GIGANTEA directly activates *Flowering Locus T* in *Arabidopsis thaliana*

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Plants perceive environmental signals such as day length and temperature to determine optimal timing for the transition from vegetative to floral stages. *Arabidopsis* flowers under long-day conditions through the *CONSTANS* (*CO*)–*FLOWERING LOCUS T* (*FT*) regulatory module. It is thought that the environmental cues for photoperiodic control of flowering are initially perceived in the leaves. We have previously shown that GIGANTEA (GI) regulates the timing of *CO* expression, together with FLAVIN-BINDING, KELCH REPEAT, F BOX protein 1. Normally, *CO* and *FT* are expressed exclusively in vascular bundles, whereas GI is expressed in various tissues. To better elucidate the role of tissue-specific expression of GI in the flowering pathway, we established transgenic lines in which GI is expressed exclusively in mesophyll, vascular bundles, epidermis, shoot apical meristem, or root. We found that GI expressed in either mesophyll or vascular bundles rescues the late-flowering phenotype of the *gi-2* loss-of-function mutant under both short-day and long-day conditions. Interestingly, GI expressed in mesophyll or vascular tissues increases *FT* expression without up-regulating *CO* expression under short-day conditions. Furthermore, we examined the interaction between GI and *FT* repressors in mesophyll. We found that GI can bind to three *FT* repressors: SHORT VEGETATIVE PHASE (SVP), TEMPRANILLO (TEM)1, and TEM2. Finally, our chromatin immunoprecipitation experiments showed that GI binds to *FT* promoter regions that are near the SVP binding sites. Taken together, our data further elucidate the multiple roles of GI in the regulation of flowering time.

Successful reproduction in higher plants depends on appropriate timing of flowering. Understanding the mechanisms underlying flowering time pathways can provide insight into the networks mediating the effects of environmental cues on developmental programs, and has important implications for crop production.

Plants use multiple environmental cues to determine the timing of flowering, such as temperature, quality and quantity of light, and day-length changes. Among these signals, day-length change is the most reliable because it occurs in regular and predictable cycles year after year. Photoperiodism refers to the rhythms of biological processes that are based on day-length changes and is found in many species including insects, birds, and mammals. Although the molecular mechanism for photoperiodism is not well-understood, biologists have identified several key elements governing this phenomenon in plants.

In the model plant *Arabidopsis thaliana*, flowering is accelerated when the length of daylight is prolonged compared with darkness (16 h of light and 8 h of darkness, designated as long day; LD). Time measurement in the photoperiodic flowering pathway is regulated by daytime expression of *CONSTANS* (*CO*) (1, 2). Under LD, *CO* expression coincides with light. CO protein is stabilized by light (a component of a process referred to as external coincidence) and it activates a downstream factor, *FLOWERING LOCUS T* (*FT*) (3). In short days (SD; 8 h of light and 16 h of darkness), the peak time of *CO* expression occurs after dusk, so that CO protein is not stabilized and is unable to activate *FT* (3, 4). Taken together, the timing of *CO* expression is crucial in the photoperiodic flowering pathway.

Previously we showed that GIGANTEA (GI) and FLAVIN-BINDING, KELCH REPEAT, F BOX protein 1 (FKF1) are important in the regulation of the timing of *CO* gene expression (5). The expression of GI is under control of the circadian clock and peaks at the end of the day. We determined that GI and FKF1 form a complex in a light-dependent manner to mediate the degradation of CYCLING DOF FACTOR 1 (CDF1), a key *CO* repressor. Specifically, under LD conditions, the expression of GI and FKF1 peak at the same time, leading to the optimal formation of the GI–FKF1 complex (internal coincidence). Under SD conditions, the expression of GI peaks a few hours before the peak of FKF1 expression, resulting in low levels of the GI–FKF1 complex and the maintenance of CDF1. Thus, sufficient levels of the GI–FKF1 complex are required for the proper induction of *CO*, which in turn triggers flowering under LD conditions (6).

In addition to the *CO–FT* module, photoperiod-responsive flowering is modulated by multiple regulatory processes. Genetic analysis has indicated that GI alone may also play a role in inducing this function, separately or in parallel to its involvement in the *CO–FT* module (7). Jung et al. reported that GI regulates *FT* expression by regulating miR172 levels. They showed that TARGET OF EAT (TOE)1, one of the targets of miR172, regulates *FT* expression independently of *CO* (8). miR172 represses flowering through targeting multiple transcription factors such as *SCHLAFMUTZE* (*SMZ*) and its paralog *SCHNARCHZAPFEN* (*SNZ*) (9). SMZ has been shown to repress *FT* directly. SMZ also regulates expression of *APETALA1* (*AP1*) and *SUPRESSOR OF CONSTANS OVEREXPRESSION* (*SOC1*), which regulate flowering time and floral development in shoot apical meristem (9). Kim et al. showed that EARLY FLOWERING 3 (ELF3) influences flowering by regulating the expression of multiple flowering genes such as *GI* and *FT*. Interestingly, the *elf3-1* mutant displays an early flowering phenotype independently of *CO* expression (10). These reports suggest that photoperiodic flowering is regulated by complex mechanisms, including uncharacterized *CO*-independent pathways.

