose. After seed stratification was performed, the plates were incubated in growth chambers under continuous LED white light conditions. The plates were then either left in white light (R: ~25 \(\mu\)mol\(\times\)m\(^{-2}\)\(\times\)sec\(^{-1}\), B: ~27 \(\mu\)mol\(\times\)m\(^{-2}\)\(\times\)sec\(^{-1}\), FR: ~5 \(\mu\)mol\(\times\)m\(^{-2}\)\(\times\)sec\(^{-1}\)) or transferred to simulated shade (R: ~25 \(\mu\)mol\(\times\)m\(^{-2}\)\(\times\)sec\(^{-1}\), B: ~27 \(\mu\)mol\(\times\)m\(^{-2}\)\(\times\)sec\(^{-1}\), FR: ~50 \(\mu\)mol\(\times\)m\(^{-2}\)\(\times\)sec\(^{-1}\)) before hypocotyl measurements were made. For EOD shade treatment, the plates were either left under short days conditions (8 hours light/16 hours dark) or transferred to shade (R: ~25 \(\mu\)mol\(\times\)m\(^{-2}\)\(\times\)sec\(^{-1}\), B: ~27 \(\mu\)mol\(\times\)m\(^{-2}\)\(\times\)sec\(^{-1}\), FR: ~50 \(\mu\)mol\(\times\)m\(^{-2}\)\(\times\)sec\(^{-1}\)) for 2 hours before darkness. At least three independent biological replicates were evaluated for the phenotypic analysis.

Measurement of hypocotyl growth kinetics via DynaPlant. Kinetics of hypocotyl growth was measured by a commercial high-throughput imaging platform, DynaPlant® (Microlens Technology, Beijing, http://www.dynaplant.cn/en). Seedlings for kinetics measurement were sown on 1/2 MS medium containing 2% phytagel (Solarbio, P8170) and grown under SD conditions and then transferred to continuous white light with plates
vertically positioned, after which the shade treatment was started or the seedlings were kept under continuous white light for 2 hours. The images of hypocotyl growth were captured by the DynaPlant® platform once every 10 min for each seedling with the physical resolution of 1.2 μm per pixel. The length of new hypocotyl growth in the time-series images were quantified by DynaPlant Analysis software which was provided by the manufacturer. The values shown indicate the means with SEMs.

**Quantitative RT-PCR analysis.** Approximately 100 mg of seedlings grown on 1/2-strength MS media supplemented with 1% agar under different light conditions were collected in Eppendorf tubes, frozen in liquid nitrogen, and ground to a fine powder. Total RNA was extracted using a Trizol kit (Promega, USA). One microgram of total RNA was used for reverse transcription using a First Strand cDNA Synthesis Kit (TIANGEN, China) according to the manufacturer’s instructions. The cDNAs were then subjected to real-time qPCR using a CFX Connect Real-Time System (Bio-Rad, USA) and SYBR Green qPCR Mix (Mei5 Biochem, China). Three biological replicates per sample were used for qRT-PCR analysis. The data are presented as the means with the SEMs of three biological replicates normalized to the expression of the reference gene \( AT2G39960 \) (Li et al., 2012). The comparative \( \Delta \Delta Ct \) method was employed to evaluate the relative quantities of each amplified product in the samples. The specificity of the qRT-PCR reactions was determined by melt curve analysis of the amplified products using the standard method of the system. The primers used are listed in Supplemental Table S1.

**Firefly luciferase complementation imaging assays.** An LCI assay was performed as described previously (Yang et al., 2018). Briefly, the fragments encoding PIF7 were amplified by PCR and ligated into a pCAMBIA2300-nLUC vector to produce nLUC-PIF7. The coding regions of \( ELF3 \) were amplified by
PCR and ligated into pCAMBIA2300-cLUC to produce ELF3-cLUC. The resulting constructs were transformed into Agrobacterium strain GV3101. Agrobacterium cells harboring different constructs were then infiltrated into N. benthamiana leaves. Three days after infiltration, luciferin (Promega, USA) (2.5 mM, 0.1% Triton X-100) was spread before LUC activity was monitored by a Tanon 5500 chemical luminescence imaging system (Tanon, China).

