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The evening complex integrates photoperiod signals to control flowering in rice

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Plants use photoperiodism to activate flowering in response to a particular daylength. In rice, flowering is accelerated in short-day conditions, and even a brief exposure to light during the dark period (night-break) is sufficient to delay flowering. Although many of the genes involved in controlling flowering in rice have been uncovered, how the long- and short-day flowering pathways are integrated, and the mechanism of photoperiod perception is not understood. While many of the signaling components controlling photoperiod-activated flowering are conserved between *Arabidopsis* and rice, flowering in these two systems is activated by opposite photoperiods. Here we establish that photoperiodism in rice is controlled by the evening complex (EC). We show that mutants in the EC genes *LUX ARRYTHMO* (*LUX*) and *EARLY FLOWERING3* (*ELF3*) paralogs abolish rice flowering. We also show that the EC directly binds and suppresses the expression of flowering repressors, including *PRR37* and *Ghd7*. We further demonstrate that light acts via phyB to cause a rapid and sustained posttranslational modification of ELF3-1. Our results suggest a mechanism by which the EC is able to control both long- and short-day flowering pathways.

rice | flowering | ELF3 | LUX | Evening Complex

Photoperiod provides seasonal information to control flowering. Rice flowering is accelerated under short-day (SD) photoperiod while other cereals such as wheat and barley flower under long-day (LD) conditions. The activation of flowering in rice is dependent on the expression of florigens *Heading date3a* (*Hd3a*) and *Rice FT1* (*RFT1*) (1). Inductive SD photoperiods lead to the up-regulation of *Hd3a*, which activates the floral transition (2). Under LD, flowering occurs later, via induction of *RFT1* (1). In *Arabidopsis* (an LD plant), the circadian- and light-controlled CCT domain-containing protein CONSTANS (CO) activates flowering in LD. *CO* expression is circadianly controlled by GIGANTEA (GI), while CO protein stability is dependent upon light-active phytochromes to induce *Arabidopsis* florigen *FLOWERING LOCUS T* (*FT*) (3, 4). The GI-CO-FT pathway is conserved in other flowering plants. The *Arabidopsis* CO homolog in barley, CO1, was demonstrated to be similarly regulated to activate *VRN3*, an *FT* ortholog, in LD (5), as are the homologs CO1 and CO2 in wheat (6). In rice, Heading date1 (Hd1), a CO homolog, has been proposed to be bifunctional: promoting flowering under SD and inhibiting it under LD (7). Besides the conserved GI-CO-FT pathway, rice has another regulatory mechanism to activate flowering through the CCT-domain flowering repressor Grain number, Plant Height, and Heading date1 (Ghd7), which in LD inhibits the flowering activator Early heading date1 (Ehd1), a B-type response regulator capable of inducing the expression of *Hd3a* and *RFT* (8, 9).

Recently, it has been shown that Ghd7 directly interacts with Hd1 (10, 11) and the LD suppression of flowering by Hd1 depends on the floral repressors Ghd7. Similarly, the presence of another CCT domain-containing protein PSEUDO-RESPONSE REGULATOR37 (PRR37) is predicted to switch the activity of Hd1 from a flowering activator to a repressor (10–12) potentially via a heteromeric protein complex (13). The expression of *Ghd7* and *PRR37* under noninductive LD is therefore a key means to regulate flowering, but the mechanism by which they perceive photoperiod is unknown. *Ghd7* expression is regulated by ELF3-1 and the phytochrome pathway (14) whereas PRR37 functions in the circadian clock (15). ELF3 and the transcription factor LUX are part of the evening complex (EC) in several plant species. In *Arabidopsis*, the EC acts as a transcriptional repressor, binding and reducing the expression of key target genes such as the flowering activator *PIF4* (16, 17). *LUX* orthologs in wheat (18), barley (19), pea (20), and soybean (21) have been implicated in the control of photoperiodic flowering. *ELF3* was also implicated in the same mechanism in barley (22, 23), pea, and lentil (24). A rice genome duplication event resulted in the establishment of two *ELF3* paralogs, *ELF3-1* and *ELF3-2* (25). Recently, the existence of a

Significance

Plants use photoperiod information to control flowering. Flowering in rice is accelerated by short days, while other plants, such as *Arabidopsis*, flower in response to long days. The molecular mechanisms controlling photoperiod-dependent flowering in rice are not fully understood. Previous experiments have demonstrated the presence of several floral repressors that confer relatively mild flowering phenotypes. Here we show that the rice evening complex of the circadian clock is essential for the activation of flowering. We find that the evening complex (EC) binds directly to the promoters of floral repressors, reducing their transcription. These findings are consistent with a key role for the EC in controlling flowering in many diverse species including soybean, barley, *Brachypodium*, and *Arabidopsis*.

