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GIGANTEA adjusts the response to shade at dusk by directly impinging on PHYTOCHROME INTERACTING FACTOR 7 function

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For plants adapted to bright light, a decrease in the amount of light received can be detrimental to their growth and survival. Consequently, in response to shade from surrounding vegetation, they initiate a suite of molecular and morphological changes known as the shade avoidance response through which stems and petioles elongate in search for light. Under sunlight–night cycles, the plant's responsiveness to shade varies across the day, being maximal at dusk time. While a role for the circadian clock in this regulation has long been proposed, mechanistic understanding of how it is achieved is incomplete. Here, we show that the clock component GIGANTEA (GI) directly interacts with the transcriptional regulator PHYTOCHROME INTERACTING FACTOR 7 (PIF7), a key player in the response to shade. GI represses PIF7 transcriptional activity and the expression of its target genes in response to shade, thereby fine-tuning the magnitude of the response to limiting light conditions. We find that under light/dark cycles, this function of GI is required to adequately modulate the gating of the response to shade at dusk. Importantly, we also show that this circuit primarily operates in epidermal cells, highlighting the relevance of tissue-specific clock-output connections for the regulation of plant development in resonance with the environment.

shade avoidance | circadian gating | GIGANTEA | PIF7 | tissue specificity

Significance

Because of the importance of light to their survival, plants have evolved sophisticated mechanisms to optimize its exploitation. An outstanding adaptive response in terms of plant plasticity in dynamic light environments is the shade avoidance response which sun-loving plants deploy to escape canopy and grow toward the light. This response is regulated by a complex signaling network in which cues from different pathways are integrated, including light, hormone, and circadian signaling. Our study provides a mechanistic model of how the circadian clock contributes to this network by modulating the sensitivity to shade signals at dusk. In light of evolution and local adaptation, this work gives insights into a mechanism through which plants may have optimized resource allocation in fluctuating environments.

Light is a key environmental cue and resource for plants, as they use it to interpret their surroundings but also rely on it to perform photosynthesis and fix carbon, which is essential for plant growth and development. In both natural and agricultural settings, the light environment is highly dynamic, and plants are constantly monitoring light quantity and quality to adapt to it accordingly. Because of the importance of light to their survival, plants have evolved exquisite mechanisms to maximize exploitation of this resource and to cope with unfavorable conditions (such as limiting or high-intensity light). For plants adapted to open environments, changes in light quality caused by neighboring vegetation are interpreted as a threat entailing competition for light and trigger an adaptive response to escape canopy known as the shade avoidance response (SAR) (1). Phenotypically, the SAR comprises a series of morphological changes which include stem and petiole elongation, leaf hyponasty, and early flowering, among others (1–3).

At the molecular level, the proximity of other plants is sensed as a change in the ratio of red to far-red (R:FR) light, which is caused by an enrichment in the FR wavelengths of the light spectrum that are reflected and transmitted through the leaves of the surrounding vegetation (4). This change in light quality is perceived by the phytochrome family of photoreceptors, especially phytochrome B (phyB) (4–6), which then transduce the signal to transcriptional networks through the regulation of the activity of the basic helix–loop–helix transcription factors PHYTOCHROME INTERACTING FACTORS (PIFs) (4). Under bright light, where the R:FR ratio is high, phyB is in its active far-red-absorbing form (Pfr) which localizes in the nucleus where it physically interacts with PIFs and promotes their phosphorylation and subsequent degradation. In the shade, the enrichment in FR light (low R:FR ratio) promotes the photoconversion of Pfr to its inactive red-absorbing form which is translocated to the cytoplasm thereby allowing PIF accumulation and activity (4). This then enables the induction of the expression of auxin biosynthesis enzymes and cell elongation genes, which promote and support shade-induced growth (7, 8). Several PIFs have been implicated in the response to shade, including PIF4 and 5 (9) and PIF7 (10, 11), which seems to play a dominant role in this pathway.

In the field, plants must adapt a sessile lifestyle under fluctuating environments and are presented with a variety of challenges on a daily basis, many of which arise from the existence of day/night cycles. These cycles generate, for example, large but predictable fluctuations in important ambient variables including light intensity. In this context, organisms have evolved circadian clocks as endogenous time-tracking mechanisms that

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enable them to anticipate these changes and to organize their physiology accordingly, precisely timing biological processes to occur at the most appropriate times and thereby maximizing resource allocation (12). An important modality through which the circadian clock delivers time-of-day information to output signaling pathways is gating. Circadian gating entails the circadian clock to adjust the sensitivity of output pathways to external and internal stimuli so that the magnitude of the response to a given signal will vary depending on the time of the day. It is assumed that this helps plants filter whether an ambient fluctuation is relevant and ensures that the elicited response is appropriate for the time of the day (12, 13).

In the case of the responsiveness to shade, it has been shown that it varies across the day. Shade light experienced at different times of the day promotes hypocotyl elongation (14–17), but it is most effective when occurring at dusk (14, 17, 18). Importantly, it was shown that the circadian clock plays a role in this temporalization (14, 18). In this regard, several clock components have been implicated in the regulation of shade signaling (14, 17–20), but only loss of function of the core clock genes *TIMING OF CAB EXPRESSION 1* and *CIRCADIAN CLOCK ASSOCIATED 1* and *LATE ELONGATED HYPOCOTYL* was shown to impair temporalization of the response as these mutants reacted equally to shade at both dusk and dawn (14, 18). The underlying molecular mechanisms, however, remain to be elucidated. More recently, it was reported that another clock gene, *EARLY FLOWERING 3*, represses PIF7 at night mediating the gating of the response at this time (17). However, it is unclear what the physiological relevance of such a finding might be, given that under natural conditions, shade poses a stress and is sensed during the light period, not in the middle of the night. We have previously shown that the clock component GIGANTEA (GI) restricts growth at dusk and during the early night by affecting PIF expression and function at multiple levels, including transcriptional and posttranslational mechanisms (21). This function of GI proved to be key to regulate growth rhythms and establish the phase of maximal hypocotyl growth at the end of the night period (21, 22). Here, we show that GI is also required to modulate growth in response to environmental changes fine-tuning the magnitude of the response to shade at dusk. GI achieves this through direct interaction with PIF7 and regulation of the responsiveness of its transcriptional targets to shade at dusk. Furthermore, we pinpoint the epidermis as the key tissue where GI function is required in this pathway, reinforcing current models on circadian clock spatial organization and tissue-level specialization for the regulation of output pathways.

Results and Discussion

***GI* Mutants Display Shade Avoidance Syndrome–Related Traits and Are Hypersensitive to Shade.** GI is a regulator of light signaling and growth and, consequently, *gi* mutants display longer hypocotyls under different light conditions (21, 23, 24). In addition to this increase in hypocotyl length, these mutants also present other traits reminiscent of those that appear in response to shade, such as hyponastic leaves (Fig. 1 *A* and *B*). We therefore examined whether the response to shade is altered in this mutant. We observed that *gi-2* mutants are indeed more responsive to shade and display longer hypocotyls when grown under constant shade conditions (low R:FR ratio < 0.7) (Fig. 1*C*).

