Negative regulatory roles of *DE-ETIOLATED1* in flowering time in *Arabidopsis*

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*Arabidopsis* flowers early under long days (LD) and late under short days (SD). The repressor of photomorphogenesis *DE-ETIOLATED1* (*DET1*) delays flowering; *det1-1* mutants flower early, especially under SD, but the molecular mechanism of DET1 regulation remains unknown. Here we examine the regulatory function of DET1 in repression of flowering. Under SD, the *det1-1* mutation causes daytime expression of *FKF1* and *CO*; however, their altered expression has only a small effect on early flowering in *det1-1* mutants. Notably, DET1 interacts with GI and binding of GI to the *FT* promoter increases in *det1-1* mutants, suggesting that DET1 mainly restricts GI function, directly promoting *FT* expression and regulate the timing of flowering. GI and FLAVIN-BINDING, KELCH REPEAT, F-BOX PROTEIN 1 (*FKF1*) form a complex and regulate the timing of *CO* expression. The diurnal expression of *GI* and *FKF1* has little overlap in SD, leading to minimal formation of the GI-FKF1 complex. By contrast, in LD, the more extensive overlap of GI and FKF1 diurnal expression leads to formation of more GI-FKF1 complex. Thus, GI acts as a flowering inducer with FKF1 in the *CO-FT* pathway mainly in LD. In a CO-independent flowering pathway, GI can also directly activate *FT* expression by binding to its promoter region, indicating that GI can directly or indirectly induce *FT* transcription in the photoperiod pathway.

In addition to regulation by the photoperiod pathway, genes involved in the autonomous and vernalization pathways also control *FT* expression. *FLOWERING LOCUS C* (*FLC*) has a central place in those two pathways and directly regulates *FT* and *SOC1* expression by binding to their promoters. Chromatin remodeling also affects *FLC* expression. For example, MULTICOPY SUPPRESSOR OF IRA1 4 (*MSI4*)/FVE, in the autonomous pathway, negatively regulates *FLC* expression via histone deacetylation of the *FLC* locus. Furthermore, MSI4/FVE interacts with DDB1 and HDA6, and mediates transcriptional silencing by histone modification of
H3K4me3 and H3K27me3. This indicates that MSI4/FVE plays a significant role in FLC expression by making a complex with various chromatin remodeling factors.

DET1, a repressor of photomorphogenesis, was first identified as a member of the CONSTITUTIVE PHOTOMORPHOGENIC/DETETIOLATED/FUSCA (COP/DET/FUS) gene family. DET1 forms a complex with COP10 and DAMAGED DNA BINDING PROTEIN 1 (DDB1) to promote the activity of ubiquitin-conjugating enzymes (E2) for repression of photomorphogenesis in the ubiquitination pathway. DET1 also acts as a pacemaker to adjust the period length of the circadian rhythm, possibly through interaction with LHY and CCA1. DET1 acts as a flowering repressor; det1-1 mutants flower slightly earlier in LD and extremely early in SD. Despite recent advances in the understanding of DET1 function, the molecular mechanism causing early flowering in det1-1 mutants remains unknown.

Here we demonstrate that DET1 delays flowering time in SD, mainly by reducing the affinity of GI binding to the FT promoter in the photoperiod pathway. DET1 also contributes to upregulating FLC expression in the autonomous pathway, possibly by weakening the activity of MSI4/FVE in histone modification of the FLC locus. These effects, in turn, lead to reduced expression of FT and SOC1. These findings provide new insights into how DET1 dynamically suppresses flowering in SD and thus plays an important role in maintaining photoperiod sensitivity in Arabidopsis.

**Results**

**The det1 mutation alters the expression of flowering-time genes.**

The det1 null mutants are lethal; to study the molecular mechanism by which DET1 functions in floral repression, we therefore used a weak allele, det1-1, and counted the rosette leaf number at bolting to measure flowering time (Fig. 1a, b). We found that det1 mutants flower early under LD and extremely early under SD, which shows that flowering in det1-1 mutants is photoperiod-insensitive. These results indicate that DET1 acts as a strong floral repressor in SD and has a key role in maintaining the photoperiod sensitivity of the regulation of flowering time in Arabidopsis.