**In vitro pull-down assays.** To detect the interaction between PIF7 and ELF3, seedlings from ELF3 transgenic plants or from PIF7-Flash transgenic plants were ground in liquid nitrogen and resuspended in extraction buffer (100 mM Tris-HCl [pH 7.5], 300 mM NaCl, 2 mM EDTA [pH 8.0], 1% Triton X-100, 10% glycerol, and protease inhibitor cocktail). The protein extracts were centrifuged at 20,000 x g for 10 min, and the resulting supernatant was incubated with preclarified PIF7-GST/ELF3-GST beads for 1 hour. GST was used as a negative control. The beads were washed in extraction buffer five times, resuspended in SDS-PAGE loading buffer and analyzed by both SDS-PAGE and immunoblotting with anti-ELF3 antibodies or anti-Myc (Sigma-Aldrich, USA). The polyclonal antibodies against ELF3 were produced by GL Biochem (Shanghai, China). The peptide CSIQEERKRYDSSKP was used to immunize rabbits to raise polyclonal antibodies. A protein affinity column was then used to purify the ELF3 antibodies. Western blotting was performed to determine the specificity of the ELF3 antibodies, as shown in Figure 3B.

**Co-IP assays.** Total protein extracts were prepared from PIF7oxELF3ox and ELF3ox seedlings. A Co-IP assay was performed as described previously (Peng et al., 2018). IP was performed using an anti-Flag affinity gel (Sigma, USA). Input and IP-resulting fractions were analyzed by Western blots using anti-Myc and anti-ELF3 antibodies. The production of anti-ELF3 antibodies has been published previously (Ding et al., 2018).
**ChIP-PCR.** ChIP was performed as previously described (Peng et al., 2018). Col-0, *PIF7oxELF3ox* and *ELF3ox* seedlings were grown under white light for 4 days and then treated with 1 hour of shade. The seedlings were then harvested and cross-linked for 15 min under vacuum in cross-linking buffer (extraction buffer 1 with 1% formaldehyde). Cross-linking was stopped with 125 mM glycine (pH 8.0) under vacuum for 5 min, and then seedlings were washed three times in double-distilled water and rapidly frozen. Bioruptor was used at high power with 30 s on/30 s off cycles fifteen times until the average chromatin size was approximately 300 bp. Anti-Flag M2 affinity gel (Sigma, USA) was used for IP. Quantitative real-time PCR was performed with a kit from Takara to determine the enrichment of DNA immunoprecipitated in the ChIP experiments using the gene-specific primers listed in Supplemental Table S1. To facilitate comparisons, fold-change values were obtained from the enrichment data, expressed as the percent input, by using *AT2G39960* as an internal reference gene.

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Yang, C., Xie, F., Jiang, Y., Li, Z., Huang, X., and Li, L. (2018). Phytochrome A Negatively Regulates the Shade Avoidance Response by Increasing Auxin/Indole Acidic Acid Protein Stability. Dev Cell 44, 29-41.
Figure S1. Transcriptional and translational level of PIF7 at the different time of day with white light or shade treatments, Related to Figure 1.

(A-B) Phosphorylation of PIF7 is regulated by light, not circadian clock. Top panel represents light treatment for the detections of phosphorylation of PIF7. PIF7ox seedlings were grown under SD conditions for 5 days and then transferred to continuous white light, after which the continuous white light (A) or the shade treatment (B) was started for 1 hour. The white, black and gray colors represent white light, darkness and shade, respectively. The bottom panels represent the phosphorylation level of PIF7. (C) Phosphorylation and protein level of PIF7 under SDs. Five-day-old SD-grown PIF7ox seedlings were kept under SD conditions. The protein level of PIF7 was detected every 3 hours from ZT0 to ZT21 using anti-Myc antibody. (D) The transcriptional level of PIF4 and PIF7 under short day. Transcriptional levels of PIF4 and PIF7 were detected every 3 hours from ZT0 to ZT21 by qRT-PCR. Data was presented as mean with SEMs of three biological replicates. The asterisk indicates statistically significant differences between mean values according to Student’s t-test (** P < 0.01, *** P < 0.001).
Figure S2. Transcriptional and translational level of ELF3 after shade treatment, Related to Figure 2.