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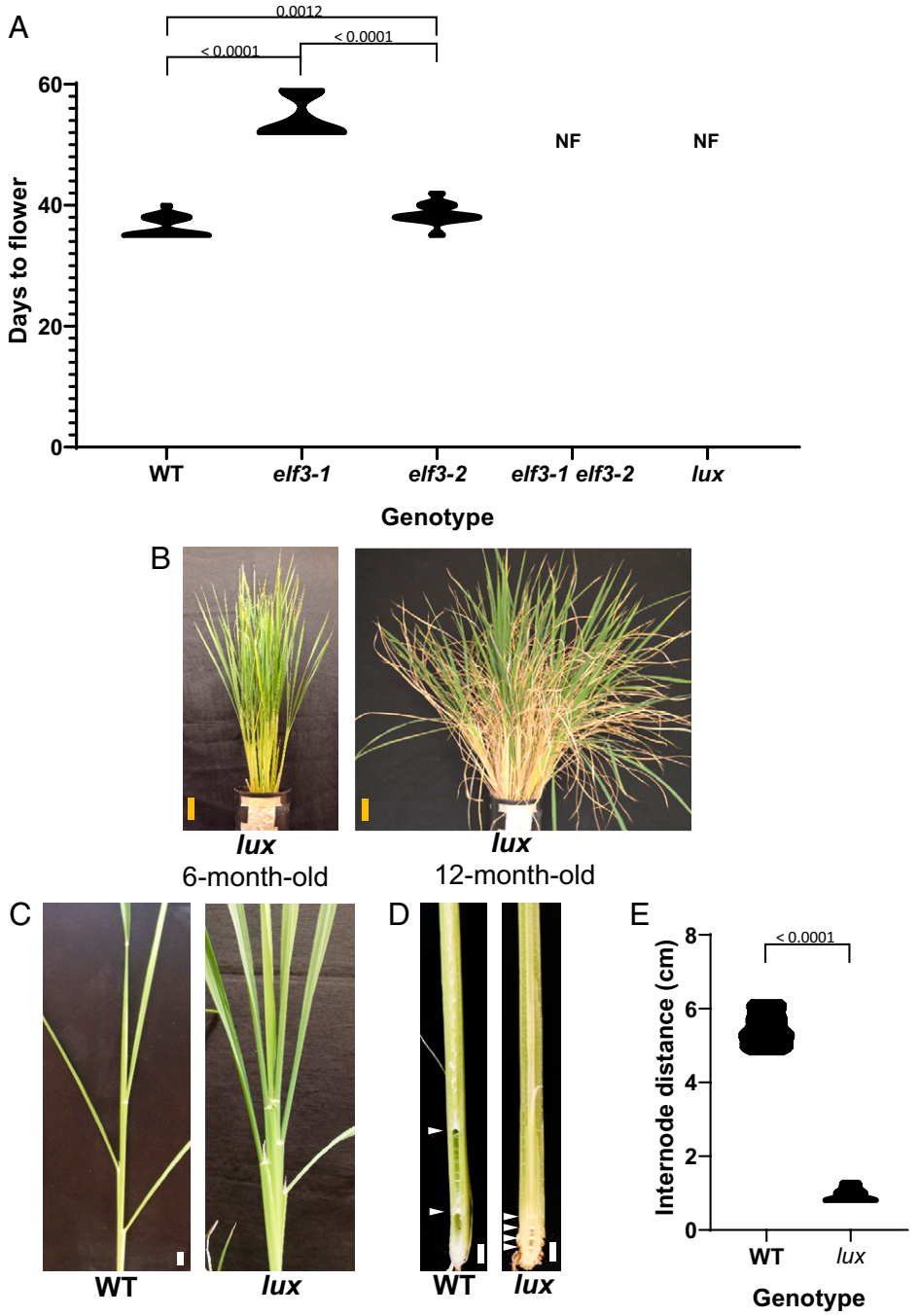

Fig. 1. EC genes are essential for flowering in rice. (*A*) Flowering time for *elf3-1, elf3-2*, and *lux* in rice from the emergence of the second leaf. Plants were grown under ND conditions (12-h day, 12-h night). Nonflowering genotypes are marked with "NF". *P* values are for two-tailed unpaired *t* test with $n = 10$. (*B*) Adult *lux* plants (line 120T7, 2 nucleotide insertion) at 6 and 12 mo growth in SD (10-h day, 14-h night). (Yellow scale bars represent 10 cm.) (*C–E*) *lux* (line 69S4b, insertion of one nucleotide in one allele and deletion of four nucleotides in the other allele) showing decreased distance between internodes. (White scale bars represent 1 cm.) *P* values are for two-tailed unpaired *t* test.

ternary EC has been confirmed in rice (26). The role of *LUX* in rice flowering is not known but mutants in *ELF3* paralogs or *ELF4* have a mild late flowering response, consistent with a role for the EC in flowering (26–28).

Results

The EC is Essential for Flowering in Rice. The EC is an important regulator of circadian programs in plants (16). To determine if it has a role in controlling photoperiodism in rice, we investigated mutants in *LUX* and two *ELF3* paralogs generated by genome editing in the rice variety Nipponbare (hereafter referred as WT) (*SI Appendix*, Fig. S1*A* and Table S1). Rice contains two *Arabidopsis ELF3* orthologs, *ELF3-1* and *ELF3-2*. Individually, they have a mild effect on flowering (27, 28), however, the double *elf3-1 elf3-2* mutant does not flower under our conditions (Fig. 1*A*). Similarly, we found that *lux* plants never flowered. This was despite growing plants under both long and short photoperiods for more than one year (Fig. 1*B* and *SI Appendix*, Fig. S1*B*). The EC mutants also exhibit short internode elongation (Fig. 1 *C–E*), a trait correlated with delayed flowering (29). *ELF3-1* and *ELF3-2* appear to both contribute to the formation of the EC, as only the double mutant shows the dramatic nonflowering phenotype of *lux*.