In previous studies, it was shown that the fast, initial hypocotyl elongation response to shade is biphasic and occurs after an initial lag phase (15). Examination of this early response to shade revealed that it is also altered in *gi-2* seedlings, which display a shorter lag

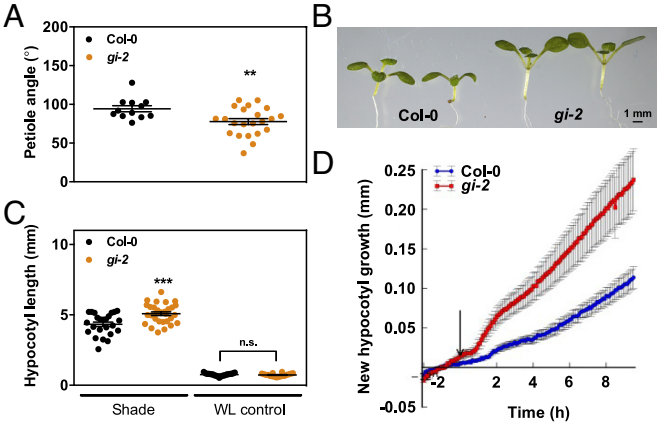

Fig. 1. GI is a negative regulator of the response to shade. (*A*) Petiole angle measurements from wild-type (Col-0) and *gi-2* seedlings grown for 10 d under SD conditions. Mean ± SEM, n = 12 to 22; $^{**}P < 0.01$ Student's *t* test. (*B*) Representative pictures of Col-0 and *gi-2* seedlings grown for 10 d under SDs. (*C*) Hypocotyl length measurements from wild-type (Col-0) and *gi-2* seedlings grown for 4 d under continuous white light and then transferred to constant shade light for 7 d (Shade) or kept in white light (WL control). Mean ± SEM, n = 18 to 30; $^{***}P < 0.001$, n.s. not significant Tukey's multiple comparison test. (*D*) New hypocotyl growth observed for Col-0 and *gi-2* seedlings exposed to supplemental FR light to give a R:FR ratio of 0.7. The arrow indicates the start of treatment (t = 0), and hypocotyl growth was monitored for 10 h after the treatment. Mean ± SEM, n = 12.

phase after which elongation occurs in a biphasic mode but at a considerably faster rate compared to wild-type seedlings (Fig. 1*D*). This indicates that *gi-2* mutants not only grow more but they also respond faster to the change in light quality.

Hormone signaling is central to the regulation of growth-related processes and, consequently, several hormones, including brassinosteroids, gibberellins (GAs), and auxin, play a role in the promotion of hypocotyl elongation in response to shade (25). Auxin, however, seems to be a key player in this pathway (4, 7, 8). With regard to hormone signaling, gene ontology analyses of genes differentially expressed in *gi-2* seedlings have shown that several hormone-related pathways are altered in these mutants, including auxin signaling (21). Additionally, a role for GI in the gating of the response to GAs was recently uncovered and characterized (26). Consequently, we inspected the relevance of both auxin and GAs for the fast response to shade in *gi* mutants (*SI Appendix*, Fig. S1). We compared the early response to shade in wild-type [Columbia 0 (Col-0)] and *gi-2* seedlings in the presence or absence of paclobutrazol (an inhibitor of GA synthesis) and N-1-naphthylphthalamic acid (an inhibitor of auxin polar transport). Although a significant effect on new hypocotyl growth rate was observed for both treatments, the effect of auxin transport blockage was more drastic, completely nullifying the response (*SI Appendix*, Fig. S1). This is consistent with previous observations that auxin plays an essential role in the promotion of hypocotyl elongation in response to shade and indicates that it is required for the function of GI in this pathway.

GI Interacts with PIF7 and Functions Upstream of It for the Regulation of the Response to Shade. PIF7 plays a prominent role in shade signal transduction as it accumulates in its dephosphorylated (active) form and increases the expression of auxin biosynthetic genes (10, 11). Given that GI interacts with and modulates the activity of several PIFs, we wondered whether interaction with PIF7 could be an underlying mechanism of GI function in the response to shade. We confirmed the interaction between GI and PIF7 through several complementary approaches including yeast two-hybrid (Y2H) assays (Fig. 2*A*), in vitro pull-downs (Fig. 2*B*), and through in vivo coimmunoprecipitations

(coIPs) in *Arabidopsis thaliana* transgenic lines expressing tagged protein versions of PIF7 and GI (Fig. 2*C*). Genetic analyses also showed that loss of *PIF7* strongly affected hypocotyl elongation specially under shade light and that it completely reduced the long hypocotyl phenotype of *gi-2* in response to shade (Fig. 2*D*). It also rescued the reduced petiole angle phenotype of *gi-2* observed under short day (8 h light/16 h darkness, SD) conditions (*SI Appendix*, Fig. S2*A*). In addition to PIF7, PIF4 and PIF5 have also been implicated in the response to shade (9, 27) and GI is known to modulate the accumulation and activity of these PIFs for the regulation of photoperiodic growth (21). In fact, the rapid response to shade observed in *gi-2* seedlings strongly resembles what occurs under SD photoperiods, where the expression of growth-promoting genes typically expressed at the end of the night is rapidly induced upon darkness in the absence of GI, resulting in the promotion of hypocotyl elongation at this time (21). We therefore wondered whether GI function in the response to shade occurred mainly through PIF7 or whether it included regulation of other PIFs such as PIF4 and PIF5. Hypocotyl length measurements showed that under SD conditions, it was the loss of *PIF3*, *PIF5*, and both *PIF4* and *PIF5* that had the strongest effect on hypocotyl elongation, strongly reducing the long hypocotyl phenotype of *gi-2* mutants (*SI Appendix*, Fig. S2*B*). Under these conditions, mutations in *PIF7* resulted in a partial suppression of the elongated hypocotyl phenotype of *gi-2* mutants, indicating its partial contribution to this process under this light regime. In response to shade light, however, it became evident that *PIF7* played a more prominent role with the *pif7-1* mutation displaying the strongest phenotype and being the one more significantly reducing the hypocotyl elongation phenotype of *gi-2* both under constant shade light (*SI Appendix*, Fig. S2*C*) or under light/dark cycles supplemented with shade (*SI Appendix*, Fig. S2 *D* and *E*). Thus, this genetic interaction supports the hypothesis that *GI* regulates the response to shade upstream of *PIF7*.

GI Represses PIF7 Activity and Affects Its Binding to Target Promoter Regions in Response to Shade. GI regulates PIF activity and accumulation through several mechanisms that include transcriptional and posttranslational regulation (21, 28). Because *PIF7* expression was observed to be largely unaffected by loss of *GI* function (*SI Appendix*, Fig. S3*A*), we focused on the mechanistic implications of the GI–PIF7 interaction at the protein level. First, we analyzed the effect of GI on PIF7 protein accumulation. We observed that coinfiltration of GI together with PIF7 in transient expression in *Nicotiana benthamiana* leaves did not have any effect on PIF7 accumulation (*SI Appendix*, Fig. S3*B*). A similar trend was observed in *Arabidopsis* lines expressing a tagged version of PIF7 driven by its own promoter (Fig. 3*A* and *SI Appendix*, Fig. S3*C*). These lines behaved like PIF7 overexpression lines (*SI Appendix*, Fig. S4), likely because they were generated in a Col-0 background. Consistent with PIF7 prominent role under shade, we observed that these lines only slightly promoted hypocotyl elongation under SD but grew considerably more than wild-type seedlings in shade (*SI Appendix*, Fig. S4*C*). Analysis of the accumulation of PIF7 in these lines (in both Col-0 and *gi-2* backgrounds) showed that although a slight increase in total PIF7 levels could be observed in shade in *gi-2*, no significant differences in the accumulation of nonphosphorylated (active) PIF7 [under both white light (WL) and shade conditions] (Fig. 3*A*) or in the ratio phosphorylated to total PIF7 (*SI Appendix*, Fig. S3*D*) could be quantified. Hence, these experiments suggest that GI interaction with PIF7 does not significantly affect its stability. This finding is not entirely surprising, as previous studies have shown that PIF7 is more stable than other PIFs and, although phosphorylated, it is not rapidly degraded in the light (10, 29). Nevertheless, we further investigated the effect of GI on PIF7 accumulation after long-term shade treatments. In this case, we did see a more significant effect of GI in promoting PIF7 degradation, as an increase in PIF7 levels could be observed in the *gi-2* background after 5 d in constant shade (*SI Appendix*, Fig. S5