(A) Light treatment for the detection of shade-induced gene expression and hypocotyl growth. Wild-type seedlings were grown under SDs for 5 days and then transferred to continuous white light, after which the shade treatment was started or the seedlings were kept under continuous white light for 1 hour. The white, black and gray colors represent white light, darkness and shade, respectively. (B) Transcriptional level of ELF3 after shade treatment. The error bars indicate the SEMs of three independent studies. (C) Detection the specificity of anti-ELF3 antibody. Seedlings were grown for 5 days under white light, the same number of seedlings were taken, total protein was extracted, and the content of the target protein ELF3 was detected by anti-ELF3 antibody. (D) Translational level of ELF3 at 16 hours and 20 hours after transfer to constant conditions. Equal loading of samples is shown by anti-H3 antibodies.
Figure S3. Effect of ELF3 on the expression level of PIF7’s targets, Related to Figure 2.  
(A) Transcriptional level of IAA29, PIL1, IAA19, YUCCA8, YUCCA9, IAA2, GH3.3, ATHB4, and HAT2 from published RNA-sequencing data of elf3-1 (Ezer et al., 2017). (B) Transcriptional levels of YUCCA8, IAA29 and PIL1 were confirmed by qRT-PCR. Five-day-old SD-grown wild-type seedlings were collected every 3 hours from ZT0 to ZT21. The error bars indicate the SEMs of three independent studies. (C) Relative transcriptional level of ELF3 in Col-0 and ELF3ox seedlings. Seedlings were grown for 5 days in white light. (D) Relative protein level of ELF3 in ELF3ox seedlings. Seedlings were grown for 5 days under white light, the same number of seedlings were taken, total protein was extracted, and the content of the target protein ELF3 was detected by anti-ELF3 antibody. ELF3-GST was purified from E. coli. (E) Relative expression of IAA29 and PIL1 in Col-0 and ELF3ox seedlings. Seedlings were grown under SD conditions for 5 days and transferred to continuous white light, and started shade treatment or kept in continuous white light for 1 hour. The white, black and gray colors represent white light, darkness and shade, respectively. The error bars indicate the SEMs of three independent studies.
Figure S4. Effect of ELF3 on the expression level of *PIF4* and *PIF7* at the different time of short day, Related to Figure 3.

Transcriptional levels of *PIF4* and *PIF7* were confirmed by qRT-PCR. Five-day-old SD-grown wild-type seedlings were collected every 3 hours from ZT9 to ZT21. The error bars indicate the SEMs of three independent studies.
Figure S5. ELF3 suppresses the DNA-binding activity of PIF7 in vivo, Related to Figure 4.
ChIP-PCR analysis using anti-Flag agarose at various chromatin regions of IAA29 and PIL1 chromatin in Col-0, PIF7ox, and PIF7oxELF3ox seedlings. ChIP assays were performed via 4-day white light-grown seedlings treated with 1 hour of shade. The top panels show a schematic of the gene structure of IAA29 and PIL1. The back boxes represent coding regions, and the white boxes represent untranslated regions. The G-box within the gene promoter is indicated. The bars labeled with numbers represent regions examined by PCR. The asterisk indicates statistically significant differences between mean values according to Student’s t-test (*** $P < 0.001$).
Figure S6. ELF3 acts upstream of PIF7 in shade-induced growth, Related to Figure 5.