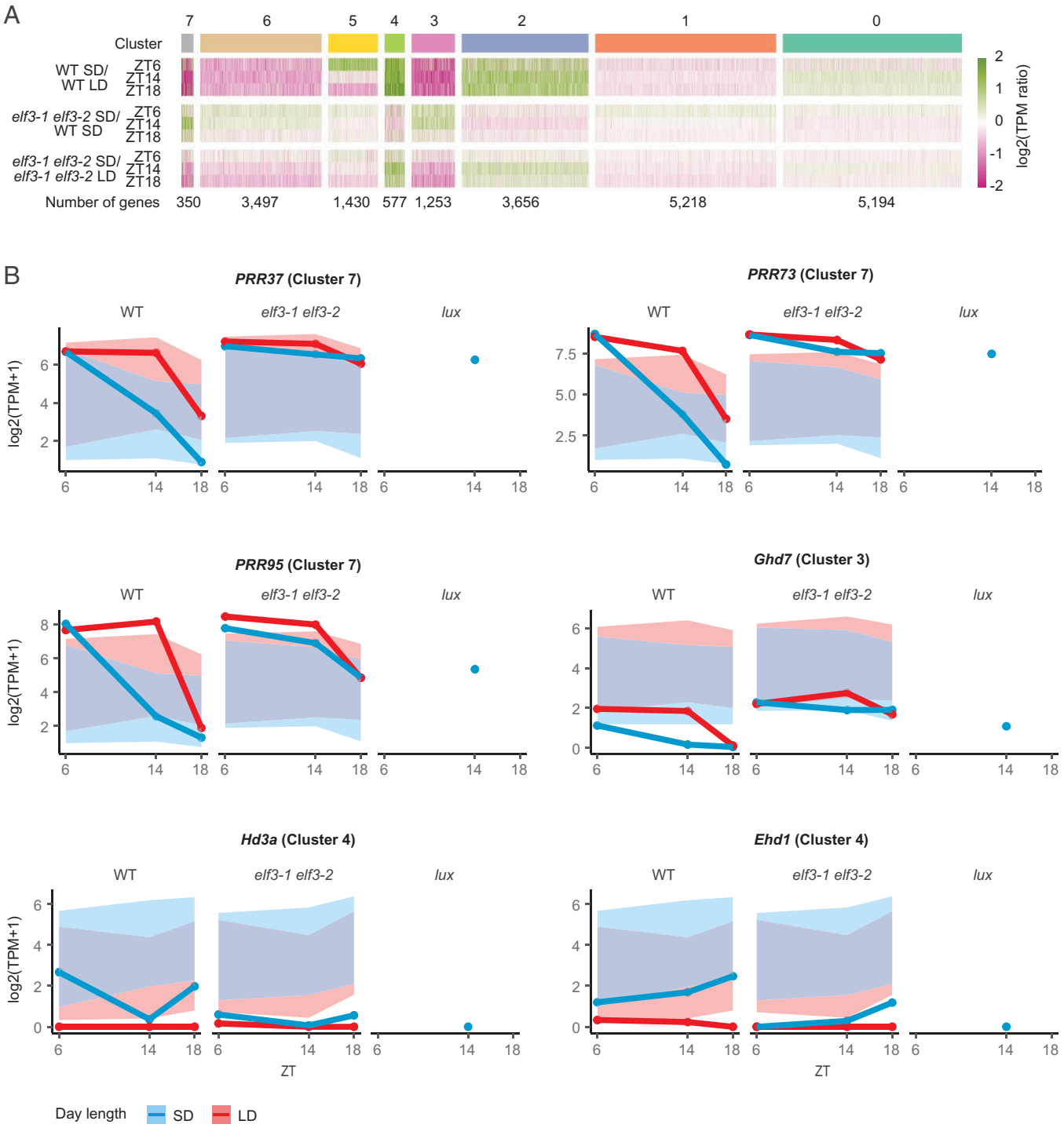

Fig. 2. EC activity is essential for the SD transcriptome. (*A*) Clustering of gene expression under SD (10-h day, 14-h night) and LD (14-h day, 10-h night) reveals groups of genes that are activated (e.g., clusters 4 and 2) and repressed (e.g., clusters 7, 6, and 3) by SD photoperiods. These clusters become largely daylength neutral in the *elf3-1 elf3-2* background (line 246U1.6a2: 1 nucleotide insertion in *ELF3-1* locus: two nucleotide deletion in *ELF3-2* locus). Values for log2(TPM ratio) > 2 or < −2 are transformed to the range of ± 2. (*B*) Examples of circadian and flowering time genes that lose their photoperiod responsiveness in *elf3-1 elf3-2* and *lux* (line 120T7, two nucleotide insertion). Blue and red ribbons denote the overall behavior of the relevant cluster in SD and LD, respectively.

Consistent with this, both ELF3-1 and ELF3-2 interact with LUX in yeast two-hybrid assays (*SI Appendix*, Fig. S1*C*) similar to previous reports (26). Taken together, these results indicate that the EC is essential for flowering in rice.

The EC Controls the Photoperiod Transcriptome. Flowering is a complex trait, and it is possible that disrupting the EC, an integral component of the circadian clock, may affect the floral transition indirectly. We therefore investigated the photoperiod-dependent transcriptome in rice by comparing inductive SD to noninductive LD conditions. We observe prominent clusters of photoperiod-dependent gene regulation at ZT6, ZT14, and ZT18, consistent with the major impact of photoperiod on rice development (Fig. 2*A*, *SI Appendix*, Fig. S2*A*, and Datasets S1 and S2). Particularly prominent are clusters 7 and 3, which show strong down-regulation in SD, and cluster 4, which is

up-regulated in response to SD. The SD repressed cluster 7 contains key floral regulators, such as *PRR37, PRR73, PRR95, GI, FKF1* and *DOF12* (Fig. 2*B*) and is enriched for GO terms associated with circadian rhythm, rhythmic process, and photosynthesis (*SI Appendix*, Fig. S15) as has been observed for evening complex targets in *Arabidopsis* (17), while cluster 3 contains the flowering regulator *Ghd7* (Fig. 2*B*) and is enriched for GO terms associated with salt stress (*SI Appendix*, Fig. S13) (26). The key outputs of flowering are found in the induced cluster 4, including *Hd3a* and *RFT1*. To investigate the potential role of the EC in this process, we examined the *elf3-1 elf3-2* SD and LD transcriptome. By performing clustering using the same gene order as for the WT photoperiod response, we observe the transcriptome has a greatly reduced response to SD in *elf3-1 elf3-2*. Specifically, the strongly down-regulated SD clusters 7 and 3 become up-regulated in *elf3-1 elf3-2*, while cluster 4, which is up-regulated in SD in WT becomes down-regulated in *elf3-1 elf3-2* (Fig. 2 *A* and *B* and *SI Appendix*, Fig. S2*B*). Indeed, similar patterns are observed across nearly all the clusters. Taken together, these results show that *elf3-1 elf3-2* mutants in SD resemble WT plants grown in LD, at both the phenotype and transcriptome level. This behavior is also seen for *lux* at ZT14 (Fig. 2*B* and *SI Appendix*, Fig. S2 *A* and *B*), suggesting that the EC is required for the correct expression of the photoperiod transcriptome (*SI Appendix*, Fig. 2*A*). Consistent with the role of the EC as a transcriptional repressor in *Arabidopsis*, we find that nearly all the genes up-regulated in *elf3-1 elf3-2* are also up-regulated in *lux* (*SI Appendix*, Fig. S3).

