Distinct Roles of GIGANTEA in Promoting Flowering and Regulating Circadian Rhythms in Arabidopsis

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The circadian clock acts as the timekeeping mechanism in photoperiodism. In Arabidopsis thaliana, a circadian clock-controlled flowering pathway comprising the genes GIGANTEA (GI), CONSTANS (CO), and FLOWERING LOCUS T (FT) promotes flowering specifically under long days. Within this pathway, GI regulates circadian rhythms and flowering and acts earlier in the hierarchy than CO and FT, suggesting that GI might regulate flowering indirectly by affecting the control of circadian rhythms. We studied the relationship between the roles of GI in flowering and the circadian clock using late elongated hypocotyl circadian clock associated1 (lec1) double mutants, which are impaired in circadian clock function, plants overexpressing GI (35S:GI), and gi mutants. These experiments demonstrated that GI acts between the circadian oscillator and CO to promote flowering by increasing CO and FT mRNA abundance. In addition, circadian rhythms in expression of genes that do not control flowering are altered in 35S:GI and gi mutant plants under continuous light and continuous darkness, and the phase of expression of these genes is changed during diurnal cycles. Therefore, GI plays a general role in controlling circadian rhythms, and this is different from its effect on the amplitude of expression of CO and FT. Functional GI:green fluorescent protein is localized to the nucleus in transgenic Arabidopsis plants, supporting the idea that GI regulates flowering in the nucleus. We propose that the effect of GI on flowering is not an indirect effect of its role in circadian clock regulation, but rather that GI also acts in the nucleus to more directly promote the expression of flowering-time genes.

INTRODUCTION

Induction of flowering in response to daylength synchronizes flowering to the changing seasons and is believed to be important in adaptation of plants to growth at different latitudes (Ray and Alexander, 1966). Physiological experiments implicated the circadian clock as the timekeeping mechanism that enables the measurement of daylength (Samach and Coupland, 2000; Yanovsky and Kay, 2003). Forward genetics in Arabidopsis thaliana identified a genetic pathway that promotes flowering specifically on exposure to long days (LDs) (Searle and Coupland, 2004), and the role of the circadian clock in photoperiodic time measurement was confirmed by demonstrating that transcription of the genes that act in this pathway is circadian clock controlled. Mutations in one of these genes, GIGANTEA (GI), both impair circadian rhythms and delay flowering. Here, we use molecular-genetic approaches to compare the role of GI in the circadian system with its function in controlling flowering.

GI, CONSTANS (CO), and FLOWERING LOCUS T (FT) were placed in the Arabidopsis photoperiod pathway based on genetic analysis (Redei, 1962; Koornneef et al., 1991, 1998). Loss-of-function mutations in each of these genes delay flowering under LDs but have little or no effect under short days (SDs). Genetic epistasis and analysis of expression of these three genes in mutant and wild-type backgrounds placed them in the functional hierarchy GI-CO-FT (Kardailsky et al., 1999; Kobayashi et al., 1999; Samach et al., 2000; Suarez-Lopez et al., 2001). GI, the earliest acting of these genes, encodes a protein of 1173 amino acids, which has no homology to proteins whose biochemical function is known (Fowler et al., 1999; Park et al., 1999). The Arabidopsis protein SPINDLY, an O-linked β-N-acetylglucosamine transferase implicated in gibberellin signaling, was shown to interact with GI in yeast, suggesting that the functions of these proteins might be related (Tseng et al., 2004). GI is highly conserved in seed plants, including monocotyledonous plants, such as rice (Oryza sativa) (Hayama et al., 2002), and gymnosperms, such as loblolly pine (Pinus taeda). By contrast, GI homologs appear to be absent from the genomes of the moss Physcomitrella, of Chlamydomonas, and of animals (Mittag et al., 2005). In onion epidermal cells, fusion proteins in which GI was fused to the marker proteins green fluorescent protein (GFP) and β-glucuronidase were localized to the nucleus (Huq et al., 2000). Furthermore, in gi mutants, the abundance of CO mRNA is reduced (Suarez-Lopez et al., 2001), suggesting that GI plays a role,
which might be direct or indirect, in promoting CO transcription. However, the biochemical function of GI protein is unknown.

The second gene, CO, encodes a nuclear zinc finger–containing protein (Putterill et al., 1995; Samach et al., 2000; Robson et al., 2001). The phase of circadian clock controlled CO expression within the day/night cycle is such that CO mRNA is expressed when plants are exposed to light under LDs but not under SDs (Suarez-Lopez et al., 2001; Yanovsky and Kay, 2002; Imaizumi et al., 2003), and exposure to light is required to activate CO protein function (Valverde et al., 2004). In response to light, CO is proposed to directly activate expression of FT (Kardailsky et al., 1999; Kobayashi et al., 1999; Samach et al., 2000; Valverde et al., 2003), which encodes a protein with homology to RAF-kinase inhibitor proteins of animals. FT strongly promotes flowering, but the biochemical function of this and related proteins in plants is not yet clear (Bradley et al., 1996; Pnueli et al., 1998, 2001; Kardailsky et al., 1999; Kobayashi et al., 1999). FT is the latest acting protein identified in this pathway and must somehow activate expression of genes involved in floral development at the apex of the plant (Schmid et al., 2003). Nevertheless, CO and FT are expressed in the phloem and will act there to promote flowering, suggesting that they may indirectly induce the floral transition at the apex (Takada and Goto, 2003; A et al., 2004).