The det1-1 mutation causes period-shortening of clock-regulated gene expression; the internal circadian periods of CAB2.LUC (encoding a luciferase) expression in det1-1 mutants were approximately 18 h in continuous darkness and 21 h in continuous light conditions. To investigate whether the circadian defect in det1-1 mutants causes extremely early flowering under SD (Fig. 1 and Table S1), we analyzed the expression modes of floral inducers by measuring the phases and amplitudes of GI, FKF1, CO, FT, and SOC1 mRNA abundance, in WT and det1-1 mutants grown in SD (Fig. 2). In WT, GI expression peaked at ZT6 (zeitgeber time; 6 h after dawn) during daytime, but the peaks of FKF1 and CO expression occurred at ZT9 and ZT12 during nighttime, respectively, resulting in no FT expression. In det1-1 mutants, GI, FKF1, CO, and FT expression did not significantly differ compared with WT (Fig. 2a). However, the peaks of FKF1 and CO expression shifted 3 h and 6 h earlier than those in WT, respectively (Fig. 2b, c). Accordingly, the peaks of GI, FKF1, and CO expression occurred at ZT6 during daytime in det1-1 mutants under SD. Thus, it appears that the daytime expression of CO and light-stabilized CO (Fig. 2c) can activate FT expression in det1-1 mutants under SD (Fig. 2d). The waveform and peak time of SOC1 expression did not change in det1-1 mutants, but SOC1 mRNA abundance increased (Fig. 2e), possibly due to daytime expression of CO and/or increased expression of FT (Fig. 2e, d). Thus, we first speculated that circadian dysfunction might cause the early flowering in det1-1 mutants, as previously reported.

To test whether circadian-period shortening causes the extremely early flowering of det1-1 mutants in SD (Fig. 1 and Table S1), we examined whether the flowering-time defect could be recovered when det1-1 mutants were entrained in SD (light:dark = 1:2) under reduced diurnal cycles, i.e. environmental time periods (T) of 24 T (8-h light:16-h dark), 21 T (7-h light:14-h dark), and 18 T (6-h light:12-h dark). Although reduced diurnal cycles of 21 T and 18 T slightly delayed flowering compared to normal cycles of 24 T, the

![Figure 1](https://example.com/figure1.png)  
**Figure 1** | Flowering-time phenotypes of det1-1 mutants. (a) Phenotypes of wild-type (WT, Col-0 ecotype) and det1-1 mutant plants. Plants were grown at 22°C under cool-white fluorescent light (90–100 μmol m⁻² s⁻¹) in LD (16-h light:8-h dark) or SD (10-h light:14-h dark), and photographed at 2 to 4 days after bolting. Scale bars = 2 cm. (b–c) Genetic analysis to show epistasis between det1-1 and flowering mutants using double (b) and triple mutants (c). The number of rosette leaves of WT (Col-0) and flowering-time mutants grown under LD (16-h light:8-h dark) and SD (10-h light:14-h dark) in (b), and LD (16-h light:8-h dark) and SD (8-h light:16-h dark) conditions in (c) (see Table S1) flowering time was measured as the number of rosette leaves at bolting. Means and standard deviations were obtained from more than 20 plants.
**det1-1** mutants still flowered much earlier than WT under SD of 24 T (Fig. 3). To investigate the cause of early flowering in **det1-1** mutants under reduced T cycles, we analyzed the phases and amplitudes of **GI**, **FKF1**, **CO**, **FT**, and **SOC1** mRNA abundance in **det1-1** mutants grown under SD of 18 T (Fig. S1). Unlike the SD of 24 T, the wave-forms and peaks of **GI**, **FKF1**, and **CO** expression in **det1-1** mutants were very similar to those of WT. However, **FT** and **SOC1** expression was still upregulated in **det1-1** mutants, suggesting that the internal period-shortening defect in **det1-1** mutants cannot fully explain the extremely early flowering under SD of 24 T. The **FKF1** and **CO** peak shifts likely produce a small effect on early flowering in **det1-1** mutants, because **fkf1-t** and **co-101** mutations delayed flowering in **det1-1** mutants under SD whereas they were almost ineffective in WT21 (Fig. 1b and Table S1). Thus, these results strongly suggest that other defects in mechanisms of floral repression lead to photoperiod-insensitive early flowering in **det1-1** mutants, rather than the circadian dysfunction in the **FKF1**-**CO**-**FT** pathway.