The EC Binds to the Promoters of Key Floral Regulators. Since the clusters of genes repressed by SD become up-regulated in the *elf3-1 elf3-2* background, we sought to determine if this is a direct effect of EC activity. We therefore investigated the genome-wide binding of ELF3-1 at ZT14. We observe enrichment for ELF3-1 binding in the promoters of cluster 7 genes (21% are bound by ELF3-1) and cluster 3 genes (7% bound) (Fig. 3*A* and Dataset S3). Cluster 7 is particularly prominent, with multiple floral regulator genes being directly bound by ELF3-1: *PRR37, PRR73, PRR95* and *GI* (Fig. 3*B*). The major floral repressor present in cluster 3, *Ghd7*, is also directly bound by ELF3-1 (Fig. 3*B* and *SI Appendix*, Fig. S4). The association of ELF3 binding loci with gene repression is consistent with the EC role as a transcriptional repressor in *Arabidopsis* (17). By contrast, clusters of genes that are repressed in the *elf3-1 elf3-2* background do not show an enrichment for ELF3-1 binding. For example, cluster 4 contains many key floral inducers, including *Hd3a, RFT1, Ehd1, FTL10* and *MADS14*, but this cluster is not enriched for ELF3-1 binding events (Fig. 3*A*). This indicates that ELF3-1 controls flowering by binding to the promoter region of floral repressors, and not by repressing the expression of the florigen encoding genes directly. Since ELF3 does not have a DNA binding domain but is recruited to target promoters by LUX in *Arabidopsis* (30), we investigated if this is also the case in rice. We observe enrichment for sequences similar to LUX Binding Site (LBS) in the ELF3-1 ChIP-seq peaks, consistent with LUX recruiting ELF3-1 (*SI Appendix*, Fig. S3 *A*–*C*). ChIP-seq of a tagged version of LUX demonstrated an enrichment at the center of the peaks identified in the ELF3-1 ChIP-seq (*SI Appendix*, Figs. S3*D* and 4*B*). These results suggest that ELF3-1 is likely recruited by LUX to repress the expression of flowering-time regulators such as *Ghd7* and *PRR37*. Since *Ghd7* and *PRR37* activity is linked to a delay in flowering in LD (9, 31), the photoperiodic-dependent expression of these genes suggests a direct mechanism to account for daylength–dependent flowering.

ELF3-1 Activity is Controlled by phyB in Response to Light. Since the EC is able to control the photoperiod transcriptome, we sought to determine how light signals are integrated by the EC. LUX protein levels rise at dusk and remain high for the next 12 h (*SI Appendix*, Fig. 6*A*), suggesting that LUX is likely not the limiting factor for EC activity. In *Arabidopsis*, ELF3 activity is controlled by temperature through a reversible phase change mediated by a prion-related domain (32). In the case of rice ELF3 homologs, we do not observe in silico evidence for such a prion. We therefore investigated if rice ELF3 protein levels are influenced by photoperiod. We observe that there is a clear difference between light and dark conditions for ELF3-1 protein behavior (Fig. 4*A*). In the light, a higher migrating protein band is present. By contrast, an additional lower sharp ELF3-1 band is visible in darkness. These are specific ELF3-1 protein bands since neither is observed in the *elf3-1* background (*SI Appendix*, Fig. 6*B*). We therefore hypothesized that under LD conditions ELF3-1 is largely in the higher, likely posttranslationally modified form, rendering it inactive. By contrast, in SD the plants experience longer nights, enabling the lower, likely active, form to accumulate during darkness. By assaying ELF3-1 in plants grown under SD and LD, we observe this is the case with the lower ELF3-1 accumulating to a higher level in SD conditions (Fig. 4*A*).

A key contributor to photoperiodism in rice is the photoreceptor phyB, with *phyb* mutants flowering earlier in LD, and being less responsive to night-break (NB) experiments (33, 34). Examining the transcriptome of the previously described *phyb* mutant (35) shows that under noninductive LD conditions, it resembles a WT transcriptome in SD, with repression of clusters 7 and 3 and up-regulation of cluster 4 (*SI Appendix*, Fig. S7 *A* and *B*). We therefore investigated if phyB may be necessary for the light-dependent behavior of ELF3-1. We observe a complete loss of responsiveness of ELF3-1 to light in *phyb* (Fig. 4*B*), with both the higher and lower forms of ELF3-1 present in day and night. This loss of responsiveness is independent of the photoperiod (Fig. 4*B*). Even short NB experiments are sufficient to greatly delay flowering in rice, a response that is dependent upon phyB (36). To determine if ELF3-1 levels respond sufficiently rapidly to account for this behavior, we assayed ELF3-1 in response to NB over a short time course. We observe loss of ELF3-1 signal within 15 min of NB, indicating this regulation is sufficiently rapid to account for the observed sensitivity of rice to even brief exposure to light during the night (Fig. 4*C*). This indicates that phyB is the photoreceptor that mediates the perception of photoperiod to control ELF3-1 activity.

Discussion

Photoperiodism enables plants to flower in the appropriate season. For temperate plants this is often spring, when daylength is increasing, while tropical plants such as rice often flower in subjective winter in response to short days. While LD and SD plants have opposite photoperiod requirements, it is noticeable that the EC has been found to regulate flowering responses in a variety of plants in both cases.