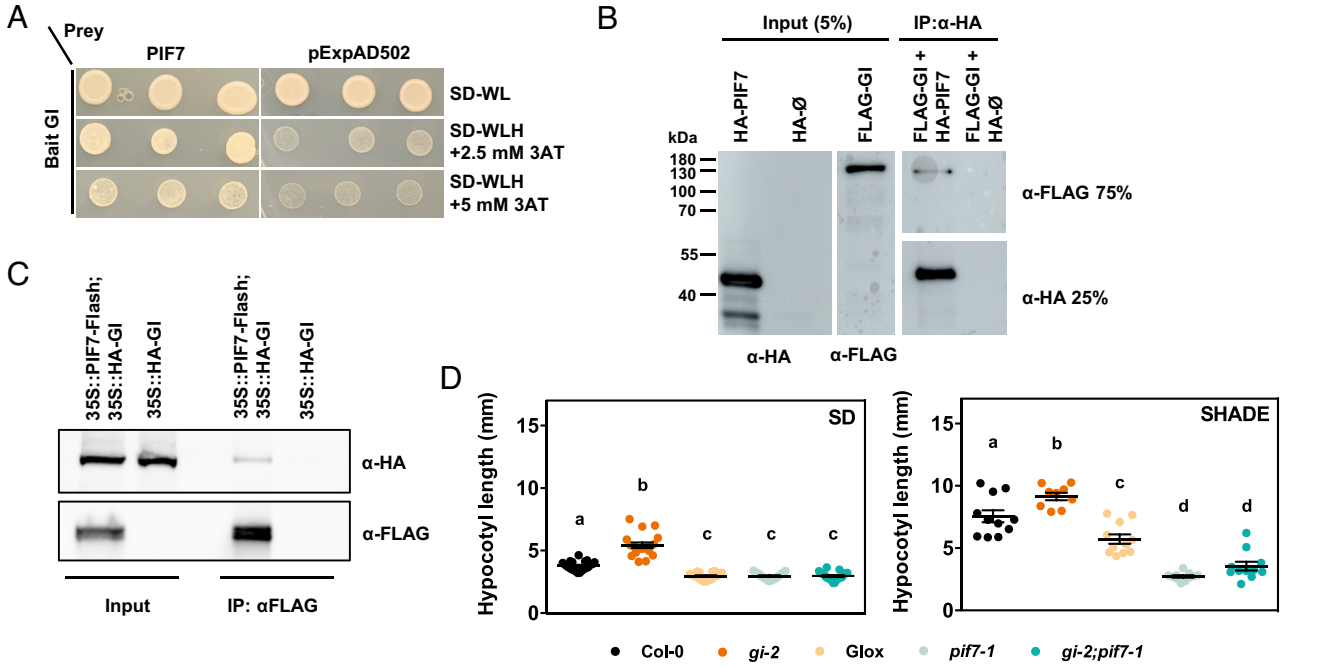

Fig. 2. GI interacts with PIF7 and functions upstream of it in the response to shade. (*A*) Y2H assays showing the interaction of GI and PIF7 proteins. Bait and prey constructs were cotransformed into yeast cells. SD-WL, minimal medium lacking Trp and Leu; SD-WLH, selective medium lacking Trp, Leu, and His, which was supplemented with 2.5 or 5 mM 3-AT. (*B*) In vitro pull-down assays showing the interaction between GI and PIF7. Proteins were expressed in an in vitro transcription and translation system. (*C*) In vivo coIPs in *Arabidopsis* transgenic seedlings expressing HA-GI and PIF7-Flash tagged protein versions expressed from the 35S promoter. Seedlings were grown for 7 d under SD conditions and harvested at ZT 8. (*D*) Hypocotyl length measurements from the indicated genotypes grown for 7 d under SD conditions (*Left*) or under continuous white light for 2 d and then transferred to constant shade light for 5 d (*Right*). Mean ± SEM, n = 9 to 21; results from Tukey's multiple comparison test are shown in compact letter display.

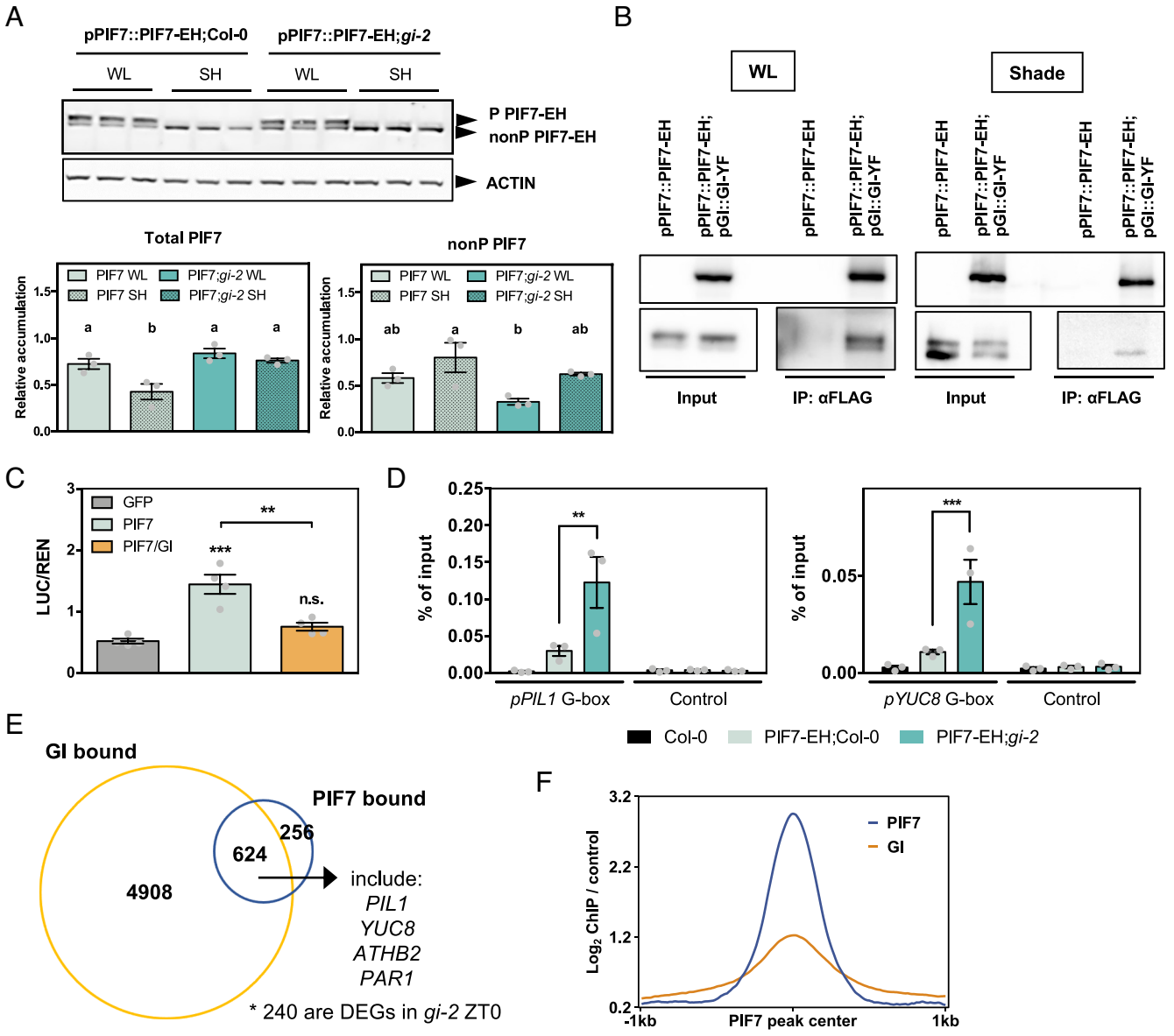

Fig. 3. GI interferes with PIF7 transcriptional activation activity. (*A*) PIF7-ECFP-HA protein accumulation at ZT 9 in the indicated backgrounds grown for 7 d under 10 h light/14 h dark conditions. Seedlings were either treated with shade for 1 h prior to harvesting (ZT 8 to 9) or kept in the light. The lower panels show the quantitation of total (*Left*) and nonphosphorylated (*Right*) PIF7 (mean ± SEM of three biological replicates, results from Tukey's multiple comparison test are shown in compact letter display). Protein levels were normalized against ACTIN levels. (*B*) CoIP assays in *Arabidopsis* transgenic seedlings expressing PIF7-ECFP-HA and GI-YPET-FLAG from their respective endogenous promoter fragments. Seedlings were grown for 7 d under 10 h light/14 h dark conditions and then either treated with shade for 1 h prior to harvesting (ZT 8 to 9) or kept in the light. (*C*) Transactivation assays in *N. benthamiana* leaves. Different effectors were coexpressed with the pPIL1::LUC reporter construct. Luminescence was measured 3 d postinfiltration and the ratio LUC/REN was calculated. Results show mean ± SEM (n = 4). ***$P < 0.001$, **$P < 0.01$, n.s. not significant Tukey's multiple comparison test. (*D*) ChIP assays of 7-d-old seedlings grown under 10 h light/14 h dark conditions and then treated with shade for 1 h prior to harvesting (ZT 8 to 9). The enrichment of the specified regions in the immunoprecipitated samples was quantified by qPCR. Values represent mean ± SEM of three biological replicates. ***$P < 0.001$, **$P < 0.01$ Tukey's multiple comparison test. (*E*) Overlap between GI and PIF7 bound genes (hypergeometric test *P* value < 4.827e-223). (*F*) Metaplot of the signal from PIF7 and GI ChIPseq plotted over the centers of PIF7 peaks.