In addition to genetic analysis, expression patterns of flowering genes indicate the spatial complexity of the regulatory pathways in flowering. Plants perceive photoperiodic changes in leaves (11); however, the expression patterns of *CO*, *FT*, *GI*, and *FKF1* are not identical throughout all leaf cell types. *CO* and *FT* are expressed exclusively in vascular bundles (12, 13), whereas *GI* and *FKF1* can be detected in several additional tissues, including mesophyll. CDFs, the only known *CO* repressors, are expressed solely in vascular bundles (14). TOE1, which functions as an *FT* repressor downstream of GI, is expressed only in vascular tissue (8). On the other hand, several *FT* repressors, namely SHORT VEGETATIVE PHASE (SVP), FLOWERING LOCUS C

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(FLC), TEMPRANILLO (TEM)1, and TEM2, were detected in both mesophyll and vascular bundles (15–17).

To better understand the molecular mechanisms of the photoperiodic flowering pathway, we investigated the effect of tissue-specific expression of GI. We show that GI expression in mesophyll can rescue the flowering deficiency in *gi-2* loss-of-function mutants. In addition, under SD conditions, GI increased *FT* expression without inducing the expression of *CO*. We also determined that GI binds to several known *FT* repressors that are expressed not only in vascular bundles but also in mesophyll. Finally, we found that GI binds to *FT* promoter regions. Taken together, our findings show that GI is capable of directly activating *FT* expression, acting alone and independently of *CO*.

Results

Effects of Tissue-Specific Expression of *GI* on Flowering Time. *GI* is expressed in many plant tissues, including vascular bundles, mesophyll, apical shoot meristem, and root (18). However, the function of GI in those tissues is not well-understood. To shed more light on how GI influences photoperiodic flowering, we expressed a GI transgene in the *gi-2* loss-of-function mutant under tissue-specific promoters. For this, we took advantage of previously defined tissue-specific promoters: *LIGHT-HARVESTING COMPLEX B2.1* (*pLhCB2.1*) and *CHLOROPHYLL A/B-BINDING PROTEIN 3* (*pCAB3*) for mesophyll specificity (19, 20), *SUCROSE TRANSPORTER 2* (*pSUC2*) and *SULFATE TRANSPORTER 1:3* (*pSultr1:3*) for expression in vascular bundles (21, 22), *ECERIFERUM 6* (*pCER6*) for epidermis expression (23), *SHOOTMERISTEMLESS* (*pSTM*) for expression in the apical shoot meristem (24), and *At3g25820/At3g25830* (*pAt3g25820/At3g25830*) for root-specific expression (25).

After selecting transformants on agar plates, we transferred T1 generation plants (first generation transformants derived from transformed plants) to soil and assayed them under long-day conditions. We recorded the total number of rosette and cauline leaves when plants started to bolt (beginning of flowering). Typically, wild-type plants form only about 15 leaves at bolting, whereas *gi-2* mutants show a significant late-flowering phenotype, forming about 60 leaves. Overexpression of *GI* under the cauliflower mosaic virus *35S* promoter promotes flowering even earlier than wild type, at about 12 leaves. In this set of experiments, we have shown that *GI* expression in mesophyll or vascular bundles rescues the late-flowering phenotype of *gi-2* (Fig. 1*A* and Fig. S1). *GI* expressed in apical shoot meristem promoted flowering time with statistical significance (Student's *t* test, $P < 0.01$). However, the effect was much weaker than that of *GI* expression in mesophyll or vascular bundles. *GI* expression in epidermis or root did not have a significant effect (Fig. 1*A*). In this study, we focused on GI function in leaf tissue because the photoperiodic signal is perceived in leaves (11).

***GI* Expression in Mesophyll or Vascular Bundles Rescues the Late-Flowering Phenotype of *gi-2* Under Both LD and SD Conditions.** To confirm the rescue of *gi-2* by *GI* expression in mesophyll or vascular bundles, we examined the flowering phenotype in homozygous T4 generations (Fig. 1 *B* and *C*). We selected two independent plant lines for both mesophyll- and vasculature-specific *GI* expression (*pLhCB2.1::GI/gi-2* #1 and #3; *pSUC2::GI/gi-2* #1 and #2). In all four lines, GI expression levels were up-regulated (Fig. S2). *GI* expression in *pLhCB2.1::GI/gi-2* #1 is about 10 times higher than in wild type at zeitgeber time (ZT) 12 (the time of lowest expression) and about 100 times higher than in wild type at ZT0 (the highest expression time point). *pLhCB2.1::GI/gi-2* #3 has 2 times higher expression of *GI* than wild type at ZT12 and about 20 times higher at ZT0. *pSUC2::GI/gi-2* #1 shows almost the same expression of *GI* as wild type at ZT20, and about 6 times higher than wild type at ZT0. *pSUC2::GI/gi-2* #3 expressed *GI* at levels 2 times higher than wild type at ZT20 and about 8 times higher than wild type at ZT0. We studied the flowering phenotype under LD and SD at both 16 °C and 23 °C, because recent findings have demonstrated the importance of ambient temperature in flowering pathways (15).