For example, the EC represses flowering in *Arabidopsis* (37–41) and other LD plants (18–23, 42). In contrast, genetic evidence from SD plants suggests the EC induces flowering (14, 21, 27, 43, 44). Here we find that *LUX* and *ELF3-1 ELF3-2* are essential for flowering to occur in rice. As seen by others (14, 27, 43, 44), *elf3-1* and *elf3-2* single mutants have mild late flowering, suggesting that ELF3-1 and ELF3-2 are partially

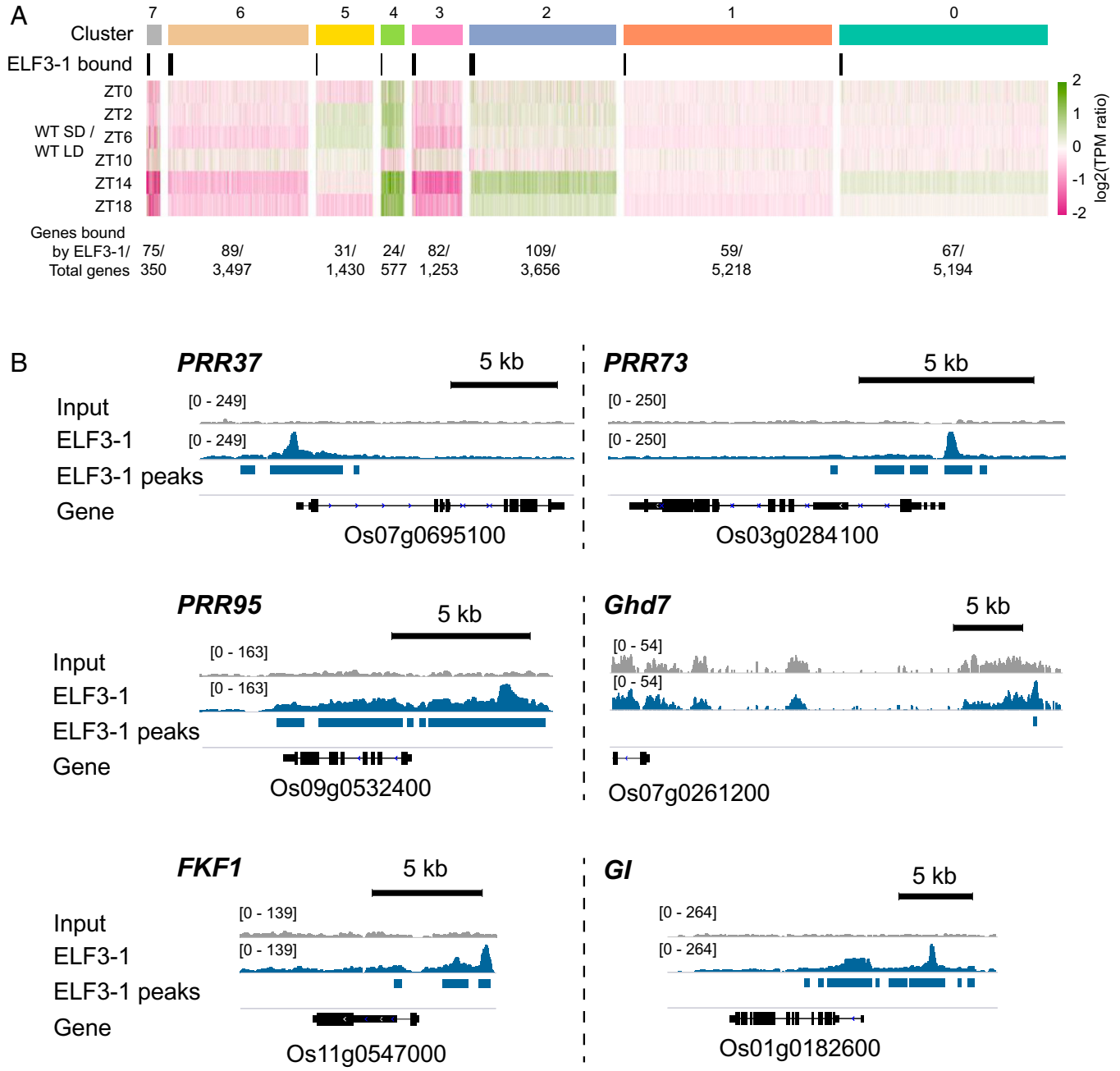

Fig. 3. ELF3-1 directly binds and represses the expression of LD responsive genes in rice. (*A*) 21.4% of the genes in cluster 7 and 6.5% of genes in cluster 3 are directly repressed by ELF3-1 at ZT14. Values for log2(TPM ratio) > 2 or < −2 are transformed to the range of ± 2. (*B*) Representative views showing ELF3-1 binding peaks at key circadian and floral regulators.

redundant in the formation of the EC. In soybean, two *LUX* homologs also act redundantly to activate flowering (21).

In *Arabidopsis*, the EC is recruited to the promoter regions of temperature, stress, and circadian clock signaling genes (17, 45, 46). We observed that the rice EC can also directly regulate gene expression. Direct targets of rice EC already implicated in the regulation of flowering include *PRR37*, *Ghd7*, *GI*, *PRR73*, and *PRR95*. It has been demonstrated that *Arabidopsis* homologs to *PRR37*, *PRR73*, and *PRR95* coordinately activate flowering in *Arabidopsis* (47), while overexpressing either rice *PRR37* or *Arabidopsis PRR5* in rice delays flowering (48, 49). Our observations are consistent with the EC having a conserved role as a transcriptional repressor (16) and explain the nonflowering phenotype of *lux* and *elf3-1 elf3-2*. We observed that EC mutants have lower levels of *Hd3a* and *RFT* transcripts, as well as *Ehd1* and *FTL10*, another *FT* ortholog in rice. We propose that the EC mutants are nonflowering due to the low levels of these flowering activators (1). Interestingly, neither Ghd7 nor PRR37 have been shown to directly interact with florigen promoters. However, Hd1, which can interact with Ghd7 and PRR37, is proven to bind and repress florigens under LD conditions (10–13). In the double mutant *ghd7 prr37* however, Hd1 acts as a flowering activator in both SD and LD conditions (12). Hence, Hd1 can act as a flowering activator in SD and as a repressor in LD, since Hd1 by itself activates gene expression, while when in a complex with Ghd7 or PRR37 it has the opposite role (10–13) (Fig. 5).