**DET1** mainly functions in the photoperiod and autonomous pathways. To test which genetic pathways of floral induction are responsible for the early flowering phenotype of **det1-1** mutants, we examined the flowering-time phenotypes of double mutants of **det1-1** and mutations with late-flowering phenotypes, specifically **cry2-1**, **fkf1-t**, **gi-1**, **co-101**, **ft-1**, and **soc1-2** (Fig. 1b and Table S1). The **cry2-1 det1-1** double mutants flowered much earlier than the **cry2-1** single mutants in both LD and SD, suggesting that **DET1** acts downstream of **CRY2**. The **fkf1-t det1-1** and **co-101 det1-1** double mutants exhibited intermediate flowering times compared with **fkf1-t**, **co-101**, and **det1-1** single mutants in both LD and SD, suggesting that although daytime expression of **FKF1** and **CO** contributes to early flowering in SD, **det1-1** mutants can flower early in the absence of **FKF1** and **CO** activity in both photoperiod conditions. In **gi-1 det1-1** and **ft-1 det1-1** mutants, the early-flowering effect of **det1-1** was almost abolished by **gi-1** or **ft-1** in both LD and SD (Fig. 1b and Table S1), indicating that **GI** and **FT** play major roles in the **DET1**-mediated flowering pathway.
As both the photoperiod and autonomous pathways regulate SOCI expression\(^\text{16}\), we further tested whether DET1 also participates in the autonomous pathway. We found that soc1-2 det1-1 double mutants showed intermediate flowering times in both LD and SD. Also, in ft-1 soc1-2 det1-1 triple mutants, the early flowering effect of det1-1 completely disappeared (Fig. 1b, c, and Table S1). These results indicate that the regulation of flowering time by DET1 does not entirely depend on the FT-mediated photoperiod pathway, but also depends on the SOCI-mediated autonomous pathway. Thus, we further examined the expression of FLC, a major gene in the autonomous pathway, in det1-1 mutants. We found that the det1-1 mutants under SD had very low levels of FLC mRNA (Fig. 2f), suggesting that DET1 induces FLC expression to repress FT and SOCI. Taking these results together, we concluded that DET1 mainly acts in the photoperiod and autonomous pathways as a strong floral repressor.

**DETI interacts with GI in vivo.** GI functions in the photoperiod pathway and det1-1 mutants did not show significant alterations in GI mRNA levels (Fig. 2a), but the gi-1 mutation nearly abolished the early flowering effect of det1-1 in gi-1 det1-1 double mutants (Table S1). Based on these observations, we postulated that DET1 mainly regulates GI at the post-translational level. Thus, we used transgenic plants expressing a tagged GI protein (pGI:GI-HA gi-2 and pGI:GI-HA gi-2 det1-1) to examine whether DET1 negatively regulates GI stability. We found that det1-1 mutants showed no significant alteration in the rhythmic accumulation of GI protein in SD (Fig. 4a). This indicates that the det1-1 mutation does not affect GI protein stability.

DETI interacts with LHY and CCA1, which regulate the circadian rhythms of expression of clock-regulated genes\(^\text{19}\). This raises the possibility that DET1 could negatively regulate GI activity by protein–protein interaction. To examine this, we performed yeast 2-hybrid assays and found that DET1 interacts with the N-terminal region of GI (amino acids [aa] 1-507) (Fig. 4b). To test the in vivo interaction of DET1 and GI, we performed bimolecular fluorescence complementation (BiFC) assays. In the onion epidermal cells, we detected reconstituted YFP fluorescence in the nucleus when nYFP-DET1 and GI-cYFP plasmids were co-transformed (Fig. 4c). To further confirm their interaction, we tested whether GI and DET1 co-immunoprecipitate from transgenic plants expressing tagged proteins. To that end, we sampled the p35S:TAP-DETI pGI:GI-HA gi-2 and p35S:TAP-GFP pGI:GI-HA gi-2 (a negative control) transgenic plants at ZT8 in SD, and used antibodies for the TAP tag to immunoprecipitate DET1. We found that HA-GI co-immunoprecipitated with TAP-DETI, but not with TAP-GFP (Fig. 4d). These results indicate that DET1 interacts directly with GI in the nucleus.