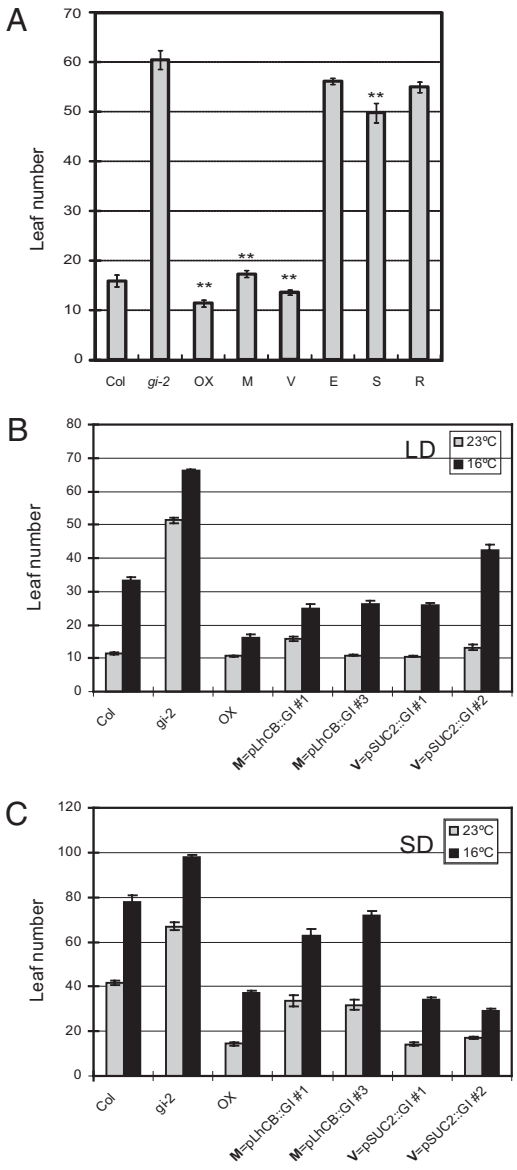

Fig. 1. Mesophyll or vascular bundle-specific expression of GI can rescue the late-flowering phenotype of *gi-2* (loss-of-function mutant). (*A*) Total leaf number was counted under LD conditions (16 h of light/8 h of darkness) for all T1 transgenic lines. Data are means ± SEM for at least 15 plants. The following lines were analyzed: wild type (Col-0), *gi-2* (loss-of-function mutant), *GI*-overexpressing line (OX), *GI* expressed in mesophyll (M; *pLhCB2.1::GI/gi-2*), *GI* expressed in vascular bundles (V; *pSUC2::GI/gi-2*), *GI* expressed in epidermis (E; *pCER6::GI/gi-2*), *GI* expressed in shoot apical meristem (S; *pSTM::GI/gi-2*), and *GI* expressed in root (R; *pAt3g25820/At3g25830::GI/gi-2*). Asterisks indicate statistical significance in comparison between *gi-2* and transgenic lines (Student's *t* test, $P < 0.01$). (*B*) Flowering phenotype under LD conditions at 16 °C or 23 °C of two independent lines expressing *GI* in mesophyll (*pLhCB2.1::GI/gi-2* #1 and #3) and two additional lines expressing *GI* in vascular bundles (*pSUC2::GI/gi-2* #1 and #2). Data are means ± SEM for 16 plants. (*C*) Flowering phenotype under SD conditions at 16 °C or 23 °C using two independent lines expressing GI in mesophyll (*pLhCB2.1::GI/gi-2* #1 and #3) and two additional lines expressing GI in vascular bundles (*pSUC2::GI/gi-2* #1 and #2). Data are means ± SEM for 16 plants.

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Under LD conditions, as shown in Fig. 1*B*, transgenic lines expressing *GI* in mesophyll or vascular bundles show phenotypes similar to the wild type at both 16 °C and 23 °C. Flowering time for all transgenic plants under LD is delayed at 16 °C compared with 23 °C. This indicates that transgenic plants that express *GI* in mesophyll or vascular tissues are still sensitive to lower temperature (typical for wild-type plants). Under SD conditions, *GI* expression in mesophyll or vascular tissues also rescued the late-flowering phenotype of *gi-2* at both temperature conditions, with temperature sensitivity preserved (Fig. 1*C*).

***FT* mRNA Levels Are Elevated in a *CO*-Independent Manner by the Ectopic Expression of *GI*.** GI was shown to influence flowering time through activation of the *CO–FT* module (26). Thus, we checked how tissue-specific expression of *GI* influences both *CO* and *FT* expression levels. To this end, we collected wild type, *gi-2* loss-of-function mutants, and our transgenic plants every 4 h over a 24-h period. These plants were grown under four different conditions (LD at 16 °C, SD at 16 °C, LD at 23 °C, and SD at 23 °C) and expression levels of *CO* and *FT* were measured by quantitative (q)PCR (Fig. 2 *A* and *B* and Fig. S3 *A* and *B*).