Phytochromes and ELF3 have antagonistic roles in flowering in many plants. For example, in the LD grass *Brachypodium distachyon*, phyC is essential for flowering, while *elf3* mutants flower early and are photoperiod insensitive (50). In rice,

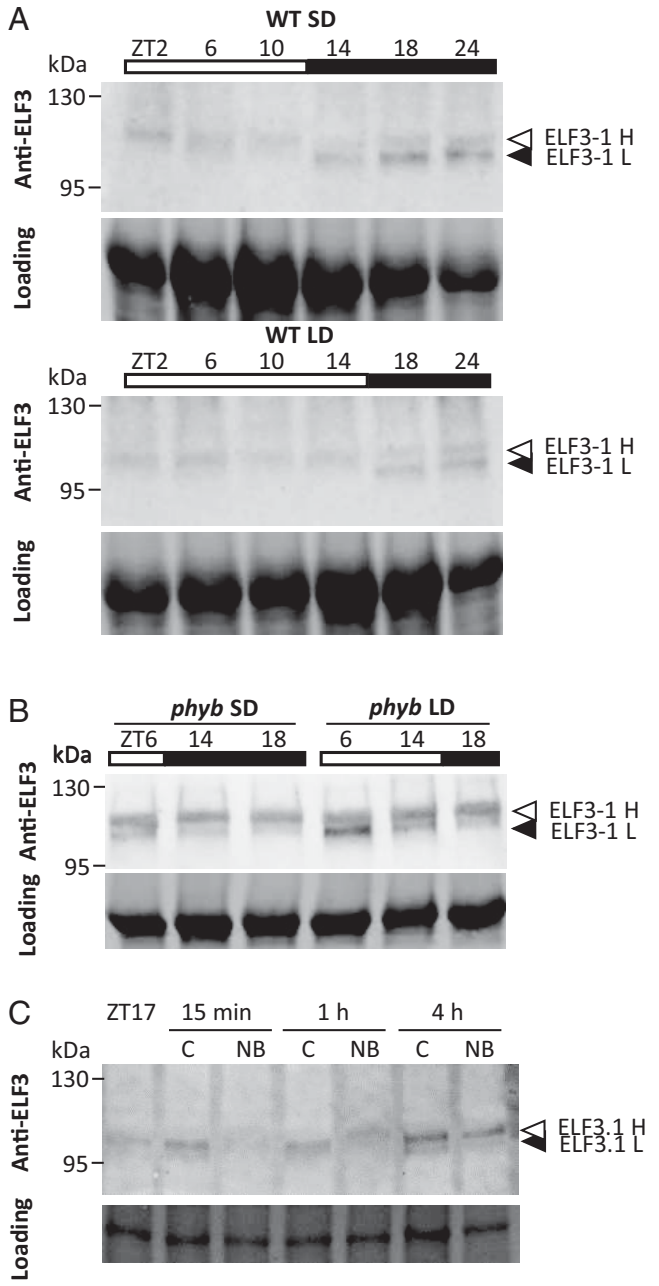

Fig. 4. ELF3-1 protein stability responds to light. (*A*) Anti-ELF3 specifically recognizes ELF3-1, which occurs in two bands during darkness. During light periods, only the higher (H) band is present. During SD (10-h day, 14-h night) the low (L) band is able to accumulate during the long night. (*B*) In the *phyb* background ELF3-1 loses photoperiod responsiveness, and both H and L bands are present in both light and dark periods. (*C*) The reduction in ELF3-1 levels in response to light is rapid, with only 15 min of NB being sufficient.

mutants in the light receptor *phyb* are early flowering (36). While NB delays flowering in rice due to increased expression of *Ghd7* (14), this effect is suppressed in *phyb* (34). Light signaling through PHYB and PHYC is also necessary for the NB response in wheat, which accelerates flowering through the upregulation of *PPD1* (50, 51). We find that ELF3-1 protein accumulates similarly in day and night in *phyb*, indicating a role for phyB in modifying ELF3-1 activity in response to light. Since phyB has been shown to bind and regulate target proteins by posttranslation modification (52–54), we suggest that light-controlled phyB is responsible for the conversion of the lower migrating ELF3-1 form to a higher form during the day, thus decreasing the accumulation of the EC complex in LD (Fig. 5). Previous results have shown that phyB can interact with ELF3-1 and ELF3-2 in vitro (14). The two distinctly migrating ELF3-1 bands are characteristic of posttranslation modification regulation. Indeed, ubiquitination of ELF3-1 by an E3 ubiquitin ligase (55) has been demonstrated, while a similar mechanism was described for ELF3-2 (56). Such posttranslation modifications independent of phyB may be responsible for the presence of the higher migrating ELF3-1 band even in *phyb* mutants. Consistent with this model, photoperiod dependent flowering in *Arabidopsis* is influenced by the ubiquitination of ELF3 and GI by the E3 ubiquitin ligase COP1 (57). Thus, while the key inputs and outputs of the photoperiod pathway are conserved (phytochrome signaling and florigens, respectively), the targets of EC repression determine the photoperiodic specificity of the response. In this way plants have been able to evolve dramatically different responses to photoperiod, facilitating adaptation to new environments.

Materials and Methods

Cloning of CRISPR/*Cas9* Transformation Cassettes. Binary vectors containing a hygromycin resistance gene driven by the cauliflower mosaic virus *35S* promoter were used to generate Cas9-mediated genomic insertion and/or deletion events at *LUX*, *ELF3-1*, and *ELF3-2* loci (*SI Appendix*, Fig. S1*A* and Table S1). The final vectors were introduced into *Agrobacterium tumefaciens* EHA105 for rice transformation (58, 59).