A and *B*). Under this condition, GI levels are similar to those under constant WL (*SI Appendix*, Fig. S5*C*). Considering the results from short and long-term shade treatments, our interpretation is that GI only moderately contributes to PIF7 destabilization under light/dark cycles, but that its function is potentiated under prolonged shade probably to attenuate PIF7 activity under this condition. We also explored whether PIF7 reciprocally affects GI stability. As shown in *SI Appendix*, Fig. S6, GI protein levels were not seen to be affected by PIF7 overexpression either in transient expression in *N. benthamiana* leaves or in stable *Arabidopsis* transgenic lines.

We next performed coIPs in double transgenic lines expressing tagged protein versions of PIF7 (fused to ENHANCED CYAN FLUORESCENT PROTEIN and the HEMAGGLUTININ tag, ECFP-HA) and GI (fused to YPet yellow fluorescent protein and a FLAG tag, YPET-FLAG) driven by endogenous promoter fragments (pPIF7::PIF7-ECFP-HA;pGI::GI-YPET-FLAG) under WL or shade conditions to investigate whether GI preferentially interacts with either the phosphorylated or nonphosphorylated form of PIF7. We found that although GI is able to interact with both forms of PIF7 under WL, it seems to preferentially interact with the dephosphorylated one in shade (Fig. 3*B*). This could be due to increased availability of this form, as it is the one that preferentially accumulates in the nucleus in shade (30). In any case, sequestration of this form, which is the active one, may be a means through which GI interferes with PIF7-mediated expression of shade-responsive genes.

To further examine the regulation of PIF7 transcriptional activity by GI, we performed transient transcriptional activation assays in *N. benthamiana* leaves using the pPIL1::LUC construct as a reporter of PIF7 transcriptional activity (21, 31). This construct contains the promoter of the well-characterized PIF target gene *PHYTOCHROME INTERACTING FACTOR 3-LIKE 1* (*PIL1*) driving the expression of the firefly luciferase gene (LUC) and it also carries the Renilla luciferase gene (REN) under control of a constitutively expressed promoter as an internal control for normalization. As expected, expression of PIF7 led to an increase in LUC reporter activity (Fig. 3*C*). Coinfiltration of GI together with PIF7, however, led to a significant reduction in pPIL1::LUC activation suggesting that GI interaction with PIF7 is indeed negatively affecting its ability to activate transcription. This observation further supports that GI may negatively regulate the response to shade by directly interacting with PIF7 and restricting its ability to activate the expression of shade-responsive genes. In fact, expression of one such gene, *YUCCA 8* (*YUC8*), is significantly up-regulated in *gi-2* mutants under SD photocycles (*SI Appendix*, Fig. S3*A*). In order to explore the relevance of these findings in vivo, we examined the effect of GI on the association of PIF7 to its genomic targets through chromatin immunoprecipitation studies in *A. thaliana* lines. Because PIF7 associates to target sites preferentially under low R:FR light (32), we analyzed the binding of PIF7 to the G-box-containing regions in the promoters of its well-known targets *PIL1* and *YUC8* (10) under these conditions in the presence and absence of GI. For both genes, we observed a significant increase in the enrichment of PIF7 target regions in the immunoprecipitated fractions in the absence of GI (*gi-2* mutant background) (Fig. 3*D*), supporting the notion that GI functions to modulate access of PIF7 to target sites in response to shade. Furthermore, a significant number of genes bound by PIF7 in shade genome-wide (32), including *PIL1* and *YUC8*, are also bound by GI (21) (Fig. 3*E*, hypergeometric test *P* value < 4.827e-223) and are genes for which the most enriched Gene Ontology (GO) categories include growth and cell wall biogenesis, response to auxin, shade avoidance, and abiotic stress responses, especially water deprivation and cold (*SI Appendix*, Fig. S7*A*). Noteworthy, GI and PIF7 occupy the same region around shared peaks (Fig. 3*F* and *SI Appendix*, Fig. S7*B*), but at dusk and in response to shade, respectively. This suggests that in addition to repression through direct interaction, GI may additionally sterically impede PIF7 binding to target genes similarly to other PIFs (21). This would provide a mechanism to modulate the responsiveness to environmental cues and their effect on plant growth. Importantly, these cues are likely not restricted to light conditions but may include others such as water availability and temperature (*SI Appendix*, Fig. S7*A*). In this regard, for example, we find that both GI and PIF7 bind the promoters of canonical genes involved in these pathways such as *DREB1A* and that *DREB1* genes, which were shown to be regulated by PIF7 under circadian clock control (33), are misexpressed in *gi-2* seedlings (Dataset S1).

GI Restricts the Shade-Responsive Expression of PIF7 Target Genes. Considering our findings on the regulation of PIF7 activity by GI, we next investigated the impact of the interaction between GI and PIF7 on the expression of shade-responsive genes genome-wide. To this end, we performed an RNA sequencing (RNAseq) experiment and identified differentially expressed genes (DEGs) in *gi-2* compared to wild-type (Col-0) plants. Comparison of these genes with a set of genes that are differentially expressed in response to low R:FR light (low R:FR vs. WL, identified from ref. 32) evidenced a significant overlap (hypergeometric test *P* value < 1.741e-121) where the expression of over 66% of genes whose expression changes in response to shade is also misregulated as a consequence of *GI* loss of function (Fig. 4*A*). Importantly, about half of these shared target genes are bound by GI at their promoter regions. In terms of GO enrichment, these genes were again found to be mainly involved in shade avoidance, stress responses, and auxin biosynthesis and signaling (*SI Appendix*, Fig. S8*A*) and include well-known PIF7 target genes such as *PIL1*, *YUC8*, *ARABIDOPSIS THALIANA HOMEOBOX PROTEIN 2* (*ATHB2*), and *PHYTOCHROME RAPIDLY REGULATED*