Under LD conditions, wild-type plants show cyclic expression of *CO*, with peaks around ZT20 at 16 °C and ZT16 at 23 °C. *FT* expression peaks at ZT16 at both 16 °C and 23 °C. In *gi-2* mutants under both temperature conditions, *CO* expression is constantly dampened and *FT* expression is not induced. Plants that overexpress *GI* showed an increase of both *CO* and *FT* expression. *GI* expression in mesophyll or vascular tissue showed a similar pattern of *CO* expression as wild-type plants at both 16 °C and 23 °C. On the other hand, *GI* expression in mesophyll or vascular tissue increased *FT* expression between ZT4 and ZT8 whereas maintaining an expression peak at ZT16 (Fig. 2*A* and Fig. S3*A*).

Under SD conditions, wild-type plants show cyclic expression of *CO* at both 16 °C and 23 °C, as is observed under LD conditions. However, *FT* is not expressed, because *CO* expression does not coincide with light under SD conditions (3). In a *gi-2* mutant, neither *CO* nor *FT* expression is induced. Plants expressing *GI* in mesophyll or vascular tissue did not show any induction of *CO*

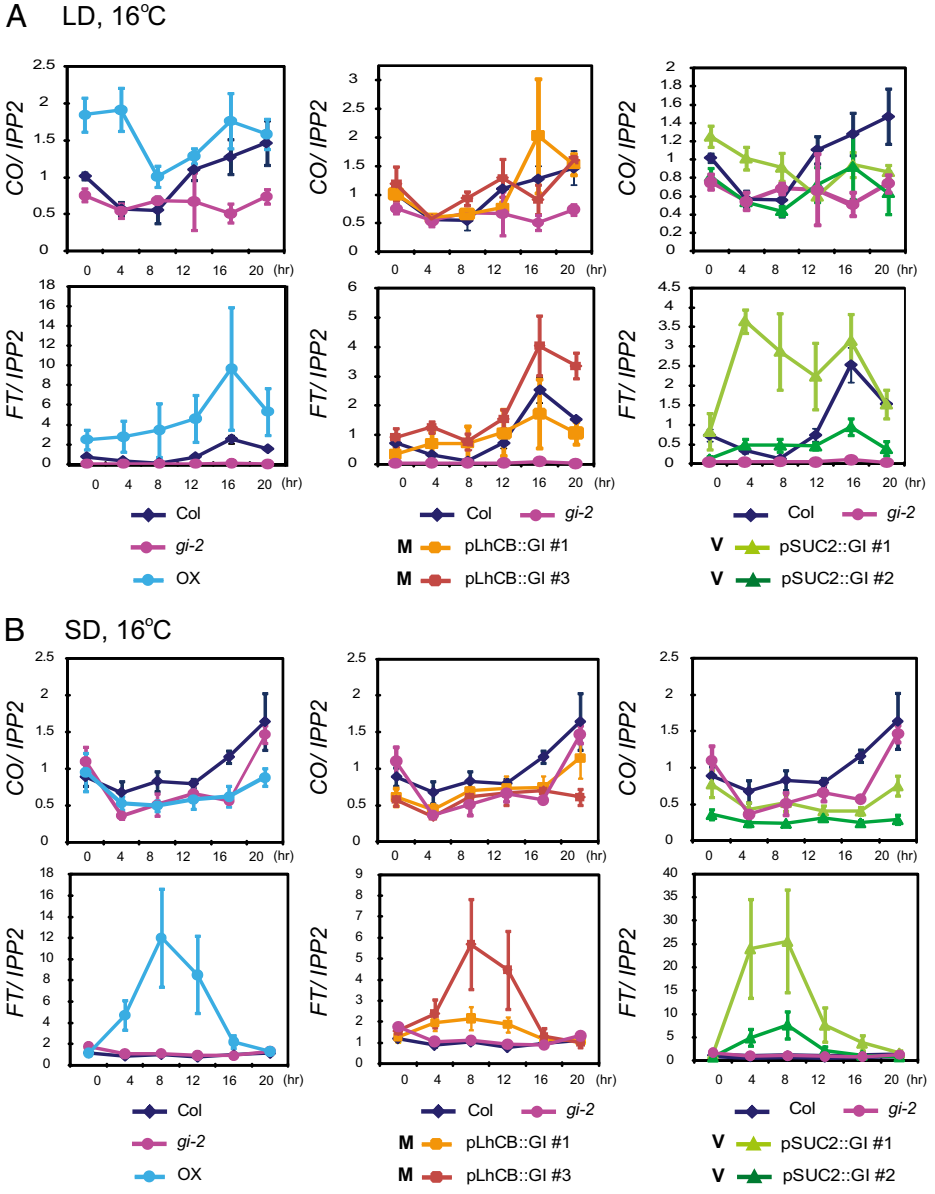

Fig. 2. Effects of tissue-specific expression of GI on *CO* and *FT* gene expression at 16 °C under LD and SD. *CO* and *FT* expression levels were analyzed by qPCR in 10-d-old seedlings on samples collected every 4 h. *ISOPENTENYL PYROPHOSPHATE:DIMETHYLALLYL PYROPHOSPHATE ISOMERASE 2* (*IPP2*) expression was used for normalization. *CO* and *FT* expression is shown relative to the average value of wild-type plant data. Plants were grown at 16 °C under LD (*A*) or SD (*B*) conditions. *CO* and *FT* expression in wild type (Col-0), loss-of-function mutant (*gi-2*), and *GI*-overexpressing line (OX) are shown in the left column. The center column shows data from wild type (Col-0), *gi-2* mutant, and two lines expressing *GI* in mesophyll (*pLhCB2.1::GI/gi-2* #1 and #3). The right column presents data from wild type (Col-0), *gi-2* mutant, and two lines expressing *GI* in vascular bundles (*pSUC2::GI/gi-2* #1 and #2).

compared with wild-type plants, or they showed a similar pattern as the *gi-2* mutant. However, these transgenic lines showed significantly high *FT* expression compared with that in wild-type plant or *gi-2* mutant. *FT* induction without increase of *CO* expression was detected at both 16 °C and 23 °C (Fig. 2*B* and Fig. S3*B*). These data indicate that the transgenic plants rescued the late-flowering phenotype under SD conditions, mostly by increasing *FT* expression in the absence of *CO* induction.

GI Expression in Mesophyll or Vascular Bundles Can Rescue the Late-Flowering Time of *co-101* Mutants. To examine the possibility that GI can induce flowering in a *CO*-independent manner, we expressed *GI* in mesophyll (*pLhCB2.1::GI*) or vascular bundles (*pSUC2::GI*) in the background of a *co* null mutation [*co-101*; T-DNA insertion line (12)]. In the absence of *CO* (*co-101*), plants show a late-flowering phenotype by producing around 48 leaves when they bolt. This late-flowering time was reduced to about 38 leaves by overexpression of *GI* under the *35S* promoter or by tissue-specific expression of *GI* in mesophyll (*pLhCB2.1::GI/co-101*) or vascular bundles (*pSUC2::GI/co-101*) (Fig. S4). This result shows that *GI* can accelerate flowering in the absence of *CO*, tested here under LD in a T1 generation. However, the rescue of the late-flowering phenotype by the ectopic expression of *GI* was less effective in *co-101* than that in the *gi-2* mutant background.