(A) Relative expression of *IAA29* and *PIL1* in *Col-0, elf3-1, elf3-1pif7-1* and *pif7-1* seedlings at 16 hours after transfer to constant conditions. The top panel represents the light treatment for the detection of shade-induced gene expression. Wild-type seedlings were grown under SD conditions for 5 days and transferred to continuous white light, after which the shade treatment was started or the seedlings were kept under continuous white light for 1 hour. The white, black and gray colors represent white light, darkness and shade, respectively. The bottom panels represent the expression of *IAA29* and *PIL1*. The error bars indicate the SEMs of three independent experiments. The asterisk indicates statistically significant differences between mean values according to Student's t-test (* *P* < 0.05, ** *P* < 0.001).

(B) Hypocotyl phenotypes *Col-0, PIF7ox, ELF3ox, and ELF3ox-PIF7ox* seedlings under SDs or EOD shade treatment. The top panel represents the light treatment for the hypocotyl measurements of seedlings grown under SDs and EOD shade conditions. The seedlings were grown for 3 days under SDs and either kept under SDs or treated for 2 hours with shade at the end of each day for 4 days. The white, black and gray colors represent white light, darkness and shade, respectively. The bottom panels represent the phenotypes of hypocotyl length. The data are presented as the means with SEMs; more than 20 seedlings were measured. The bars marked with different letters denote significant differences (*P* < 0.05), calculated by Student's t-test. The scale bar represents 2 mm.
### Table S1. Primers used in this study, Related to Figure 1, Figure2, Figure3, Figure4, and Figure5.

| Primers for genotyping | CCGTTTCATGGTCTTAGGCCG |
|------------------------|------------------------|
| *pif7-1* LP            | CATCCTCTGGTTTATCCCTACAGGC |
| *pif7-1* RP            | TGGTGGTCAGTCTTCCTCGGA |
| *elf3-1* -F            | TCCCTACTGTGCTTACAGGGG |
| *elf3-1* -R            | ATCCGGTTTGGAATCTTCTGTTTCCCACATG |
| *cca1-1* LP            | CTCTGTGGGCTGCCGAAACTTATAGA |
| *cca1-1* RP            | AACCTGACATGCAAAGATGTTCCG |
| *prr5-11* LP           | GTCCGTTTGTGTTCCATAGA |
| *prr5-11* RP           | TCTCAGAAGCATTAGGTCTTT |
| *WT toc1-2*            | TCCCTTCAGTTCTTATTACAG |
| *MT toc1-2*            | TCCCTTCAGTTCTTATTACACA |
| *toc1-2* R             | TCAAGCTTTCGCAAGCCTCA |

| Primers for clone | cgg ggtacc ATGAAGAGAGGGGAAGATGAGG |
|-------------------|----------------------------------|
| *cYFP-ELF3-Kpn1*  | gcc ggtacc ATGAAGAGAGGGGAAGATGAGG |
| *cYFP-ELF3-BamH1* | nYFP-PIF7-BamH1 -F |
| *nYFP-PIF7-BamH1* | gcc ggtacc ATGTCGAATTATGGAGTTAA |
| *nYFP-PIF7-Pst1*  | acg ctgcag CTAATCCTTCTTTTCATGAT |
| *nLUC-PIF7-BamH1* | gcc ggtacc ATGTCGAATTATGGAGTTAA |
| *nLUC-PIF7-SalI*  | aaa gtgcac ATCTCCTTTTCATGAT |
| *cLUC-ELF3-Sal1*  | cgg ggtacc ATGAAGAGAGGGGAAGATGAGG |
| *Pcambia2302-ELF3* | cgg ggtacc ATGAAGAGAGGGGAAGATGAGG |
| *Pcambia2302-ELF3-BamH1* | gcc ggtacc ATGAAGAGAGGGGAAGATGAGG |
| *pGEX4T-2-ELF3-BamH1* | gcc ggtacc ATGAAGAGAGGGGAAGATGAGG |
| *pGEX4T-2-ELF3-Sal1* | gcc ggtacc ATGAAGAGAGGGGAAGATGAGG |