CO and FT appear to be specific to flowering-time control, and mutations that impair the function of these proteins have no reported effect apart from delayed flowering. By contrast, gi mutants show several phenotypes. They are late flowering and exhibit reduced CO mRNA abundance (Suarez-Lopez et al., 2001), are altered in the period length of circadian rhythms (Park et al., 1999), impaired in phytochrome B (phyB) signaling in response to red light (Huq et al., 2000), are resistant to paraquat (Kurepa et al., 1998), and show increased accumulation of starch in the leaves during the photoperiod (Eimert et al., 1995). Whether there is a relationship between the role of GI in promoting flowering and the other processes that are impaired in the mutant is unclear. The circadian system (Somers et al., 1998b; McWatters et al., 2000; Covington et al., 2001; Mizoguchi et al., 2002), phytochrome signaling (Cerdan and Chory, 2003; Halliday et al., 2003), and sugar metabolism (Perilleux and Bernier, 2002) all have established roles in flowering-time control, suggesting that the effect of GI on flowering may be caused by its effect on one or all of these processes.

Because the circadian system has dramatic effects on flowering time, which it regulates through the CO and FT genes (Suarez-Lopez et al., 2001; Yanovsky and Kay, 2002), we studied possible connections between the role of GI in controlling circadian rhythms and its function in promoting flowering. GI is circadian clock regulated with a peak in mRNA abundance around 10 h after dawn (Fowler et al., 1999; Park et al., 1999; Hayama et al., 2002). The gi-1 and gi-2 mutations reduce the period length in circadian rhythms in leaf movements, and gi-1 causes a similar effect in expression of the CHLOROPHYLL A/B BINDING PROTEIN (CAB) gene, whereas gi-2 lengthens the period of the latter rhythm (Park et al., 1999). All gi alleles cause late flowering under LDs, in contrast with other mutations that cause short period rhythms, such as timing of cab expression1-1 (toc1-1) or late elongated hypocotyl-11 (hyl-11), which cause early flowering under SDs (Somers et al., 1998b; Mizoguchi et al., 2002). Some gi mutant alleles also cause a long hypocotyl phenotype in deetiolated seedlings, particularly under red light, indicating impaired phyB signaling (Huq et al., 2000), and this suggests that the basis of the circadian period phenotype is impaired input to the oscillator from phytochrome. Similarly, in gi-1 mutants, circadian period length does not respond to increasing light intensity as sensitively as that of wild-type plants (Park et al., 1999). Furthermore, in gi mutants, the amplitude of expression of the LHY and CIRCADIAN CLOCK ASSOCIATED1 (CCA1) mRNAs is reduced (Fowler et al., 1999; Park et al., 1999; Mizoguchi et al., 2002). The proteins encoded by these genes are MYB-like transcription factors that were proposed to act in a negative feedback loop with TOC1, thereby forming part of the oscillator of the central clock mechanism (Wang et al., 1997; Schaffer et al., 1998; Alabadi et al., 2001; Mizoguchi et al., 2002). LHY/CCA1 are proposed to repress TOC1 expression, and TOC1 in turn promotes LHY/CCA1 expression (Alabadi et al., 2001). The promotion of LHY/CCA1 expression by GI suggests that GI might also play a role in such a feedback mechanism (Mizoguchi et al., 2002).

Here, we describe a genetic and molecular analysis of GI function in transgenic plants overexpressing GI from the 35S promoter and in early flowering hyl-11 cca1-1 plants in which GI is misexpressed due to impaired control of circadian and diurnal rhythms. We conclude that GI has at least two distinct functions: a general effect on circadian rhythms and a role as an activator of output pathways that promote flowering, including one that acts through CO and FT.

RESULTS

GI Is Required for Early Flowering of hyl-11 cca1-1 Double Mutants

The hyl-11 cca1-1 double mutant is impaired in circadian clock function under free-running conditions, flowers extremely early under SDs, and exhibits a shift in the phase of expression of circadian clock–regulated genes under diurnal cycles of light and dark (Mizoguchi et al., 2002). To test whether the photoperiodic flowering pathway is required for early flowering of hyl-11 cca1-1, mutations that impair the pathway and thereby cause late flowering were introduced into the double mutant. The flowering times of the triple mutants gi-3 hyl-11 cca1-1, co-2 hyl-11 cca1-1, and ft-1 hyl-11 cca1-1 were scored under both LDs and SDs (Figure 1A). Under SDs, the gi-3 hyl-11 cca1-1 triple mutant flowered with a similar number of leaves to the wild type and gi-3 mutant controls and produced >30 leaves more than the hyl-11 cca1-1 double mutant. By contrast, the co-2 hyl-11 cca1-1 and ft-1 hyl-11 cca1-1 triple mutants flowered under SDs with only ~10 leaves more than hyl-11 cca1-1 double mutants and ~20 leaves fewer than the wild-type control (Figure 1A). The effect of gi-3 on the early-flowering phenotype of hyl-11 cca1-1 was also more severe than the effect of co-2 and ft-1 under LDs (Figure 1A).