GI Expression in Mesophyll Induces *FT* Expression in This Tissue. *FT* is activated by *CO* and expressed exclusively in vascular bundles (12). However, *FT* expression is regulated in both mesophyll and vasculature by *FT* repressors (e.g., SVP) (15). In our hands, *GI* expression in mesophyll rescued the late-flowering phenotype under SD conditions, possibly by up-regulating *FT* expression. Therefore, we examined whether *FT* expression is induced in mesophyll in transgenic lines that express *GI* in mesophyll. For this, we expressed *β-Glucuronidase* (*GUS*) under the control of the 7-kbp promoter of *FT* in the *pLhCB2.1::GI/gi-2* #1 and #3 plant lines. It was shown that the 5.7-kbp sequence upstream of the *FT* translational start site contains all regulatory elements required to mediate spatial and temporal expression of *FT* in response to photoperiod (27). We performed GUS staining of 3-wk-old T1 plants grown under SD conditions (Fig. 3*A*). In wild-type plants, *FT* expression was observed in vascular bundles only. In transgenic lines that express *GI* in mesophyll, *FT* was coexpressed in this tissue, confirming that the expression of GI protein in mesophyll can induce *FT* expression in the same tissue.

Interactions Between GI and *FT* Repressors. To further examine the possibility that GI regulates *FT* expression in mesophyll, we examined direct interactions between GI and *FT* repressors. We focused on SVP, FLC, TEM1, and TEM2, because these four repressors were shown to be expressed not only in vascular bundles but also in mesophyll, and they all bind to *FT* promoter regions (15–17). We first examined interactions by yeast two-hybrid assays (Fig. S5). We found that the GI N-terminal region interacts with all of the *FT* repressors. We next used a tobacco transient expression assay to examine the physical interactions of these proteins in vivo. Briefly, we coexpressed HA-tagged GI with GFP-tagged SVP, FLC, TEM1, or TEM2 under the *35S* promoter. After harvesting tissues, we fractionated plant lysates to separate cytosolic and nuclear-enriched material. We used anti-GFP antibody to immunoprecipitate tagged repressors and examined whether HA-GI coprecipitated with anti-HA antibody. We found that three out of the four repressors, namely SVP, TEM1, and TEM2, did coprecipitate HA-GI in nuclear but not in cytosolic fractions (Fig. 3 *B* and *C*). FLC did not coprecipitate HA-GI (Fig. 3*B*, both fractions tested). SVP, TEM1, and TEM2 repress *FT* expression by binding *FT* promoter regions (15–17), which suggests that GI binds directly to these *FT* repressors in the nucleus, where they function.