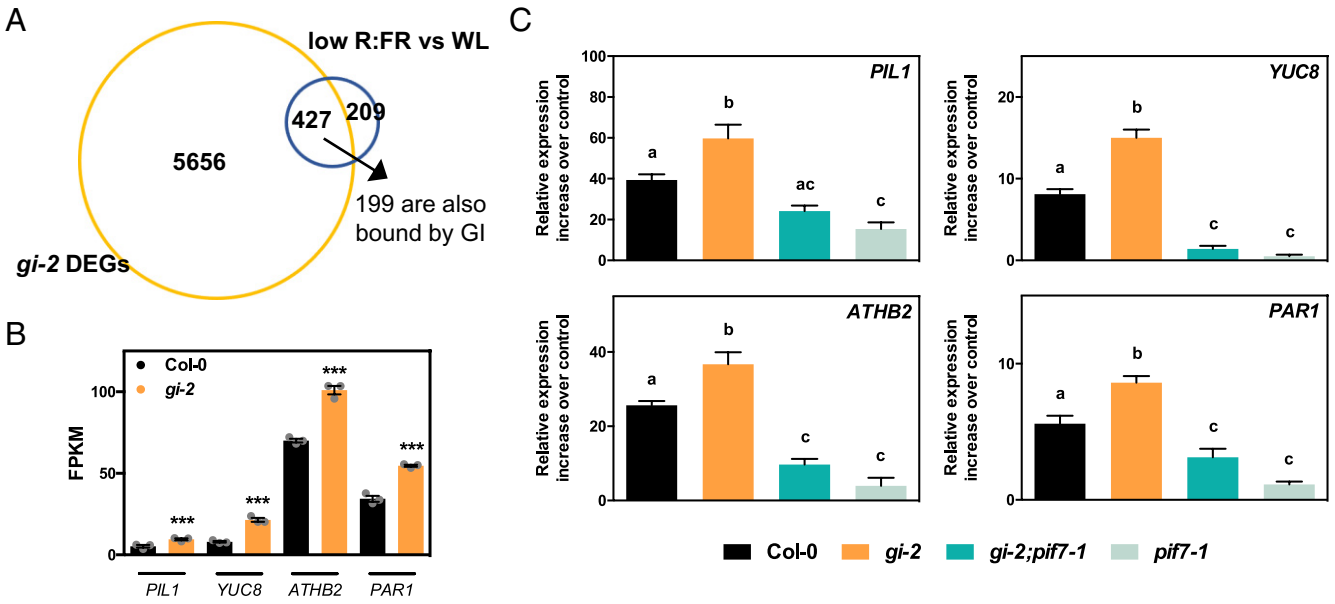

Fig. 4. GI restricts the shade-induced expression of PIF7 target genes. (*A*) Overlap between DEGs in *gi-2* and genes misregulated in response to low R:FR light (intersection *P* value < 1.741e-121). (*B*) Expression levels in FPKM of several PIF7 target genes as identified by RNAseq (***$P < 0.001$). (*C*) Increase in *PIL1*, *YUC8*, *ATHB2*, and *PAR1* expression in the indicated backgrounds in response to a 1 h long treatment with shade at dusk (ZT 8 to 9) relative to nontreated seedlings. Seedlings were grown for 7 d under 10 h light/14 h dark photocycles. Mean ± SEM of three biological replicates. The results from Tukey's multiple comparison test are shown in compact letter display.

1 (*PAR1*) (10, 34). These are genes typically induced upon shade and, consistent with the repressive effect of GI on PIF7 transcriptional activity, they appeared to be significantly up-regulated in *gi-2* (Fig. 4*B*). Given the effect of GI on the hypocotyl elongation response to shade, we next investigated whether the shade-promoted induction of their expression was also affected in *gi-2*. To this end, we grew *Arabidopsis* seedlings under light/dark photocycles (10 h light/14 h darkness) and performed a 1 h long shade treatment around dusk [Zeitgeber Time (ZT) 8]. This is the time when *Arabidopsis* is more responsive to shade (18) and GI is more highly expressed (21, 35, 36). We observed that indeed, these genes are more strongly induced by shade in *gi-2* and that this induction is dependent on PIF7 function (Fig. 4*C*). Interestingly, these genes were still slightly induced in *gi-2;pif7-1* mutants, most likely due to the participation of other factors controlled by GI in their regulation upon shade, such as PIF4 and 5 (20, 21). Noteworthy, the expression of neither *PIF7* nor *GI* was induced, further supporting the notion that the induction of PIF7 target genes is the consequence of a mechanism operating posttranscriptionally (Fig. 4*C* and *SI Appendix*, Fig. S8*B*). Altogether, our findings point to a function of GI in modulating the magnitude of the response to shade by regulating PIF7-mediated transcriptional activation.

GI Gates the Sensitivity to Shade at Dusk and Functions in the Epidermis to Regulate Shade Signaling. It has previously been shown that the response to shade is maximal around dusk and a role for light and circadian signaling components was proposed (14, 17, 18). Hence, we next examined the physiological relevance of GI function in shade signaling and evaluated its role in the gating of the response to shade. Consistent with previous observations, shade events occurring in the morning of light/dark cycles were ineffective, while shade perception in the afternoon had a significant effect on hypocotyl elongation (*SI Appendix*, Fig. S9*A*). In this context, we observed that *gi-2* mutants were hypersensitive to shade at dusk and grew significantly more than Col-0 seedlings. Moreover, this phenotype was observed to be dependent on PIF7 function (*SI Appendix*, Fig. S9*A*). In order to take a closer look at the response at dusk, we performed 2 h shade treatments at different ZTs around dusk time (ZT 6, 7, 8, 9, and 10; seedlings grown under 10 h light/14 h darkness photocycles) and quantified the effect of the treatment on hypocotyl elongation in *gi-2* compared to Col-0 seedlings (Fig. 5*A*). These experiments allowed us to confirm that GI is required to modulate the magnitude of the response to shade and that its function is more relevant at dusk, at ZT 8 to 10 (Fig. 5*A*). To confirm the circadian function of GI in delivering time information to the response to shade independently of signals associated to light/dark cycles, we further performed similar 2 h shade treatments at either subjective dawn or subjective dusk under free-running conditions (i.e., constant WL). The results confirmed that GI is indeed involved in the gating of the response to shade and is required to modulate its magnitude at dusk (*SI Appendix*, Fig. S9*B*).

In *Arabidopsis* seedlings, shade sensed in the cotyledons triggers a transcriptional response to promote auxin biosynthesis, which then travels to the hypocotyl to induce cell elongation in response to the perceived changes in light quality (37, 38). For this, the epidermis was shown to play an important role, as in this tissue auxin functions, at least partially, to induce brassinosteroid-mediated cell elongation (39). In recent years, it is becoming evident that the circadian system in *Arabidopsis* is spatially organized, with circadian clocks differentially processing specific environmental signals in different tissues to coordinate individual physiological responses (40, 41). To gain insights into the spatial characteristics of GI-mediated modulation of the response to shade, we investigated the effect of tissue-specific (TS) expression of GI on hypocotyl elongation under both SD photoperiods and in response to shade. To this end, we transformed *gi-2* null mutants with a suite of constructs in which the coding sequence of *GI* is expressed from an endogenous promoter fragment (pGI) or from different TS promoters (39, 42) and analyzed their ability to complement the mutant phenotype in terms of hypocotyl elongation. The TS promoters used comprised pCAB3 (mesophyll), pCER6 (epidermis), pCO2 (cortex), pSCR (endodermis), pSHR (stele), and pSUC2 (phloem companion cells) (39, 42). As expected, we observed that *GI* expressed from its endogenous promoter rescued the long hypocotyl phenotype of *gi-2* in SD photoperiods and in response to shade (Fig. 5*B*). Noteworthy, from all TS promoters tested, only *GI* expressed in the epidermis behaved like the endogenous promoter line (fully rescuing the long hypocotyl phenotype of *gi-2*) (Fig. 5*B*), even though *GI* was expressed at considerable levels in all lines (*SI Appendix*, Fig. S9*C*). All other TS expression of GI either had no effect or only partially rescued the phenotype. Interestingly, the ability of epidermal GI to repress hypocotyl elongation was stronger under SD conditions, opening the possibility of intrinsic mechanistic differences underlying both processes which may differ in their spatial characteristics. With regard to the GI–PIF7 interaction, investigation of the double transgenic lines pPIF7::PIF7-ECFP-HA;pGI::GI-YPET-FLAG by confocal microscopy revealed that both GI and PIF7 primarily colocalize in the nuclei of epidermal cells, especially in the cotyledons and in response to shade light (Fig. 5*C* and *SI Appendix*, Fig. S10). While GI is present in the nuclei of several cell types (Fig. 5*C* and *SI Appendix*, Fig. S11), we observed that PIF7 accumulates at highest levels in the nuclei of epidermal cells in cotyledons in response to shade (Fig. 5*C* and *SI Appendix*, Fig. S10). This is consistent with previous observations on TS expression of *PIF7* where β-glucuronidase (GUS) expression under the control of the *PIF7* promoter was detected in cotyledons and rosette leaves but not in roots or hypocotyls (33). Nevertheless, in our confocal microscopy inspections, PIF7-ECFP-HA signal could still be observed in some nuclei in cotyledons under WL and in hypocotyls under shade light (*SI Appendix*, Fig. S10). This detection was likely possible due to the higher sensitivity of the confocal microscopy technique (as opposed to GUS staining) and indicates that PIF7 is expressed in both organs, but is found at higher levels in cotyledons. This was further confirmed by western blot analyses of PIF7 protein levels in cotyledons and in hypocotyls (*SI Appendix*, Fig. S12). Hence, PIF7 likely functions locally in the hypocotyl to promote growth in response to shade as earlier proposed (38, 43, 44), but may play a major role in cotyledons, where it can be rapidly activated in response to changes in light quality and promote auxin biosynthesis. In fact, we observed that nuclear accumulation of PIF7 is enhanced by shade light (in both organs, but more significantly in cotyledons), consistent with previous observations by Huang et al. who reported that inactive phosphorylated PIF7 is retained in the cytoplasm by 14-3-3 proteins and relocates to the nucleus upon dephosphorylation, which is promoted by shade light (30). In this regard, the ratio PIF7/GI was significantly increased in the nuclei of cotyledons in response to shade (*SI Appendix*, Fig. S13). A more detailed inspection of PIF7 and GI subnuclear localization under high and low R:FR light further revealed the subcellular dynamics of the interaction. We observed that a fraction of PIF7-ECFP-HA is recruited to nuclear speckles under WL, while it is homogeneously distributed in the nucleoplasm under shade light (*SI Appendix*, Fig. S14*A*), consistent with previous reports (32, 45). As for GI and PIF7 colocalization, it was only seen in the nucleoplasm under both conditions (*SI Appendix*, Fig. S14*B*).