With respect to flowering time, gi-3 is therefore epistatic to hyl-11 cca1-1, indicating that the extreme early flowering of hyl-11 cca1-1 double mutants is almost completely dependent on GI activity, particularly under SDs. Genes that act later in the photoperiod pathway, such as CO and FT, are required to a lesser extent for this phenotype.
The Expression Patterns of GI, CO, and FT Are Altered in the lhy-11 cca1-1 Double Mutant

In wild-type plants grown under SDs, the CO mRNA only accumulates during the night, and FT is not expressed under these conditions. A peak in the abundance of CO mRNA at an earlier phase under SDs in lhy-11 cca1-1 mutants may in part be responsible for the early flowering of these plants, as was shown for toc1-1 mutants (Blázquez et al., 2002; Yanovsky and Kay, 2002). To test whether CO mRNA is expressed at an earlier phase in lhy-11 cca1-1 double mutant plants than in the wild type, RNA was extracted at intervals of 4 h for 24 h from plants growing under SDs of 10 h light, and CO mRNA abundance was analyzed...
by RT-PCR (Figure 2B). In wild-type plants, CO mRNA abundance rose 8 to 12 h after dawn and continued into the dark period with high expression also occurring 20 h after dawn. By contrast, CO mRNA abundance rose earlier in lhy-11 cca1-1 plants, rising sharply between 4 and 8 h after dawn with a second peak in expression 20 h after dawn.

CO is proposed to directly activate FT expression in a light-dependent manner (Suarez-Lopez et al., 2001; Yanovsky and Kay, 2002; Valverde et al., 2004); therefore, whether the altered pattern of CO expression in lhy-11 cca1-1 double mutants correlated with earlier expression of FT under these conditions was also tested. In the double mutant plants, the abundance of

Figure 2. Abundance of the mRNAs of Flowering Time and Circadian Clock–Regulated Genes in lhy-11 cca1-1 Plants Grown under SDs.

The expression of the GI (A), CO (B), FT (C), CCR2 (D), and FKFI (E) genes was analyzed by RT-PCR in lhy-11 cca1-1, gi-3 lhy-11 cca1-1, gi-3, or Ler plants grown in SDs. Results are presented as a proportion of the highest value after standardization with respect to TUBULIN2 levels (TUB). Open and closed bars along the horizontal axis represent light and dark periods, respectively; these are measured in hours from dawn (zeitgeber time [ZT]). Each experiment was done at least twice with similar results.
Expression of GI from the Cauliflower mosaic virus 35S Promoter Causes Early Flowering

Analysis of lhy-1 lhy-1 cca1-1 double mutants suggested that expression of GI early during the light phase caused early flowering under SDs by activation of expression of CO and FT. However, many circadian clock-regulated genes are expressed at an earlier phase in plants impaired in LHY and CCA1 function (Alabadi et al., 2002; Mizoguchi et al., 2002). To assess whether misexpression of GI was sufficient to induce early flowering, a fusion of GI to the viral Cauliflower mosaic virus 35S promoter was constructed and introduced into both gi-3 mutant and wild-type plants. In 35S:GI plants, the GI mRNA was present throughout the daily cycle. At each time point, GI mRNA was more abundant in 35S:GI than in wild-type plants (Figure 3A). Transgenic wild-type plants or gi-3 mutants carrying 35S:GI flowered earlier than control wild-type plants (Figure 1B; data not shown for gi-3). This difference was most pronounced under SDs, where 35S:GI plants flowered with 35 fewer leaves than the wild type. These data indicate that constant overexpression of GI is sufficient to promote early flowering, even under noninductive SD conditions.

In addition to the lhy-1 lhy-1 cca1-1 double mutant, lhy-1, a dominant gain of function allele of LHY, was described as impaired in photoperiodic flowering and showed reduced GI expression under LDs (Schaffer et al., 1998; Fowler et al., 1999). Flowering time of the lhy-1 mutant was measured under LDs and SDs (Figure 1B). The mutant appeared almost day-neutral, flowering later than the wild type under LDs and earlier than the wild type under SDs. 35S:GI promotes early flowering in the lhy-1 mutant (Figure 1B), consistent with GI playing a major role in the promotion of flowering downstream of LHY. 35S:GI lhy-1 plants were also earlier flowering than lhy-1 under SDs and under these conditions slightly earlier flowering than 35S:GI, suggesting that a part of the flowering phenotype of lhy-1 is additive to the effect of 35S:GI.