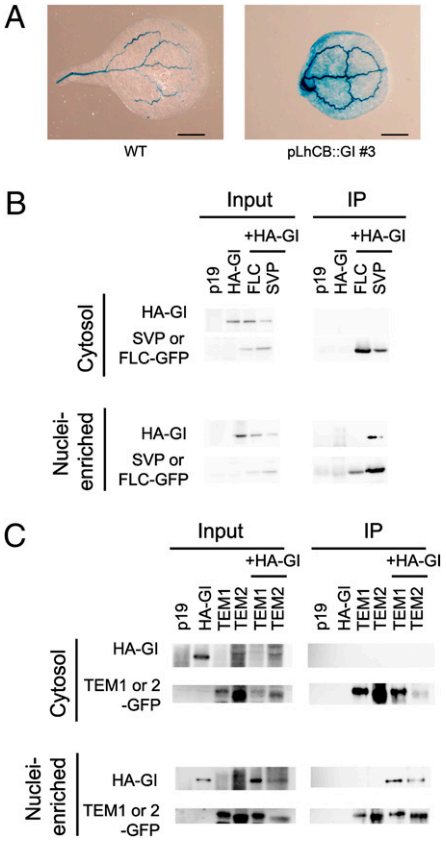

Fig. 3. GI up-regulates *FT* expression in mesophyll and binds to *FT* repressors. (*A*) GUS staining. *FT* is up-regulated in mesophyll in *pLhCB2.1::GI/gi-2* #3. *pFT::GUS* expression in wild type (*Left*) and in a plant line that expresses *GI* in mesophyll (*pLhCB2.1::GI/gi-2* #3) (*Right*). (Scale bars, 1 mm.) (*B*) GI interacts with SVP in vivo. *35S::HA-GI* and *35S::GFP-SVP* were expressed in *Nicotiana benthamiana* and extracted for coimmunoprecipitation experiments. The upper two lanes are input (*Left*) and coimmunoprecipitated samples (IP) (*Right*) from cytosolic fractions. The lower two lanes are from nuclei-enriched fractions. HA-GI is coimmunoprecipitated with GFP-SVP in nuclei-enriched fractions. (*C*) GI interacts with TEM1 and TEM2. *35S::HA-GI* and *35S::GFP-TEM1* or *35S::GFP-TEM2* were expressed in *N. benthamiana*. The upper two lanes are input (*Left*) and coimmunoprecipitated samples (*Right*) from cytosolic fractions. The lower two lanes are from nuclei-enriched fractions. HA-GI is coimmunoprecipitated with GFP-TEM1 and GFP-TEM2 in nuclei-enriched fractions.

GI Is Recruited to *FT* Promoter Regions. To further elucidate how GI regulates *FT* expression in a *CO*-independent manner, we examined the physical interaction of GI protein with *FT* promoter regions. We performed chromatin immunoprecipitation assays (ChIP) using endogenous GI promoter-driven GFP-tagged GI lines (*pGI::GFP-GI/gi-2* #27). Plant tissues were collected at ZT13 under LD. To detect the enrichment of promoter regions, we used primer pairs to amplify the five genomic regions as reported previously (15) (Fig. 4*C*). *FT* repressors SVP, FLC, TEM1, and TEM2 were shown to bind to *FT* promoter regions, including the 5′ UTR regions (15–17). We determined that amplicons 4 and 5, which are close to or include the 5′ UTR region, were significantly enriched (Fig. 4*A*), indicating that GI may bind there. This result suggests that GI activates *FT* gene expression independently of *CO* through direct binding to *FT* promoter regions (alone or in a complex with another protein). In addition, similarities between the chromatin-binding pattern of GI and that of *FT* repressors suggests that GI could interfere with *FT* repressors by regulating their access to the *FT* promoter or their activity and/or stability.