**DETI negatively regulates GI binding to the FT promoter.** The det1-1 mutation does not alter GI mRNA expression (Fig. 2a) or GI protein levels (Fig. 4a) but gi-1 shows nearly complete epistasis to det1-1 in flowering time (Fig. 1b and Table S1). Based on this observation, we hypothesized that in the photoperiod pathway, DET1 negatively regulates the activity of GI, which directly upregulates FT expression through a CO-independent pathway\(^\text{7}\). To test whether det1-1 mutation affects the GI-FT module, we performed chromatin immunoprecipitation (ChIP) assays, using pGEI-HA gi-2 and pGI:GI-HA gi-2 det1-1 seedlings entrained in SD, to test whether det1-1 affects the ability of GI to bind to the FT promoter. We collected tissues from 10-day-old seedlings at ZT8 and detected relative enrichment of the promoter regions by PCR with primers for six regions of the FT promoter, as described previously\(^\text{8}\). When we compared GI binding affinity to the FT promoter regions, the amplicons close to the 5’ untranslated region (UTR) were significantly more enriched in ChIP from det1-1 mutants (Fig. 5b). This result strongly supports the notion that DET1 plays an important role in the suppression of FT transcription by preventing GI binding to the FT promoter, and thus contributing to late flowering in SD conditions.

**DETI positively regulates FLC expression to delay flowering time in SD.** In the autonomous pathway, FLC functions as a key floral repressor and downregulates the transcription of FT and SOCI\(^\text{14-26}\). As the transcript levels of FT and SOCI were upregulated in det1-1 mutants under SD (Fig. 2d, e), and FLC expression was almost absent in det1-1 mutants entrained in SD (Fig. 2f), we reasoned that DET1 also functions to delay flowering in the autonomous pathway by upregulating expression of FLC. A previous report showed that the COP10-DET1-DD1 complex interacts with CUL4 and the DDB1–CUL4 complex interacts with MSI4/FVE to induce FLC transcription\(^\text{14}\). Thus, we asked if DET1 interacts with MSI4 to form a DET1-MSI4 complex to regulate FLC mRNA levels. To test this, we examined the in vivo interaction of MSI4-DET1 by BiFC assays (Fig. 6a). We detected strong GFP fluorescence in the nuclei of cells co-transformed with plasmids expressing DET1-nYFP and cYFP-MSI4, indicating that DET1 interacts with MSI4, which directly binds to the FLC promoter to repress FLC transcription.

Since MSI4 binds to the FLC promoter and alters histone modification, specifically H3K27me3 and H3K4me3, at the FLC locus\(^\text{15,16}\), we further examined the histone methylation levels of the FLC locus, using anti-H3K27me3 and anti-H3K4me3 antibodies in WT and det1-1 mutants. The ChIP analysis revealed that det1-1 mutants maintained higher levels of H3K27me3 and lower levels of H3K4me3 at the FLC locus than did WT (Fig. 6b), consistent with the histone modification states observed in the early-flowering host1-3 mutants\(^\text{28}\). Taking these results together, we suggest that the DET1-MSI4/FVE complex likely contributes to late flowering in SD by altering histone modification of the FLC locus in the autonomous pathway.

**Discussion**

DETI is involved in repression of photomorphogenesis in the ubiquitination pathway\(^\text{16,17,29}\), light-response regulatory pathway\(^\text{30}\), and circadian period\(^\text{18,19}\). However, the function of DET1 in the regulation of flowering time remains unclear. In this study, we provide evidence showing how DET1 regulates photoperiod sensitivity by delaying flowering time in SD. For example, det1-1 mutants showed increased GI activity (Fig. 5) and epigenetic silencing of FLC expression (Fig. 6), resulting in upregulation of FT and SOCI. Thus, we propose a model for the regulatory role of DET1 in both photoperiod and autonomous pathways (Fig. 7).