Extreme Early Flowering of 35S:GI Plants Requires CO and FT

Late-flowering gi mutants contain lower levels of CO mRNA (Suarez-Lopez et al., 2001), suggesting that early flowering 35S:GI plants might show increased expression of CO mRNA that in turn caused increased abundance of FT mRNA. The effect of 35S:GI on the abundance of CO and FT mRNA was therefore measured in 35S:GI plants grown under SDs (Figures 3B and 3C). FT mRNA was present at high abundance 4, 8, and 12 h after dawn, suggesting that 35S:GI was sufficient to induce FT expression under SDs, where no FT expression occurs in wild-type plants. Similarly, CO mRNA abundance was higher in 35S:GI than in wild-type plants, and this effect was most pronounced early in the day when in wild-type plants CO mRNA abundance is at trough levels (Figure 3B). Previously, FKF1 was proposed to activate the expression of CO mRNA (Imaizumi et al., 2003). However, FKF1 mRNA levels were not affected by 35S:GI (Figure 3D), indicating that GI does not regulate CO mRNA abundance through the regulation of FKF1 mRNA. These data suggest that constant overexpression of GI from the Cauliflower mosaic virus 35S promoter leads to increased CO mRNA.
expression during the photoperiod in SDs and that CO then activates expression of FT.

To test whether CO and FT are required for early flowering of 35S:GI plants, mutations in these genes were introduced into the 35S:GI background. The resulting lines were scored for flowering time under LDs and SDs. Under both day lengths, the co-2 35S:GI and ft-1 35S:GI plants flowered at a time intermediate between the original mutant and 35S:GI (Figure 1B). Especially under SDs, the early flowering caused by 35S:GI was still apparent in the mutant lines, which flowered significantly earlier than wild-type plants (Figure 1B). This suggests that the extreme early flowering of 35S:GI under SDs requires functional CO and FT genes but that GI can also promote flowering independently of these genes.

35S:GI Delays the Phase of Circadian Clock–Controlled Gene Expression and Shortens Circadian Period under Continuous Light

35S:GI plants flower early under SDs and show increased abundance of the CO and FT mRNAs (Figures 1B, 3B, and 3C). Whether a similar effect is observed on the patterns of expression of other circadian clock–regulated genes was tested in SD-grown plants (Figures 4A and 4B). A CCR2:LUCIFERASE (CCR2:LUC) transgene was introduced into 35S:GI and gi-3 backgrounds, and five transformants in each genetic background were isolated. Luminescence of CCR2:LUC plants showed a strong diurnal rhythm, which was followed for 72 h under SDs (Figure 4B). Under these conditions, luminescence of gi-3 CCR2:LUC and 35S:GI CCR2:LUC peaked 11.6 and 12.5 h after dawn, respectively (Figure 4B). Similarly, the abundance of the LHY mRNA appeared to fall to trough levels more slowly in the morning and to rise in expression later in the evening, which is also consistent with a delayed phase of expression (Figure 4A). These experiments indicate that under SDs, the phase of expression of circadian clock–regulated genes is delayed by 35S:GI and gi-3. However, these effects are much less dramatic than those observed on the amplitude of diurnal rhythms in expression of mRNAs of the flowering-time genes CO and FT (Figures 3B and 3C) and do not correlate with flowering.
Figure 4. Circadian Clock–Regulated Gene Expression in 35S:GI and gi-3 Plants under SDs, LL, or DD.
time because gi-3 and 35S:GI had similar effects on phase under SDs but opposite effects on flowering time (Figures 1 and 4B).

Mutations in GI generally shorten free-running rhythms in gene expression under continuous light (LL), reduce the amplitude of expression of the LHY and CCA1 genes, and cause late flowering (Fowler et al., 1999; Park et al., 1999; Mizoguchi et al., 2002). The early-flowering phenotype of toc1-1 mutants can be explained by their short-period phenotype (Yanovsky and Kay, 2002). Therefore free-running rhythms in 35S:GI plants were tested to assess whether alteration of these rhythms might underlie the early-flowering phenotype. 35S:GI, gi-3 mutant, and Landsberg erecta (Ler) plants carrying the CCR2::LUC transgene were entrained to LD cycles of 16 h light/8 h dark and shifted to LL, and their luminescence was measured for 120 h (Figure 4D). Almost all plants of each genotype were rhythmic throughout the experiment (Figure 4E), although the rhythms dampened more rapidly in gi-3 and 35S:GI than in Ler (Figure 4D). In 35S:GI, the phase of the first peak that occurs after ZT 24 h in LL, and therefore the peak after the first subjective night, was more severely delayed compared with the wild type under entraining conditions, indicating that the phase of the circadian rhythm in CCR2::LUC expression is delayed in 35S:GI. By contrast, in gi-3 mutants, the first peak in LL occurred earlier than that of the wild type, which is probably due to the shorter period of gi-3 mutants under free-running conditions (see below).

The period lengths of the rhythms under LL were more variable for gi-3 and 35S:GI, but mean period length was shorter for both genotypes (Figures 4D and 4E). Therefore, under LL, both gi-3 and 35S:GI mutants exhibit a shorter period length than is observed in Ler, and 35S:GI causes a significant delay in phase.

The pattern of expression of the mRNA of LHY was also analyzed at 4-h intervals for 80 h under LL in Ler, gi-3, and 35S:GI plants. The amplitude of the circadian rhythm in expression of LHY mRNA was delayed compared with that in LHY plants, whereas in 35S:GI, LHY mRNA showed a robust rhythm, and no increase in amplitude was observed despite the reduced amplitude observed in gi-3. A period length could not be extracted from these data with certainty using fast Fourier transform-nonlinear least squares (FFT-NLLS). However, visual inspection of the pattern of LHY mRNA abundance suggested that the effect of 35S:GI on phase was similar to that observed with CCR2::LUC because, for example, at 72 to 80 h in LL, the timing of the peak in LHY mRNA was delayed compared with that in Ler.