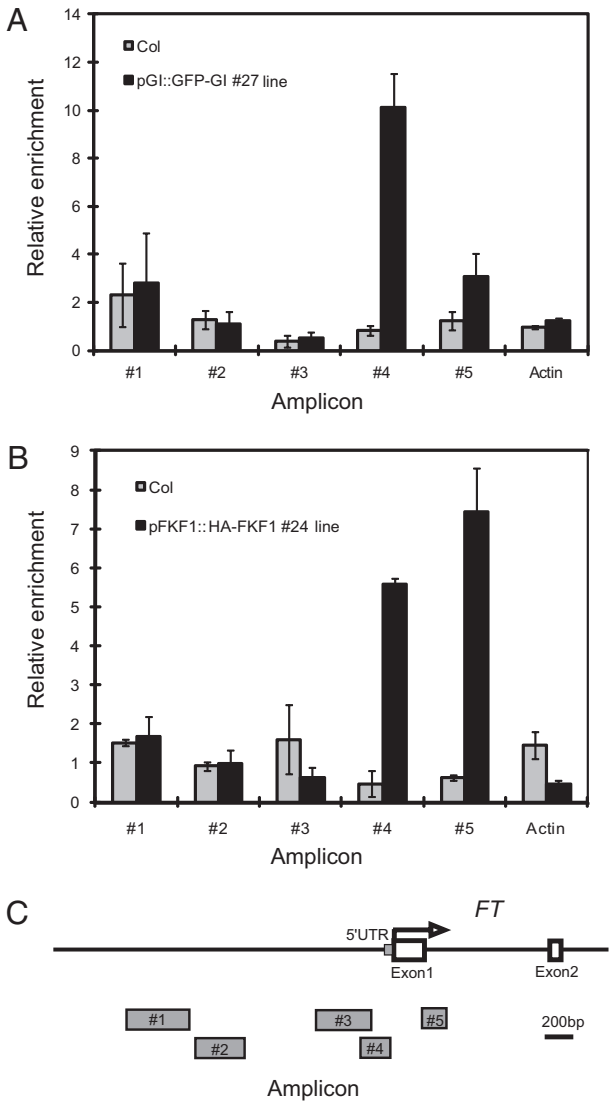

Fig. 4. GI binds to *FT* promoter regions. (*A*) *FT* chromatin regions associated with the GFP-GI protein. Plants were harvested 13 h after light onset (ZT13) on day 10 under LD conditions. Relative enrichment in the wild-type sample and in the *pGI::GFP-GI/gi-2* sample are shown. Data are an average of five independent ChIP analyses. *ACTIN2* (*ACT2*) gene was used as a negative control. (*B*) *FT* chromatin regions associated with the HA-FKF1 protein. Plants were harvested at ZT13 on day 10 under LD conditions. (*C*) Schematic drawing of the *FT* locus and the amplicon locations for the ChIP analyses in *A* and *B*. Five amplicon locations are shown. White and light gray boxes represent exons and 5′ UTRs, respectively.

Involvement of FKF1 in *FT* Gene Regulation. Models based on qPCR data predict that FKF1 influences *FT* expression in a *CO*-independent way (28). FKF1 functions by forming a complex with GI to regulate the timing of *CO* gene expression (5). To examine whether FKF1 is also involved in the direct activation of *FT* gene expression by GI, we performed a series of ChIP experiments with *pFKF1::HA-FKF1/fkf1* #24 to examine whether FKF1 can bind to *FT* promoter regions. These experiments show that FKF1 does bind to *FT* promoter regions, in a pattern similar to that of GI binding (Fig. 4*B*).

Next, using yeast two-hybrid assays, we examined interactions between FKF1 and four *FT* repressors (SVP, FLC, TEM1, and TEM2). We could not detect obvious interactions between FKF1 protein and the *FT* repressors we assayed (Fig. S6). It is possible that FKF1 has different molecular targets from those that bind to GI. Ultimately, the molecular mechanism by which FKF1 potentially activates *FT* expression in a *CO*-independent manner requires further analysis.

Discussion

We have shown that targeted expression of GI in mesophyll or in vascular bundles rescues the late-flowering phenotype in *gi-2* loss-of-function mutants. In our hands, GI activated *FT* expression in a *CO*-independent manner under SD conditions. Ectopic expression of GI in mesophyll triggered *FT* expression in this tissue. In addition, we demonstrated that GI binds directly to SVP, TEM1, and TEM2 in vivo. Finally, our ChIP experiments showed that GI physically associates with *FT* promoter regions, the same regions that are binding sites for *FT* repressors SVP, TEM1, and TEM2. Taken together, we suggest a unique model where GI directly regulates *FT* expression through neutralization of *FT* repressors by blocking their access to *FT* promoter regions and/or by effecting their stability and/or activity.

GI Activates *FT* in a *CO*-Independent Manner. Our data showed that *GI* expression in mesophyll (*pLhCB2.1::GI/gi-2*) and in vascular tissues (*pSUC2::GI/gi-2*) can activate *FT* gene expression without up-regulating *CO* under SD conditions at both 16 °C and 23 °C (Fig. 2 and Fig. S3).

One explanation for why the strong *CO*-independent *FT* induction by GI was detected under SD conditions is that under LD conditions, expression of GI and FKF1 peak at the same time, a sufficient amount of GI–FKF1 complex is formed, *CO* is up-regulated, and *FT* is induced. However, under SD conditions, maximal expression of FKF1 does not coincide with maximal GI expression, the amount of GI–FKF1 complex is smaller, and *CO* is not significantly up-regulated. In contrast, under LD conditions, some *CO*-independent activation of *FT* by GI likely occurs, because we detected the physical interaction of GI with the *FT* promoter in plants grown under LD (Fig. 4*A*). As additional evidence for our model that GI can induce *FT* without the involvement of CO, we determined that expression of *GI* in these tissues rescued the *co-101* late-flowering phenotype (Fig. S4). We consider *CO*-dependent *FT* activation to be prominent in *Arabidopsis*, because the effect of ectopic *GI* expression in *co-101* mutant was relatively weak.