In this study, we showed that gi-1 and ft-1 nearly completely suppressed the early flowering of det1-1 mutants and that DET1 directly interacts with GI in vitro and in vivo (Fig. 4). However, DET1 does not interact with the light-input components PHYA, PHYB, CRY1 C-terminus (CCT1), or CRY2 C-terminus (CCT2), or the floral inducers CO or FKF1 (Fig. S3), indicating that DET1 has a unique role in the posttranslational regulation of GI activity in the photoperiod pathway. A previous study revealed that EARLY FLOWERING4 (ELF4), one of the circadian-clock components\(^\text{30}\), acts upstream of GI\(^\text{15}\). ELF4 represses GI binding to the CO promoter to control flowering\(^\text{32}\). Our results revealed that co1-101 det1-1 mutants showed intermediate flowering-time phenotypes, but in ft-1 det1-1 mutants, the early flowering phenotype of det1-1 almost completely disappeared under LD (Fig. 1b), indicating that DET1 function in the regulation of photoperiodic flowering mainly depends on FT expression. Thus, we hypothesized that DET1 regulates GI binding to the FT promoter to delay flowering time and showed that GI binding to the FT promoter significantly increased in the det1-1 mutant background (Fig. 5). This result indicates that DET1 represses FT expression via direct regulation of GI binding to the FT promoter.

DETI functions as a repressor of photomorphogenesis in darkness by forming a complex with COP10 and DDB1 and promoting the
activity of ubiquitin-conjugating E2 enzymes in the ubiquitination pathway. The RING-type E3 ubiquitin ligase COP1, a member of the COP/DET/FUS family, also represses photomorphogenesis in darkness; cop1-4 mutants display very similar phenotypes to det1-1 mutants, such as short hypocotyls and opened cotyledons. This implies a potential functional connection between DET1 and COP1. Indeed, COP1 interacts with COP10, but not with DET1, suggesting that COP1 could interact with the COP10-DET1-DDB1 (CDD) complex to repress photomorphogenesis. In addition, cop1-4 mutants flower extremely early under SD, similar to det1-1 mutants. Thus, the CDD complex may function with COP1 in regulation of flowering time, although we have no direct evidence because the det1-1 cop1-4 double mutant is lethal. COP1 directly controls GI stability by interacting with GI in the presence of ELF3 for photoperiodic flowering. However, DET1 does not regulate GI stability but does negatively affect GI binding to the FT promoter (Fig. 4a). Therefore, although DET1 and COP1 have very similar mutant phenotypes and post-translational behavior, they seem to regulate GI function independently through distinct molecular mechanisms.

Other negative regulators of FT transcription, including FLC, SVP, TEM1, and TEM2, bind to the regions near the 5' UTR of FT. In single mutants of these regulatory genes, FT mRNA expression increases to levels similar to those seen in det1-1 mutants. Notably, SVP, TEM1, and TEM2 interact with GI to regulate FT expression, although the regulatory function of their interaction is not clearly understood. Therefore, DET1 could be involved in the function of these FT repressors. To investigate this possibility, we examined the interaction of DET1 with these four FT repressors by yeast 2-hybrid assays, which revealed that DET1 does not interact with FLC, SVP, TEM1, or TEM2. This result strongly suggests that DET1 may regulate the GI-FT module independent of these known FT repressors.

In addition, we revealed that DET1 regulates the expression of FLC, a key component in the autonomous pathway. We found that...
the det1-1 mutants showed a remarkable decrease in FLC mRNA levels and had altered levels of H3K4me3 and H3K27me3 (Figs. 2f and 6b), as observed in the early-flowering hos1-3 mutants28. Furthermore, our examination of the components of the CDD complex showed that in addition to interacting with DDB1, DET1 also interacts with MSI4/FVE, which repress FLC expression in the autonomous pathway (Fig. 6a)14. This indicates that DET1 represses FLC expression possibly through direct interaction with MSI4/FVE. Meanwhile, FLC negatively regulates not only FT but also the downstream factor SOC1, which encodes a MADS box transcription factor37. In genetic analysis, ft-1 was completely epistatic to det1-1 in LD, but in SD the ft-1 det1-1 double mutants showed an intermediate phenotype, indicating incomplete epistasis. Consistent with this, SOC1 expression was upregulated in det1-1 mutants (Fig. 2e), but soc1-2 did not rescue the early flowering of det1-1 (Fig. 1b and Table S1). Notably, the ft-1 soc1-2 det1-1 triple mutants showed complete suppression of the early flowering of det1-1 in both photoperiods. This supports the idea that DET1 suppresses both FT and SOC1 via promoting FLC expression in the autonomous pathway.