Taken together, these experiments indicate that 35S:GI and gi-3 mutations shorten circadian period and that 35S:GI delays circadian phase, as measured accurately for CCR2::LUC, but that 35S:GI, unlike gi-3, does not influence the amplitude of LHY expression.

Effects of 35S:GI and gi-3 on Circadian Regulation of CCR2 in Continuous Darkness

The effect of GI mutations on circadian rhythms may be partly a consequence of impaired light input to the oscillator (Park et al., 1999). To follow the effect of GI overexpression and of impaired GI function on circadian rhythms in continuous dark (DD), the luminescence of 35S:GI, gi-3, and Ler plants carrying CCR2::LUC was compared for 4 d in DD after entrainment in LDs (Figure 4F). In contrast with wild-type plants, in gi-3 and 35S:GI plants, rhythms were of lower amplitude and the period length of the rhythms varied more widely between individuals (Figures 4F and 4G). CCR2::LUC appeared arrhythmic in both gi-3 and 35S:GI after the first 30 h in DD (Figure 4E). Therefore, in DD, GI is required to maintain at least a subset of circadian rhythms represented by CCR2::LUC, suggesting that the effect of GI in the circadian system is not only in light input.

The Effects of GI and lhy-11 cca1-1 on Seedling Deetiolation

Mutations in GI were previously shown to impair phyB signaling during seedling deetiolation in red light (Huq et al., 2000), and because phyB also regulates flowering time and circadian clock entrainment, the effects of 35S:GI and lhy-11 cca1-1 on hypocotyl length were compared in a similar way as shown above for flowering time (Figure 5). To test whether the expression of GI is limiting for red light responsiveness, the hypocotyls of 35S:GI

Figure 4. (continued).

(A) and (C) LHY and CCR2::LUC expression was analyzed in gi-3, 35S:GI-A, 35S:GI-B, or Ler plants. The expression of the LHY gene was analyzed by RNA gel blotting of RNA isolated from plants grown under SDs (8 h light/16 h dark) (A) or LL (C). Results are presented as a proportion of the highest value after standardization with respect to TUB levels. Numbers on the horizontal axis represent the time in hours after dawn (ZT) in SD (A) and at the start of the LL treatment (C). Open and closed boxes on the horizontal axis indicate light and dark, respectively (A), and subjective day and subjective night, respectively (C).

(B), (D), and (F) The expression of the CCR2 gene was followed by the luminescence of transgenic plants carrying the CCR2::LUC transgene and grown under SDs (8 h light/16 h dark) (B), LL (D), or DD (F). The results are presented as normalized luminescence. Data are the means ± SE of the luminescence of ~20 individual seedlings. Error bars are shown every fifth data point for clarity. Five independently transformed wild-type and mutant lines were analyzed under LL and DD with similar results, and under SDs two transformants were analyzed. Numbers on the horizontal axis represent the time in hours after dawn (ZT) in SD (B), after the start of the LL treatment (D), and in hours in darkness in DD treatment (F). Open and closed boxes on the horizontal axis indicate light and dark, respectively (B), subjective day and subjective night, respectively (D), and light and dark boxes on horizontal axis represent subjective day and subjective night, respectively (F).

(E) and (G) Plots showing the FFT-NLLS analysis of the CCR2::LUC data plotted in (D) and (F), respectively. A strong circadian expression of CCR2::LUC is reflected by the clustering of data points with low relative amplitude error values, which indicate robust rhythms. Scattered data points with relative amplitude error values closer to 1 indicate weaker rhythms. All plants in (D) were rhythmic, whereas in (F), more wild-type Ler seedlings were rhythmic (15/18) than gi-3 (12/19) and 35S:GI-B (11/20) seedlings.

Each experiment was done at least twice with similar results.
Figure 5. The hypocotyl length of *lhy-11 cca1-1* and 35S:GI plants under different intensities of red light and under SDs.
plants were measured under different intensities of red light (Figure 5A). The hypocotyls of 35S:GI plants were shorter in red light than those of wild-type plants (P < 0.05). The difference was most pronounced at ~10 and 1 μmol m$^{-2}$ s$^{-1}$. These results indicate that 35S:GI plants are hypersensitive to red light and, therefore, that the expression of GI is limiting on red light responses, particularly at high intensities.

The early flowering of lhy-11 cca1-1 double mutants may be explained by misexpression of GI (Figure 2A); therefore, the mechanism of early flowering is closely related to that of 35S:GI plants. Previously, lhy cca1 mutants were shown to have short hypocotyls under high intensity red light (Hall et al., 2003; Mas et al., 2003). To determine whether this effect requires GI and to compare the phenotype with that of 35S:GI, fluence response curves for hypocotyl elongation of lhy-11 cca1-1 and gi-3 lhy-11 cca1-1 seedlings in red light were made (Figure 5B). Under these conditions, the hypocotyls of lhy-11 cca1-1 plants, which in contrast with the previously studied lhy cca1 lines are in the Ler background, were shorter than the wild type at ~10 μmol m$^{-2}$ s$^{-1}$, but overall, no significant difference in hypocotyl length between Ler and lhy-11 cca1-1 was found under red light. The gi-3 mutant exhibited a longer hypocotyl than Ler (P < 0.05), whereas the hypocotyls of gi-3 lhy-11 cca1-1 plants were shorter than gi-3 and slightly longer than lhy-11 cca1-1 (P < 0.05). The intermediate phenotype of the triple mutant suggests a complex interaction between gi-3 and lhy-11 cca1-1 in regulating hypocotyl length under red light.