Cloning of LUX Genomic Tagged Transformation Cassette. For generating a genomic tagged line of *LUX*, a 2,935 bp sequence comprising 2,000 bp of the promoter sequence, 217 bp of 5′UTR, and 717 bp of *LUX* coding sequence was fused to a 5xMYC tag followed by a 500 bp fragment downstream of *LUX* stop codon (3′UTR) by PCR. The final vector was introduced into *Agrobacterium tumefaciens* EHA105 for rice transformation.

Rice *calli* Culture and Transformation. Stable transformations were generated using wild-type rice cv. Nipponbare following established methods (60).

Identification of Tagged LUX, *lux, elf3-1, elf3-2,* and *elf3-1 elf3-2* Mutants. Plant lines obtained from tissue culture harboring the hygromycin cassette were further tested. The *LUX* tagged lines were validated by testing the expression of *LUX::MYC* by RT-qPCR. *Cas9* transformed lines were then tested for the presence

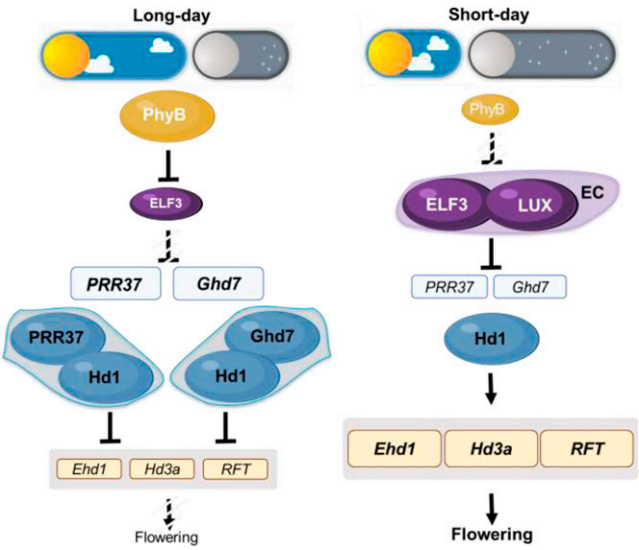

Fig. 5. The EC integrates photoperiod information in rice. ELF3 activity increases during darkness, enabling the EC to reduce expression of key floral repressors such as *PRR37* and *Ghd7* in SD conditions. Activation of PhyB under LD conditions enables repression of ELF3-1, resulting in the enhanced expression of the transcriptional regulators that, in concert with Hd1, repress florigen genes *Hd3a* and *RFT*, therefore delaying flowering.

of insertions or deletions (InDels) in the target loci using PCR and Sanger sequencing (61).

Rice Growth Conditions. For the measurement of flowering-time, wild-type, *elf3-1* (line 246U2.7a1), *elf3-2* (line 246U2.7a1), and *elf3-1 elf3-2* (line 246U2.7a2). Germinated seedlings were planted in containers with soil (soil:peat:vermiculite in 2:2:1 volume ratio) in controlled conditions at 28 °C under 12 h/12 h light/dark photoperiod (ND conditions) at 70% humidity. *lux* mutants were grown under similar conditions to compare flowering time, while initial plant material used was prepared from *calli*, so time to flower was counted from the emergence of the second leaf. Additionally, WT and *lux* rice (from *calli* culture) were grown under LD conditions (14-h day, 10-h night) and SD conditions (10-h day, 14-h night).

For gene expression or protein accumulation, wild-type, *phyb-1* [generated elsewhere (36)], *elf3-1* (line 246U2.7a1), *elf3-2* (line 246U2.7a1) and *elf3-1 elf3-2* (line 246U2.7a2) rice seeds were surface sterilized and germinated under dark for 3 d at 28 °C. Rice *lux* mutants (line 75Z3a) tillers were obtained by a donor plant and grown under similar conditions in order to compare results. Seedlings were grown on 1/2 MS solid medium. Pools of four to five plants were collected 21 d after germination at specific time-points: wild-type plants were collected at indicated times.

For the identification of EC direct targets, wild-type and *LUX* genomic 5xMYC tagged line seeds were surface sterilized and germinated under dark for 3 d at 28 °C. Germinated seedlings were then transferred to tubes containing sterile 1/2 MS solid medium and placed in a growth chamber in SD conditions for 14 d.

For the NB experiment, wild-type and *phyb-1* rice seeds were germinated as described above but placed in a growth chamber at SD conditions for 10 d. Part of the plants was exposed to a NB of 300 μmol photons $m^{-2}s^{-1}$ of photoactive radiation for 15 min in the middle of the night (ZT17) while others remained under dark.

Extraction of Total RNA. Total RNA was extracted from WT, *phyb-1*, *lux* (line 75Z3a), and *elf3.1 elf3.2* (line 246U2.7a2), sampled at multiple points in the day under SD or LD conditions, by homogenizing samples to powder using a freeze cold mortar and pestle followed by total RNA extraction using the RNeasy plant kit (Qiagen), followed by DNase I treatment (Ambion). Total RNA was quantified using Qubit4 (Thermo Fisher Scientific) and tested for integrity using an RNA screentape in an Agilent2200 tape station (Agilent).

Chromatin Immunoprecipitation. Three grams of plant material for each genotype was fixed in 1× phosphate buffered saline (10 mM PO_4^{3-}, 137 mM NaCl, and 2.7 mM KCl) containing 0.5% formaldehyde (Sigma). The reaction was quenched with glycine to a final concentration of 62 mM. Chromatin immunoprecipitation (ChIP) was performed as described (62), with the exception that 100 μL of anti-cMyc agarose affinity gel (A7470-1ML) was used per sample or custom anti-ELF3 (Agrisera, AS184168, lot# 1808). Three microliters of anti-ELF3 was preincubated 1 h with Novex DYNAL Dynabeads Protein A and G (50 μL each. Sequencing libraries were prepared using TruSeq ChIP Sample Preparation Kit (Illumina). Libraries were sequenced on a NextSeq-500 (Illumina; single end, 75 bp reads).