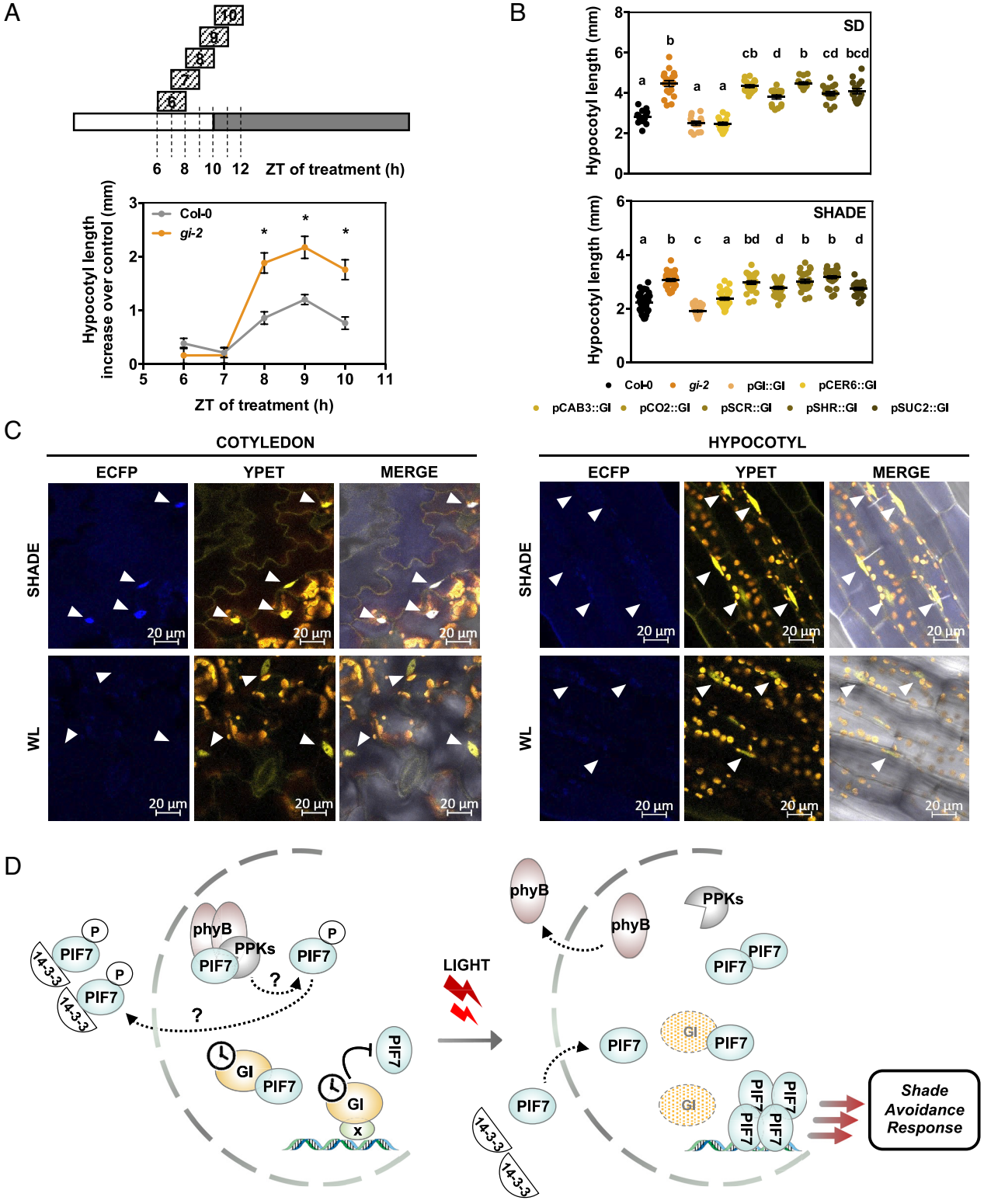

Fig. 5. GI functions in the epidermis to modulate the response to shade at dusk. (*A*) Hypocotyl length increase (measured as the difference between shade-treated and WL–kept seedlings) of 2-d-old seedlings grown under 10 h light/14 h dark conditions and either exposed to shade for 2 h at the times indicated or kept in the light for three consecutive days (mean ± SEM, n = 15 to 23) (*$P < 0.005$ Tukey's multiple comparison test). A scheme of the experimental setup is shown in the *Upper* panel. (*B*) Hypocotyl length measurements from the indicated genotypes grown for 7 d under SD conditions (*Upper*) or under continuous WL for 2 d and then transferred to constant shade light for 2 d (*Lower*). Mean ± SEM, n = 14 to 37; the results from Tukey's multiple comparison test are shown in compact letter display. (*C*) Subcellular localization of PIF7-ECFP-HA and GI-YPET-FLAG expressed from their endogenous promoters in cotyledons and hypocotyls of *Arabidopsis* seedlings in response to shade or under WL. Double pPIF7::PIF7-ECFP-HA;pGI::GI-YPET-FLAG transgenic seedlings were grown for 7 d under 10 h light/14 h dark photocycles and then either transferred to shade for 1 h at ZT8 or kept in WL before imaging. Images of the ECFP and YPET signal were obtained using confocal microscopy. White arrows indicate observed nuclei. (*D*) Model depicting GI function in the response to shade at dusk. At this time, GI-mediated repression of PIF7 (through direct interaction and likely also through occupation of PIF7 genomic targets) provides a means through which the clock controls the magnitude of the response to the changes in light quality in a timely fashion.

Thus, considering our observations and available data from the literature, a molecular framework arises (Fig. 5*D*) in which, under WL (high R:FR ratio), nonphosphorylated PIF7 is recruited by phyB to nuclear speckles (termed photobodies, PBs) and this interferes with PIF7 ability to bind DNA (45). It is possible that this recruitment to PBs further causes PIF7 phosphorylation, as it has recently been identified that these condensates contain PHOTOREGULATORY PROTEIN KINASES, known to phosphorylate PIFs (46). Whether phosphorylation of PIF7 promotes its disassociation from PBs and its relocation to the cytoplasm remains to be explored. Noteworthy, a fraction of PIF7 can still be detected in the nucleoplasm under high R:FR light (*SI Appendix*, Fig. S14*A*). We propose that this is the fraction repressed by GI, as GI and PIF7 were only seen to colocalize in the nucleoplasm (*SI Appendix*, Fig. S14*B*). It would be interesting to investigate whether this nucleoplasmic PIF7 is a fraction of nonphosphorylated PIF7 not recruited to PBs. Our data suggest that this could be the case because GI preferentially binds nonphosphorylated PIF7 (Fig. 3*B*). This would mean that under WL, an equilibrium is maintained through which a fraction of nonphosphorylated PIF7 is recruited to PBs (and possibly phosphorylated, disassociated from speckles, and translocated to the nucleoplasm), while another fraction remains in the nucleoplasm and is repressed by GI through direct binding, with its transcriptional activity being further impaired by GI occupation of genomic targets. In response to shade, the disassembly of phyB PBs would tilt the equilibrium toward a high accumulation of nonphosphorylated PIF7 in the nucleoplasm, which can then activate transcription as GI is slowly degraded. Furthermore, it is possible that cytoplasmic PIF7 is also shuttled to the nucleus under this condition (due to its dephosphorylation through an unknown mechanism) (30), which would further contribute to increase its nuclear levels. Because active PIF7 rapidly accumulates in the nucleus in response to shade, GI-mediated repression would act as a "molecular brake" to ensure that the response is elicited by a significant environmental cue and not a momentary fluctuation in the light conditions. A full response would then be elicited by sustained exposure to shade, likely due to sustained high levels of active PIF7 in the nucleus and GI degradation. In this context, GI function seems to be primarily required in the epidermis to modulate responsiveness and trigger appropriate levels of hypocotyl elongation under varying light conditions. This is in line with previous observations about TS functions of the clock which showed that the clock in the epidermis is essential for cell elongation in response to temperature (41).