DET1 interacts with LHY/CCA1 and is required for transcriptional repression of CCA1/LHY target genes such as TOC119. These observations indicate that DET1 functions with LHY/CCA1 to regulate the circadian rhythms of evening genes. Moreover, DET1 could act with LHY/CCA1 to negatively regulate GI binding to the FT promoter mainly in SD, because lhy cca1 double mutants also exhibit photoperiod-insensitive early flowering26. To prove this hypothesis will require further analysis, such as examination of the in vivo interaction of CCA1-GI or LHY-GI, and GI binding activity to the FT promoter in either lhy cca1 double mutants or LHY or CCA1 overexpressors.

Based on these data, we propose a model for the molecular mechanism by which DET1 represses flowering in non-inductive SD conditions (Fig. 7). In WT plants, the absence of FT expression under SD conditions
way. Whether DET1 acts in the CDD complex to delay flowering binding activity to the ways (Fig. 7); DET1 suppresses flowering mainly by decreasing GI cating that DET1 mainly regulates flowering via GI.

Figure 7 | Working model of DET1 function in floral repression in Arabidopsis. DET1 suppresses FT and SOC1 expression through the photoperiod and autonomous pathways of flowering. In the photoperiod pathway, DET1 mainly represses flowering by modulating GI-mediated floral induction at the transcriptional and post-translational levels during daytime under SD. DET1 represses the function of daytime-expressed GI by preventing GI from binding to the FT promoter in a CO-independent pathway. In the autonomous pathway, DET1 interacts with MS4/VE and possibly modulates trimethylation of FLC chromatin to epigenetically induce FLC expression. Genes and proteins are represented as rectangles and ovals, respectively.

can be explained by the incongruity of peak expression of FKF1 and GI; GI peaks in the late afternoon but FKF1 peaks at night, leading to reduced expression of CO and FT during daytime. As GI also directly induces FT expression in a CO-independent pathway, we wondered why GI, which is expressed in the afternoon, is not capable of inducing FT expression under SD (Fig. 2a, d). In this study, we found that DET1 suppresses FT transcription by repressing GI binding activity to the FT promoter (Fig. 5b). This model is further supported by genetic analysis showing that gi-1 and ft-1 are almost completely epistatic to det1-1 (Fig. 1b and Table S1), indicating that DET1 mainly regulates flowering via GI.

In conclusion, we propose that DET1 functions as a strong represor of flowering, acting in both photoperiodic and autonomous pathways (Fig. 7); DET1 suppresses flowering mainly by decreasing GI binding activity to the FT promoter in the photoperiod pathway and epigenetically upregulating FLC expression in the autonomous pathway. Whether DET1 acts in the CDD complex to delay flowering under SD in Arabidopsis remains to be elucidated.

**Methods**

**Plant materials and growth conditions.** All the Arabidopsis thaliana lines used in this study are in the Columbia (Col-0) genetic background. Flowering-time mutants were obtained from the Arabidopsis Biological Resource Center (USA), except for det1-1 which was kindly provided by Joanne Chory, cry2-1 (CS3752), gi-1 (CS3123), soc1-2 and f-1, f1a-1, and col-101 were used for genetic analysis. To create double and triple mutants, F1 heterozygotes were obtained by crossing the det1-1 mutant as the female plant with other flowering-time mutants as pollen donors. To select correct transformants, the plants showing the det1-1 morphological phenotype were isolated from F3 plants, and flowering-time mutations were finally confirmed by PCR-based genotyping. Plants were grown on soil at a constant 22°C under white fluorescent light (90-100 μmol m−2 s−1) in LD (16 h light:8 h dark) and SD (10 h light:14 h dark) or SD (8 h light:16 h dark).

**Analysis of flowering time.** The bolting date was measured as the number of days from seed sowing to opening of the first flower and as the total number of rosette leaves at bolting. Data were obtained from three experimental replications (20 to 60 plants per replication).

**RNA preparation and quantitative real-time PCR analysis.** Tissue samples were collected every 3 h from 3-week-old seedlings. Total RNA was extracted with the Plant Total RNA Isolation Kit (Macrogen). For each sample, 2 μg of total mRNA was reverse transcribed using M-MLV reverse transcriptase (Promega). The level of the transcripts was measured by real-time PCR, using GoTaq qPCR Master Mix (Promega) and the Light Cycler 2.0 instrument (Roche). Each PCR was repeated at least three times using biologically independent samples. The amount of each RNA level was determined using specific primers. The primers used for real-time PCR are listed in Table S2.