Hypocotyl lengths were also measured under SDs (Figure 5C). Under this condition, the hypocotyls of lhy-11 cca1-1 plants were significantly shorter than those of the wild type. By contrast, the gi-3 mutant exhibited a longer hypocotyl than wild-type plants. Under SDs, the hypocotyls of gi-3 lhy-11 cca1-1 triple mutants were a similar length to those of lhy cca1-1 mutants. The extreme short-hypocotyl phenotype of lhy-11 cca1-1 double mutants under SDs does not therefore depend on GI activity, and genes that act later in or independent of the photoperiod pathway, such as CO, FT, and FCA, are also not required for the shorter hypocotyl phenotype (data not shown).

Taken together, our analysis indicates that in wild-type plants GI levels are limiting on seedling deetiolation under red light and SDs. In addition, in contrast with its early-flowering phenotype, the short-hypocotyl phenotype of lhy-11 cca1-1 under SDs does not require GI.

GI:GFP Promotes Flowering and Is Located in Nuclei in Leaves and Hypocotyls of Arabidopsis

The nuclear location of GI protein was previously demonstrated using GUS:GI and GFP:GI fusion proteins in transient assays performed in onion epidermal cells (Huq et al., 2000). To test this in Arabidopsis and to determine the functionality of the fusion protein, 35S:GI:GFP and 35S:GFP:GI transgenes were made and introduced into gi-3 mutants. Transgenic plants containing 35S:GFP:GI were late flowering and not obviously earlier flowering than the gi-3 progenitor. By contrast, the gi-3 mutants containing 35S:GI:GFP flowered early and at a similar time to 35S:GI plants, indicating that the GI:GFP fusion protein was functional and promoted flowering.

Confocal microscopy was used to analyze the cellular location of GI:GFP protein in the transgenic plants (Figure 6). In epidermal cells of the hypocotyl and leaf stomatal guard cells, the fusion protein was only detected in nuclei. This demonstrates that GI:GFP, which is functionally active in promoting flowering, is localized to the nucleus, strongly suggesting that GI acts in the nucleus to control flowering time.
DISCUSSION

The phenotype of gi mutants suggested that GI plays important roles in red light signaling, regulation of circadian rhythms, flowering-time control, and starch accumulation in the leaves. However, it is unclear whether these effects are interrelated or represent independent functions of GI. We studied the relationship between the roles of GI in controlling circadian rhythms and promoting flowering. 35S:GI and gi-3 altered circadian rhythms under DD as well as LL, demonstrating that the effects of GI on the circadian system are not only due to its role in light signaling. Furthermore, under diurnal day/night cycles, 35S:GI delayed the phase of expression of circadian clock-controlled genes CCR2 and LHY, whereas gi-3 delayed the phase of CCR2 and reduced the amplitude of LHY expression. By contrast, 35S:GI and gi-3 cause early and late flowering, respectively, and their effects on the timing and amplitude of expression of the flowering-time genes CO and FT are much more dramatic than on the expression of other clock-controlled genes. We propose therefore that GI plays a significant role in controlling at least a subset of circadian rhythms in light and dark with an effect on phase in diurnal cycles but that its effect on flowering is distinct from its function in regulating these circadian rhythms. In the regulation of flowering, GI is proposed to act downstream of the putative clock components LHY/CCA1 to promote the expression of CO and FT and probably other flowering-time genes (Figure 7).

Role of GI in Promoting Flowering

The circadian clock regulates flowering through an output pathway that includes CO. Abundance of CO mRNA is reduced in gi mutants, and the 35S:CO transgene suppresses the late flowering of gi mutants (Suarez-Lopez et al., 2001). We found that the gi-3 mutation was epistatic to the early-flowering phenotype caused by lhy-11 cca1-1 under SDs and reduced CO expression in this background. This supports the idea that GI triggers flowering by acting between the oscillator, which involves LHY/CCA1, and CO. The epistasis of gi to the flowering phenotype of lhy-11 cca1-1 suggests that GI is essential for circadian clock-controlled flowering. Also, 35S:GI plants show a severe early-flowering phenotype and enhanced expression of CO and FT, demonstrating that GI expression is limiting on flowering-time gene expression and that its misexpression is sufficient to promote early flowering. The effect of 35S:GI and lhy-11 cca1-1 on flowering was partially suppressed by co-2 and ft-1.

Figure 7. Dual Role for GI in Regulating Circadian Rhythms and Flowering Time.