Altogether, our findings support a model (Fig. 5*D*) where the circadian clock, through the function of GI, fine-tunes the magnitude of the response to changes in light quality at dusk by directly impinging on key transcriptional regulators of the shade signaling pathway and highlights the relevance of TS circadian function for the regulation of specific output processes in response to environmental cues. Importantly, this tissue specificity seems to be determined by the availability of clock-regulated output partners, rather than a differential wiring of the oscillator itself.

Concluding Remarks. The circadian clock is a complex molecular network that confers plants (and other organisms) the ability to phase biological processes to the most appropriate time of the day and year in resonance with the environment. Importantly, it also modulates the sensitivity of specific signaling pathways to internal and external cues at particular phases. The ability of the circadian network to integrate multiple signals together with its robust rhythmicity is thought to help plants discern noise from the key environmental signals, thereby reducing the effects of noninformative environmental variability, such as stochastic variation in the daily light intensity (47).

Like many other physiological processes, the response to shade is under circadian control, and, in terms of phasing, the clock seems to function to temporalize the sensitivity to shade signals gating it toward dusk (14, 18). Although several clock components have been shown to affect the SAR (17, 19, 20), how gating of the response is achieved at dusk time was poorly understood at the molecular level. Here, we provide evidence of a mechanistic link between the central oscillator and the response to shade and show how it functions primarily in the epidermis to modulate the response to shade at dusk. Hence, our work uncovers an important mechanism by which a TS clock-output circuit modulates plastic growth in dynamic environments.

Limitations of the Study. In this study, we inspected the subcellular localization of PIF7-ECFP-HA and GI-YPET-FLAG under high and low R:FR light. This allowed us to confirm the recruitment of PIF7 to nuclear speckles under high R:FR light and its nucleoplasmic distribution under low R:FR light as previously reported (32, 45). As for GI, we only detected it in the nucleoplasm under both conditions. Because GI was previously reported to be dynamically recruited to nuclear speckles (48), a more in-depth investigation with higher resolution is needed to fully unravel the impact of GI subnuclear localization on its function. With regard to our results on PIF7-ECFP-HA subcellular localization, we observed a higher signal from nuclear PIF7-ECFP-HA in response to shade, which is consistent with previous reports (30). Because western blot analyses of PIF7-ECFP-HA showed similar protein levels under both WL and shade, we hypothesize that the lower signal in WL could be the result of phosphorylated PIF7 translocation to the cytoplasm, as previously shown (30). However, we cannot confirm this with our confocal data because we did not have enough sensitivity. The use of lines expressing PIF7 from its endogenous promoter makes it barely detectable in hypocotyls, which would be a more amenable organ for such confocal inspection, as previously reported (30).

Materials and Methods

Wild-type, mutant, and transgenic lines used in this study were *Arabidopsis thaliana* ecotype Col-0. *gi-2* (35), 35S::HA-GI;*gi-2* (36), Glox (21), pGI::GI-YPET-FLAG;*gi-2* (21), 35S::PIF7-Flash;*pif7-2* (10), *pif7-1* (SALK_062756/SALK_037763) (29), *pif7-2* (Syngenta collection of sequenced T-DNA insertional mutants, line 622) (29), *pif3-1* (SALK_030753) (49), *pif4-101* (Garlic_114_G06) (9), *pif5-1* (SALK_087012) (50), *gi-2;pif3-1* (21), *gi-2;pif5-1* (21), and *gi-2; pif4-101;pif5-1* (21) have been previously described. Seeds were chlorine gas sterilized and plated on 0.5× Murashige and Skoog medium (Caisson Laboratories) with 0.8% agar (Sigma). After stratification in the dark at 4 °C for 3 d, plates were transferred to a Percival incubator (Percival-scientific.com) set to the indicated light conditions with light supplied at 80 μmol m^{-2} s^{-1} by cool-white fluorescent bulbs and a constant temperature of 22 °C. Unless otherwise specified, shade light treatments of seedlings grown on plates were performed as described in refs. 37 and 51. Specifically, WL was supplied with white LEDs at 50 μmol m^{-2} s^{-1} (R:FR > 1.2), and simulated shade was supplied with red, far-red, and blue LEDs at 13 μmol m^2 s^{-1}, 20.2 μmol m^2 s^{-1}, and 1.23 μmol m^2 s^{-1}, respectively (R:FR < 0.7).

Y2H analyses were performed using the Clontech matchmaker GAL4 System according to the manufacturer's instructions. For the in vitro pull-down assays, proteins were coexpressed using the TnT® SP6 High-Yield Wheat Germ Protein Expression System (Promega) as per the manufacturer's instructions.

For gene expression analyses, total RNA was isolated with the GeneJET Plant RNA Purification Kit (Thermo Scientific), and 1 μg of total RNA was digested with DNase I (Roche) and reverse transcribed using the NZY First-Strand cDNA Synthesis Kit (nzytech). Synthesized cDNA was amplified by real-time qPCR with TB Green Premix Ex Taq (Tli RNaseH Plus) (Takara) using the QuantStudio 3 system (Applied Biosystems). *PROTEIN PHOSPHATASE 2A* (AT1G13320) was used as the normalization control in *SI Appendix*, Fig. S3 *A* and *E*, and *ISOPENTENYL PYROPHOSPHATE:DIMETHYLALLYL PYROPHOSPHATE ISOMERASE 2* (AT3G02780) was used in Fig. 4*C* and *SI Appendix*, Figs. S4*B*, S8*B*, and S9*B*. Primer sequences are listed in Dataset S2. To analyze hypocotyl length, evenly spaced seedlings were grown on plates under the indicated light conditions and photoperiod. At the specified time, seedlings were scanned, and images were analyzed using NIH ImageJ software (https://imagej.nih.gov/ij/).

A detailed description of the methods used for *Protein–Protein Interaction Assays*, *Protein Detection and Quantitation*, *Gene Expression Analyses*, *Chromatin Immunoprecipitation*, *Hypocotyl Length Measurements*, and *Confocal Imaging* can be found in *SI Appendix, Materials and Methods*.

Accession Codes. High-throughput sequencing data generated by this study can be accessed through the Gene Expression Omnibus (GEO) database with accession number GSE237670.

Data, Materials, and Software Availability. RNAseq data have been deposited in GEO (GSE237670). Previously published data were used for this work refs. 21 and 32.