Messenger RNA and DNA Library Preparation and Sequencing. Ultrapure total RNA from wild-type, *phyb-1*, *lux* (line 75Z3a), and *elf3-1 elf3-2* (line 246U2.7a2) rice genotypes was used to prepare high-throughput mRNA sequencing libraries using NEBNext Ultra II Directional RNA Library Prep Kit (New England Biolabs). ChIP DNA libraries were performed using wild-type and *LUX* tagged line via TruSeq ChIP Sample Preparation Kit (Illumina). Nucleotide sequencing was performed in a NEXTSEQ550 (Illumina) using a high-throughput flow cell.

RNA-seq and ChIP-seq Data Processing. HISAT2 with parameters '–no-mixed, –rna-strandness RF –dta –fr' was used for aligning the raw RNA-seq reads to the rice genome assemblies (63). Transcripts per million (TPM) values for genes were calculated by StringTie with default settings directed by gene annotation file IRGSP-1.0 (64). Mean TPM values were calculated from replicates.

For processing ChIP-seq fastq files, BWA was used to map raw reads to rice genome IRGSP-1.0. Unmapped reads, mate unmapped reads, nonprimary alignment and duplicate reads were removed. Peaks were identified using MACS2 and filtered by q value < 0.05. BigWig files for IGV tracks were generated using deepTools function *bamCoverage* and normalized using RPKM.

The accession number for the raw and processed data from RNA-seq and ChIP-seq in this paper is Gene Expression Omnibus (GEO): GSE181836.

RNA-seq and ChIP-seq Data Analysis. TPM values were transformed into log2(TPM+1). Genes with the maximum log2(TPM+1) > 2 were kept. Of 37,849 reference genes, 21,175 were kept. To perform clustering of transcriptomic data, a time-course perturbation matrices was constructed between SD and LD in WT, for example, $\log2\left(\frac{TPM_S D_2 T6+1}{TPM_L D_2 T6+1}\right)$. The selected perturbation matrices are described in the *SI Appendix*.

ELF3 and LUX bound genes were determined if ChIP-seq peaks overlap with the genomic regions of gene body extended by 2 kb upstream and downstream, respectively.

Motifs were predicted using HOMER2 (de novo and known motifs), using the genomic regions of 100 bp upstream and downstream of peak summits as target sequences and permuted sequences (excluding target sequences) as background. R package *motifStack* was used for generating *SI Appendix*, Fig. S5 and motifs were filtered using the following criteria: P value < 1e-5 for known motifs, P value < 1e-10 for de novo motifs.

Software Used for Analysis. Graphpad Prism 8.0.2; Geneious Prime 2020.2.2; HISAT2 version 2.2.1; StringTie version 2.1.1; bwa version: 0.7.17-r1188; macs2 version 2.2.7.1; deeptools version 3.5.0; homer version 4.11; samtools version 1.11; bedtools version 2.30.0; R version 4.1.0;

Custom code for using R packages are deposited at https://github.com/yl-lu/Rice_EC.

Validation of Sequencing Data Using RT-PCR. To validate the RNA-seq results, the same samples were used for RNA extraction using RNeasy Plant Mini Kit (Qiagen). cDNA was made from 1 μg of RNA in the Transcriptor First Strand cDNA synthesis kit (Roche Diagnostics). cDNA was diluted to 1:100 to obtain a working concentration. The PCR mix was performed using 3 μL of diluted cDNA, gene-specific primers (available in *SI Appendix*, Table S2), and LightCycler 480 SYBR Green I Master (Roche Diagnostics) set up according to the manufacturer's instructions. The cDNA was then amplified using a LightCycler 480 machine (Roche Diagnostics). Ubiquitin-conjugating enzyme E2 (UBC2) was used as an internal normalization. The experiment was done in triplicate for each sample. The results are given as a normalization of the target of interest/UBC2 amplification using double delta Ct analysis (65).

To validate ChIP-seq results, ChIP DNA and input DNA were de-crosslinked by submitting the samples to 65 °C for 8 h, followed by a cleaning step using Ampure beads according to manual. The obtained DNA was diluted to 1:100 to obtain a working concentration. The PCR mix was performed using 2 μL of diluted DNA, region-specific primers (available in *SI Appendix*, Table S2), and LightCycler 480 SYBR Green I Master (Roche Diagnostics) set up according to the manufacturer's instructions. The DNA was then amplified using a LightCycler 480 machine (Roche Diagnostics). A region showing no particular enrichment was used as an internal control. The experiment was done in triplicate for each sample. Results are given as fold change enrichment relative to the internal control region using double delta Ct analysis (65).

Protein Extraction and Analyses. Protein extracts were prepared from wild-type, *elf3-1* (line 246U2.7a1), *elf3-2* (line 246U2.7a1), *elf3-1 elf3-2* (line 246U2.7a2), *phyb-1*, and *LUX*::MYC. Whole plants were homogenized to powder using a freeze cold mortar. For each 50 mg of plant homogenized material was added 150 μL of freshly made 2 × Laemmli buffer (Bio-Rad) supplemented with 50 mM dithiothreitol. Samples were subjected to gel electrophoresis and immunoblotted as described in the *SI Appendix*.

Yeast Two-Hybrid. To test in vitro interaction, the coding sequence of *ELF3-1* and *ELF3-2* were cloned into pGADT7 vector (Clontech) whereas the coding sequence of *LUX* was cloned in pGBKT7 (Clontech). *LUX* in pGBKT7 and either *ELF3* in pGADT7 vectors were used to transform *Saccharomyces cerevisiae* Y2HGold (Clontech). Positively transformed yeast grew in a synthetic defined medium lacking the amino acids leucine and tryptophan (SD). Interaction screening was performed in SD without adenine (-Ade) and histidine (-His).

Data Availability. All the sequencing data in this study is publicly available in GEO with the reference GSE181836.

All code used in this study for data analysis is publicly available on GitHub (https://github.com/yl-lu/Rice_EC).

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