The central oscillator of the Arabidopsis circadian clock was proposed to consist of a negative feedback loop comprising LHY/CCA1 and TOC1 (Alabadi et al., 2002). Within this loop, TOC1 acts in the evening to promote expression of LHY/CCA1 in the morning, and LHY/CCA1 repress TOC1 expression. LHY and CCA1 are also shown as negative regulators of GI based on the earlier phase of GI expression detected in a lhy-11 cca1-1 double mutant (Mizoguchi et al., 2002); however, overexpression of CCA1 causes an increase in GI expression, which may suggest a more complex pattern of regulation (Fowler et al., 1999). GI may play a role in the evening related to that of TOC1 because it is also required for high amplitude expression of LHY/CCA1, is expressed in a similar phase as TOC1, and both gi mutations as well as 35S:GI have effects on circadian phase and period length. In the control of flowering time, GI increases the amplitude of CO and FT expression, which are both increased by 35S:GI and decreased by gi mutations. In addition, 35S:GI and gi mutations have opposite effects on flowering time. GI is therefore proposed to play dual roles acting within the circadian clock to regulate period length and circadian phase, while also more directly promoting expression of a circadian clock output pathway that includes CO and FT and promotes flowering. The effect of GI on flowering probably includes another pathway, indicated with an X, because co mutations only partially suppress the early flowering caused by 35S:GI or lhy cca1. FT activates SOC1 downstream of CO (Schmid et al., 2003; Michaels et al., 2005). In the diagram, the square illustrates the circadian oscillator that generates circadian rhythms, white illustrates daytime, and gray shading illustrates nighttime. The flowering pathway is one of many output pathways controlled by the circadian clock, and three other pathways expressed at different times of the day are illustrated. The genes shown in gray on the right-hand side of the figure are those that promote flowering in response to LDs and delay flowering when inactivated.
mutations, supporting the idea that the mechanism by which GI promotes early flowering includes CO and its target gene FT. Therefore, our data support a functional hierarchy of GI-CO-FT in the flowering output pathway from the oscillator, and this also reflects the sequence with which these genes are expressed during the daily cycle.

Early flowering of the toc1-1 mutant under SDs is caused by expression of CO at an earlier phase, allowing activation of CO by exposure to light under these conditions (Blázquez et al., 2002; Yanovsky and Kay, 2002). This may also explain the early flowering of lhy-11 cca1-1 double mutants. Consistent with this model, in lhy-11 cca1-1 double mutants, CO was expressed at an earlier phase under SDs, and this correlated with expression of the CO target gene FT (Figure 2). GI expression was also shifted to an earlier phase in lhy-11 cca1-1 double mutants under SDs and was required for the expression of CO. This suggests that the phase shift in GI expression may be the primary cause of early flowering under SDs in lhy-11 cca1-1 plants. This would be slightly different to early flowering toc1-1 mutants, in which CO but not GI was expressed at an earlier phase under SDs (Somers et al., 1998b; Yanovsky and Kay, 2002). Furthermore, although 35S:GI plants flower early, circadian phase is delayed, indicating that the early flowering of 35S:GI plants could be a more direct effect of early flowering of GI activating CO expression and not an indirect effect of altering circadian clock regulation. By contrast, GI probably affects LHY/CCA1 mRNA abundance indirectly because although the amplitude of LHY/CCA1 expression is reduced in gi mutants, it is not increased in 35S:GI plants, and LHY is expressed in an earlier phase than GI.

GI also appears to promote flowering by a second mechanism that is independent of CO and FT (Figure 1B). The delay in flowering of lhy-11 cca1-1 caused by co-2 and ft-1 was weaker than that caused by gi-3, suggesting that as well as promoting flowering by activating CO and FT, GI promotes flowering independently of these genes (Figure 1A). Similarly, co-2 and ft-1 only partially suppressed the early flowering of 35S:GI plants. The late-flowering phenotype of co-2 is similar to that of co-8 mutants, which carry a large deletion within the CO gene (Robson et al., 2001), and co-8 had a similar effect on flowering time of lhy-12 cca1-1 to co-2. This suggests the importance of CO function only partially suppresses the early flowering phenotype of lhy-12 cca1-1. The second mechanism by which GI promotes flowering could involve SOC1. However, SOC1 has an established role in promoting flowering downstream of CO (Bomer et al., 2000; Lee et al., 2000; Onouchi et al., 2000; Samach et al., 2000). Therefore, we propose that in wild-type plants, GI regulates at least two circadian clock–controlled output pathways that promote flowering, one that includes CO, FT, and SOC1 and a second that promotes flowering independently of these genes (Figure 7).

Apart from GI, the only proteins shown to promote CO expression are FKF1 and phyA (Tepperman et al., 2001; Imaizumi et al., 2003). The FKF1 and GI genes are expressed in a similar phase (Nelson et al., 2000). However, we found that GI is not required to activate FKF1 expression, and in 35S:GI plants, FKF1 mRNA expression is not increased (Figures 2E, 2F, 3D, and 3E). Therefore, GI does not promote CO expression and flowering by activating FKF1 transcription. The role of GI in activation of CO is probably conserved in rice because overexpression of the rice ortholog of GI (OsGI) was shown to increase the expression of the rice ortholog of CO (HEADING DATE 1) (Hayama et al., 2003), but its effect on circadian rhythms was not tested. Although the involvement of GI in flowering-time control and in the activation of CO expression is likely to be widely conserved and GI protein is present in the nucleus, the mechanism by which it regulates CO mRNA abundance is unclear.