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1. J. J. Casal, Shade avoidance. *Arabidopsis Book* **10**, e0157 (2012).
2. A. S. Fiorucci, C. Fankhauser, Plant strategies for enhancing access to sunlight. *Curr. Biol.* **27**, R931–R940 (2017).
3. C. L. Ballare, R. Pierik, The shade-avoidance syndrome: Multiple signals and ecological consequences. *Plant Cell Environ.* **40**, 2530–2543 (2017).
4. J. J. Casal, Photoreceptor signaling networks in plant responses to shade. *Annu. Rev. Plant Biol.* **64**, 403–427 (2013).
5. J. W. Reed, P. Nagpal, D. S. Poole, M. Furuya, J. Chory, Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. *Plant Cell* **5**, 147–157 (1993).
6. K. A. Franklin, G. C. Whitelam, Phytochromes and shade-avoidance responses in plants. *Ann. Bot.* **96**, 169–175 (2005).
7. M. J. Iglesias, R. Sellaro, M. D. Zurbriggen, J. J. Casal, Multiple links between shade avoidance and auxin networks. *J. Exp. Bot.* **69**, 213–228 (2018).
8. L. Ma, G. Li, Auxin-dependent cell elongation during the shade avoidance response. *Front. Plant Sci.* **10**, 914 (2019).
9. S. Lorrain, T. Allen, P. D. Duek, G. C. Whitelam, C. Fankhauser, Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. *Plant J.* **53**, 312–323 (2008).
10. L. Li *et al.*, Linking photoreceptor excitation to changes in plant architecture. *Genes Dev.* **26**, 785–790 (2012).
11. T. Mizuno, H. Oka, F. Yoshimura, K. Ishida, T. Yamashino, Insight into the mechanism of end-of-day far-red light (EODFR)-induced shade avoidance responses in Arabidopsis thaliana. *Biosci. Biotechnol. Biochem.* **79**, 1987–1994 (2015).
12. A. J. Millar, The intracellular dynamics of circadian clocks reach for the light of ecology and evolution. *Annu. Rev. Plant Biol.* **67**, 595–618 (2016).
13. K. Greenham, C. R. McClung, Integrating circadian dynamics with physiological processes in plants. *Nat. Rev. Genet.* **16**, 598–610 (2015).
14. M. G. Salter, K. A. Franklin, G. C. Whitelam, Gating of the rapid shade-avoidance response by the circadian clock in plants. *Nature* **426**, 680–683 (2003).
15. B. Cole, S. A. Kay, J. Chory, Automated analysis of hypocotyl growth dynamics during shade avoidance in Arabidopsis. *Plant J.* **65**, 991–1000 (2011).
16. M. de Wit *et al.*, Changes in resource partitioning between and within organs support growth adjustment to neighbor proximity in Brassicaceae seedlings. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E9953–E9961 (2018).
17. Y. Jiang *et al.*, The ELF3-PIF7 interaction mediates the circadian gating of the shade response in Arabidopsis. *iScience* **22**, 288–298 (2019).
18. R. Sellaro, M. Pacin, J. J. Casal, Diurnal dependence of growth responses to shade in Arabidopsis: Role of hormone, clock, and light signaling. *Mol. Plant* **5**, 619–628 (2012).
19. M. Takase, T. Mizoguchi, T. Kozuka, H. Tsukaya, The unique function of the Arabidopsis circadian clock gene PRR5 in the regulation of shade avoidance response. *Plant Signal. Behav.* **8**, e23534 (2013).
20. Y. Zhang *et al.*, Central clock components modulate plant shade avoidance by directly repressing transcriptional activation activity of PIF proteins. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 3261–3269 (2020).
21. M. A. Nohales *et al.*, Multi-level modulation of light signaling by GIGANTEA regulates both the output and pace of the circadian clock. *Dev. Cell* **49**, 840–851.e8 (2019).
22. K. Nozue *et al.*, Rhythmic growth explained by coincidence between internal and external cues. *Nature* **448**, 358–361 (2007).
23. E. L. Martin-Tryon, J. A. Kreps, S. L. Harmer, GIGANTEA acts in blue light signaling and has biochemically separable roles in circadian clock and flowering time regulation. *Plant Physiol.* **143**, 473–486 (2007).
24. E. Huq, J. M. Tepperman, P. H. Quail, GIGANTEA is a nuclear protein involved in phytochrome signaling in Arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 9789–9794 (2000).
25. M. de Wit, V. C. Galvao, C. Fankhauser, Light-mediated hormonal regulation of plant growth and development. *Annu. Rev. Plant Biol.* **67**, 513–537 (2016).
26. M. A. Nohales, S. A. Kay, GIGANTEA gates gibberellin signaling through stabilization of the DELLA proteins in Arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 21893–21899 (2019), 10.1073/pnas.1913532116.
27. P. Hornitschek *et al.*, Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. *Plant J.* **71**, 699–711 (2012).
28. F. Fornara *et al.*, The GI-CDF module of Arabidopsis affects freezing tolerance and growth as well as flowering. *Plant J.* **81**, 695–706 (2015).
29. P. Leivar *et al.*, The Arabidopsis phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels. *Plant Cell* **20**, 337–352 (2008).
30. X. Huang *et al.*, Shade-induced nuclear localization of PIF7 is regulated by phosphorylation and 14-3-3 proteins in Arabidopsis. *Elife* **7**, e31636 (2018).
31. C. Nieto, V. Lopez-Salmeron, J. M. Daviere, S. Prat, ELF3-PIF4 interaction regulates plant growth independently of the Evening Complex. *Curr. Biol.* **25**, 187–193 (2015).
32. B. C. Willige *et al.*, PHYTOCHROME-INTERACTING FACTORs trigger environmentally responsive chromatin dynamics in plants. *Nat. Genet.* **53**, 955–961 (2021).
33. S. Kidokoro *et al.*, The phytochrome-interacting factor PIF7 negatively regulates DREB1 expression under circadian control in Arabidopsis. *Plant Physiol.* **151**, 2046–2057 (2009).
34. P. Leivar *et al.*, Dynamic antagonism between phytochromes and PIF family basic helix-loop-helix factors induces selective reciprocal responses to light and shade in a rapidly responsive transcriptional network in Arabidopsis. *Plant Cell* **24**, 1398–1419 (2012).
35. S. Fowler *et al.*, GIGANTEA: A circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane-spanning domains. *EMBO J.* **18**, 4679–4688 (1999).
36. K. M. David, U. Armbruster, N. Tama, J. Putterill, Arabidopsis GIGANTEA protein is post-transcriptionally regulated by light and dark. *FEBS Lett.* **580**, 1193–1197 (2006).
37. Y. Tao *et al.*, Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell* **133**, 164–176 (2008).
38. C. Procko, C. M. Crenshaw, K. Ljung, J. P. Noel, J. Chory, Cotyledon-generated auxin is required for shade-induced hypocotyl growth in brassica rapa. *Plant Physiol.* **165**, 1285–1301 (2014).
39. C. Procko *et al.*, The epidermis coordinates auxin-induced stem growth in response to shade. *Genes Dev.* **30**, 1529–1541 (2016).
40. M. Endo, H. Shimizu, M. A. Nohales, T. Araki, S. A. Kay, Tissue-specific clocks in Arabidopsis show asymmetric coupling. *Nature* **515**, 419–422 (2014).
41. H. Shimizu *et al.*, Decentralized circadian clocks process thermal and photoperiodic cues in specific tissues. *Nat. Plants* **1**, 15163 (2015).
42. M. D. M. Marques-Bueno *et al.*, A versatile multisite gateway-compatible promoter and transgenic line collection for cell type-specific functional genomics in Arabidopsis. *Plant J.* **85**, 320–333 (2016).
43. Z. Zheng *et al.*, Local auxin metabolism regulates environment-induced hypocotyl elongation. *Nat. Plants* **2**, 16025 (2016).
44. M. V. Kohnen *et al.*, Neighbor detection induces organ-specific transcriptomes, revealing patterns underlying hypocotyl-specific growth. *Plant Cell* **28**, 2889–2904 (2016).
45. Y. Xie *et al.*, Phytochrome B inhibits the activity of phytochrome-interacting factor 7 involving phase separation. *Cell Rep.* **42**, 113562 (2023).
46. C. Kim *et al.*, Phytochrome B photobodies are comprised of phytochrome B and its primary and secondary interacting proteins. *Nat. Commun.* **14**, 1708 (2023).
47. M. Domijan, D. A. Rand, Balance equations can buffer noisy and sustained environmental perturbations of circadian clocks. *Interface Focus* **1**, 177–186 (2011).
48. Y. Kim *et al.*, ELF4 regulates GIGANTEA chromatin access through subnuclear sequestration. *Cell Rep.* **3**, 671–677 (2013).
49. J. Kim, H. Yi, G. Choi, B. Shin, P. S. Song, Functional characterization of phytochrome interacting factor 3 in phytochrome-mediated light signal transduction. *Plant Cell* **15**, 2399–2407 (2003).
50. T. Fujimori, T. Yamashino, T. Kato, T. Mizuno, Circadian-controlled basic/helix-loop-helix factor, PIL6, implicated in light-signal transduction in Arabidopsis thaliana. *Plant Cell Physiol.* **45**, 1078–1086 (2004).
51. U. V. Pedmale *et al.*, Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. *Cell* **164**, 233–245 (2016).