**Yeast 2-hybrid assays.** The full-length cDNAs of DET1, GI, PHYA, PHYB, CCT1, CCT2, CO, FKF1, FLC, SVP, TEM1, and TEM2 were amplified from wild-type total RNA using RT-PCR. GI was divided into three parts: GI N-terminal (aa 1-507), GI middle (aa 401-907), and GI C-terminal (aa 801-1173) regions. The PCR products were cloned into pGBK7 and pGADT7 vectors (MATCHMAKER GAL4 TWO-hybrid system 3, Clontech) to get the bait and prey clones. For the interaction study, plasmids containing fusion proteins were transformed into Saccharomyces cerevisiae AH109 and grown on media lacking adenine, leucine, histidine, and tryptophan. Galactosidase activity assays were performed according to the manufacturer’s protocol.

**In vivo pull-down assays.** TAP-DET1 and TAP-GFP were from Xing Wang Deng. pGGEI-GHA-g2 det1-1 was obtained by crossing pGGEI-GHA-g2 and det1-1. For DET1-GI binding assays, TAP-DET1 pGGI-GHA-g2 and TAP-GFP pGGEI-GHA-g2 were plants were grown on MS medium in SD (8 h light:16 h dark) for 10 days and then vacuum infiltrated for 7–10 min in 1X MS (Duchefa) liquid medium supplemented with 50 mM MG132 (Sigma) for proteasome inhibitor treatment. After that, plants were incubated for 10 h under light conditions. These plants were homogenized and total proteins were extracted in total protein extract buffer [50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 10 mM MgCl2, 1 mM EDTA (pH 8.0), 10% glycerol, 1 mM PMSF, 1 mM DTT]. These experiments were performed with IgG beads for TAP-IP. After washing, the immunoprecipitated fractions were analyzed by immunoblotting. The TAP-DET1 and GI fusion proteins were detected by using anti-HA antibody.

**Bimolecular fluorescence complementation assays.** Each cDNA of GI, ELF3, DET1, and MS1 was cloned into the BiFC gateway vectors to examine their in vivo interactions. For partial YFP-tagged DET1 and MS1 constructs, the cDNA of the gene was obtained by RT-PCR from wild-type (WT, Col-0) plants and fused into four BiFC plasmid sets, pSAT5-DEST-cEYFP(175-end)-C1 (pE3130), pSAT5(A)-DEST-cEYFP(175-end)-N1 (pE3132), pSAT4-DEST-nEYFP(1-174)-N1 (pE3134), and pSAT4-DEST-nEYFP(1-174)-C1 (pE3135). Partial YFP-tagged ELF3 and GI constructs were previously described. Each pair of recombinant plasmids encoding nEYFP and cEYFP fusions was mixed 1:1 (w/w), co-bombarded into onion protoplasts, and cotransformed into Arabidopsis plants using a particle gun (Bio-Rad). The cotransformed plants were grown on MS medium at 22°C under light or dark incubation, followed by observation and image analysis using a confocal laser scanning microscope (Carl Zeiss LSM710).

**Chromatin immunoprecipitation assay.** For the ChiP assay, Col-0, pGGEI-GHA-g2, and pGGEI-GHA-g2 det1-1 plants were grown for 10 days under SD (8 h light:16 h dark) conditions and collected at ZT8. The samples were cross-linked with 1% formaldehyde, ground to powder in liquid nitrogen, and then sonicated. The sonicated chromatin complexes were bound with anti-HA antibody (ab9110, Abcam) for immunoprecipitation. The amount of DNA fragment was analyzed by quantitative real-time PCR (qPCR) using specific primers. UBI10 was used as an internal standard for normalization. The primers used for qPCR are listed in Table S2. For another ChiP assay, Col-0 and det1-1 plants were grown for 14 days under SD (8 h light:16 h dark) conditions and collected at ZT8. For immunoprecipitation, we used the anti-trimethyl H3K4 (07-473, Millipore), and anti-trimethyl H3K27 (07-449, Millipore). FUS3 was used as an internal standard for normalization. Experiments were performed with three biological repeats.

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