***GI* Expressed in Mesophyll Acts Locally.** We have shown that *GI* expression in mesophyll (*pLhCB2.1::GI/gi-2*) rescues the late-flowering phenotype of the *gi-2* mutant. GI is more broadly expressed than *FT* [mesophyll, vascular bundles, root, etc. vs. vascular bundles only (12)]. The fact that mesophyll-targeted expression of GI rescues the *gi-2* flowering phenotype could mean that GI induced *FT* in mesophyll or that GI is transported to vascular bundles to activate *FT* there. The first possibility seems more likely, mainly because GI expressed in mesophyll alone induced *FT* expression there (Fig. 3*A*). Also, GI is a 125-kDa protein. Due to the large size, it is likely that intercellular movement is inefficient (29, 30). In addition, Adrian et al. showed that *35S::CO* does not induce *FT* expression in mesophyll (27). This report supports our model that *GI* induces *FT* expression in mesophyll in a *CO*-independent manner.

Unique Role of GI in the Flowering Pathway. *FT* expression is regulated by multiple factors. One mode of activation involves CO expression in vascular bundles (13, 31). On the other hand, *FT* expression is repressed by SVP, FLC, TEM1, and TEM2 in both vascular bundles and mesophyll (15–17). We found that GI interacts with SVP, TEM1, and TEM2 in vivo. In addition, our ChIP experiments showed that GI associates with *FT* promoter regions, where *FT* repressors bind (15–17). *CO*-independent activation of *FT* was inferred in previous studies (7, 10). Kim et al. presented a model of multiple parallel pathways within the *CO*–

FT pathway. Mizoguchi et al. have also suggested that GI may act in a *CO*-independent fashion. As a function of GI in a *CO*-independent pathway, Jung et al. showed that GI regulates miR172 levels, presumably through its processing pathway (8). miR172 has multiple targets such as *TOE1* that repress *FT* in vascular bundles. Our findings suggest that GI is involved in *FT* regulation in a more direct way, such as regulating the stability of *FT* repressors or their accessibility to *FT* promoter regions. This is a unique function of GI in the circuits regulating flowering time.

GI in Mesophyll Might Modulate Environmental and Developmental Signals. In this series of experiments, we show that GI interacts with *FT* repressors such as SVP, TEM1, and TEM2, which are normally expressed in both mesophyll and vascular tissues (Fig. 3 *B* and *C*). In addition, GI associates with the *FT* promoter regions where SVP, TEM1, and TEM2 bind (Fig. 4*A*) (15, 17). These data support the hypothesis that GI activates *FT* in mesophyll. Expression of SVP is influenced by temperature (15). TEM1 and TEM2 expression modulates *FT* expression during development (17). Based on the interactions between GI and those proteins and their binding to overlapping regions of the *FT* promoter, we speculate that GI may interact with *FT* repressors to transduce temperature and/or developmental signaling.

GI Expression in Vascular Bundles Is Most Effective in Promoting Flowering. We have shown that GI expression in mesophyll or vascular tissues can rescue the late-flowering phenotype of a *gi-2* mutant. Under SD conditions, vascular expression of *GI* (*pSUC2::GI/gi-2*) rescued the *gi-2* flowering phenotype more efficiently than mesophyll expression (*pLhCB2.1::GI/gi-2*). These data indicate that *FT* expression is regulated differently in mesophyll and vascular tissues. Alternatively, it is possible that FT protein is less stable in mesophyll or that transport of FT from mesophyll to vascular tissues somehow decreases its effect on the induction of flowering. It is also possible that GI moves to the vascular bundles. The size of GI reduces the efficiency of activating flowering. Efficiency of the internal transport of proteins such as FT and GI within the plant and/or the stability of the proteins in mesophyll needs to be examined to explain why the expression of GI in mesophyll is less effective at triggering flowering than expressing it in the vasculature.

Materials and Methods

Plant Materials and Growth Conditions. Details on the construction of the binary vectors, plant lines, and plant growth conditions are provided in *SI Materials and Methods*. Primer sets used in the construction are listed in Table S1.

RNA Preparation and Quantitative PCR. Whole plants were collected every 4 h. RNA isolation, cDNA preparation, and qPCR techniques are described in *SI Materials and Methods*. Each PCR was repeated atleast three times using biologically independent samples.

GUS Staining. Details on the construction of *pFT::GUS* construct and experimental procedures are described in *SI Materials and Methods*. Primer pairs used to clone *FT* promoter are listed in Table S1.

Coimmunoprecipitation Experiments. Details on the constructions and antibodies used in this study are included in *SI Materials and Methods*. A list of primer sequences used in construction are found in Table S1.

ChIP Assays. ChIP assays were conducted as described previously (5). Anti-GFP antibody (A11122; Invitrogen) and anti-HA antibody (3F10; Roche) were used for immunoprecipitation. The primers used for amplification were described previously (15).

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