| Primers for qRT-PCR | TGAAACAAAACAACCCACGA |
|---------------------|----------------------|
| *YUCCA8*-F         | TGGACTAATTCCAAACACTCCTT |
| *PIL1*-F           | GCCAGATCCCAAACACCCACGA |
| *IAA29*-F          | TCCGATTTGAACGCCTACCTT |
| *IAA29*-R          | ACCGTGTGCCATATAAAGATGTTT |

| Primers for ChIP-PCR | GCCATATGGATATGGTCCT |
|----------------------|---------------------|
| *IAA29*-ChIP-1F      | GAAATATCACAAGTCTGTT |
| *IAA29*-ChIP-1R      | ATGGAGTTGATCTTGCTTATC |
| *IAA29*-ChIP-2F      | ATCCCTAACCACACCGTCG |
| *PIL1*-ChIP-1F       | TGAGATGGAGACGCCG |
| *PIL1*-ChIP-1R       | GACCGGAAGAACCTTACAG |
| *PIL1*-ChIP-2F       | TAGATGTCTCAGG |
| *PIL1*-ChIP-2R       | TTAGATCTCAGGTCAGTCC |

| Primers for clone | cgg ggtacc ATGAAGAGAGGGGAAGATGAGG |
|-------------------|----------------------------------|
| *cYFP-ELF3-Kpn1*  | gcc ggtacc ATGAAGAGAGGGGAAGATGAGG |
| *cYFP-ELF3-BamH1* | nYFP-PIF7-BamH1 -F |
| *nYFP-PIF7-BamH1* | gcc ggtacc ATGTCGAATTATGGAGTTAA |
| *nYFP-PIF7-Pst1*  | acg ctgcag CTAATCCTTCTTTTCATGAT |
| *nLUC-PIF7-BamH1* | gcc ggtacc ATGTCGAATTATGGAGTTAA |
| *nLUC-PIF7-SalI*  | aaa gtgcac ATCTCCTTTTCATGAT |
| *cLUC-ELF3-Sal1*  | cgg ggtacc ATGAAGAGAGGGGAAGATGAGG |
| *Pcambia2302-ELF3* | cgg ggtacc ATGAAGAGAGGGGAAGATGAGG |
| *Pcambia2302-ELF3-BamH1* | gcc ggtacc ATGAAGAGAGGGGAAGATGAGG |
| *pGEX4T-2-ELF3-BamH1* | gcc ggtacc ATGAAGAGAGGGGAAGATGAGG |
| *pGEX4T-2-ELF3-Sal1* | gcc ggtacc ATGAAGAGAGGGGAAGATGAGG |

| Primers for qRT-PCR | TGAAACAAAACAACCCACGA |
|---------------------|----------------------|
| *YUCCA8*-F         | TGGACTAATTCCAAACACTCCTT |
| *PIL1*-F           | GCCAGATCCCAAACACCCACGA |
| *IAA29*-F          | TCCGATTTGAACGCCTACCTT |
| *IAA29*-R          | ACCGTGTGCCATATAAAGATGTTT |

| Primers for ChIP-PCR | GCCATATGGATATGGTCCT |
|----------------------|---------------------|
| *IAA29*-ChIP-1F      | GAAATATCACAAGTCTGTT |
| *IAA29*-ChIP-1R      | ATGGAGTTGATCTTGCTTATC |
| *IAA29*-ChIP-2F      | ATCCCTAACCACACCGTCG |
| *PIL1*-ChIP-1F       | TGAGATGGAGACGCCG |
| *PIL1*-ChIP-1R       | GACCGGAAGAACCTTACAG |
| *PIL1*-ChIP-2F       | TAGATGTCTCAGG |
| *PIL1*-ChIP-2R       | TTAGATCTCAGGTCAGTCC |