Role of GI in Circadian Clock Function

GI regulates the period length of circadian rhythms in expression of genes that are not involved in flowering control. Mutations in GI generally shorten circadian period, although the gi-2 allele lengthens the period of CAB:LUC expression (Park et al., 1999). We supported these observations by demonstrating that under LL the gi-3 mutation and 35S:GI shorten circadian period of CCR2:LUC (Figure 4). The effect of GI on circadian rhythms in DD has not been extensively studied and was based on RNA analysis of GI and LHY expression in the gi-1 and gi-2 mutants (Park et al., 1999). However, these genes are not ideal markers for the effect of GI on rhythms in DD because LHY expression dampens rapidly in gi mutant backgrounds (Park et al., 1999; Mizoguchi et al., 2002), and GI expression can also be reduced by some gi alleles (Fowler et al., 1999). We therefore extended these data by following expression in gi-3 and 35S:GI backgrounds of a CCR2:LUC transgene, which shows robust circadian rhythms in wild-type plants under DD (Doyle et al., 2002; Mas et al., 2003). The CCR2 gene is not involved in flowering and is not part of the same circadian output pathway as CO and FT. Under DD, circadian rhythm in CCR2:LUC expression damped rapidly in gi-3 and 35S:GI plants, and these appeared arrhythmic after 30 h in DD. The altered rhythms in gi-3 mutants and 35S:GI plants indicate that the effect of GI on circadian rhythms is not limited to input from light signaling (Figure 4E). Indeed, the effect of 35S:GI and gi-3 is stronger under DD than under LL. This observation indicates that in addition to promoting expression of the output pathway controlling flowering through CO and FT, GI plays a general role in controlling circadian rhythms under LL and DD.

The TOC1 and EARLY FLOWERING4 (ELF4) genes, which are circadian clock regulated and expressed in a similar phase to GI, are also involved in the regulation of circadian rhythms in DD (Doyle et al., 2002; Mas et al., 2003). In plants overexpressing TOC1 (35S:TOC1), circadian rhythms are strongly impaired in LL and in DD. In 35S:TOC1 plants grown in LL, circadian rhythms in CAB2, GI, and CCR2 expression were undetectable, whereas LHY and CCA1 mRNAs showed delayed and lower amplitude rhythms (Makino et al., 2002; Mas et al., 2003). In plants with strongly reduced TOC1 expression, rhythms in CCR2:LUC expression were abolished under DD and showed a severe short period phenotype under LL (Mas et al., 2003). Similarly, mutations in ELF4 strongly suppressed rhythms under LL or DD (Doyle et al., 2002). The strong effects observed by loss of TOC1 and ELF4 function indicate that these genes are essential for circadian rhythms under DD and that there is no redundancy in their biochemical function. Nevertheless, GI may play a related role in the circadian system to these genes because 35S:GI and gi-3
Role of GI in Light Signaling

GI was previously implicated in phyB signaling during seedling deetiolation in red light (Huq et al., 2000), and because phyB is also involved in circadian clock entrainment and the control of flowering time (Somers et al., 1998a; Cerdan and Chory, 2003), this could provide a basis for several of the phenotypes associated with GI. Our observation that 3SS:GI seedlings show shorter hypocotyls than wild-type seedlings under red light also indicates that GI expression is limiting on red light signaling (Figure 5B). However, we propose that this represents a function of GI that is largely independent of its role in circadian clock function or flowering-time control because phyB does not reduce CO transcription (Cerdan and Chory, 2003), as was shown for gi-3, and although an impairment of red light signaling might contribute to the effect of 3SS:GI on clock regulation in the light, it would not explain the effect of 3SS:GI on clock regulation in the dark.

Seedlings of lhy-11 cca1-1 double mutants show a short hypocotyl during deetiolation under red light or after growth in SDs (Mas et al., 2003), and this may be due to impaired circadian clock function because hypocotyl growth in Arabidopsis is circadian clock regulated (Dowson-Day and Millar, 1999). To test whether misexpression of GI is responsible for this phenotype, as described above for the early flowering of lhy-11 cca1-1, the hypocotyls of gi-3 lhy-11 cca1-1, lhy-11 cca1-1, and Ler plants were measured under four intensities of red light and under SDs (Figures 5B and 5C). Under red light, a complex interaction between gi-3 and lhy-11 cca1-1 was observed. However, under SDs, lhy-11 cca1-1 was epistatic to gi-3 with respect to hypocotyl elongation. This relationship was in contrast with the effect on flowering time, in which gi-3 was epistatic to the early-flowering phenotype caused by lhy-11 cca1-1. Therefore, GI and LHY/CCA1 interact differently in controlling hypocotyl length than in the regulation of flowering.

Conclusion

The biochemical function of GI is unknown, but we propose that at least some of the pleiotropic phenotypes of gi mutants represent separable roles for the protein in distinct processes and not simply indirect effects of impairing a single process. For example, the effect of GI on flowering time is not a secondary consequence of its general role in controlling circadian rhythms but is more specifically associated with promoting the expression of genes in circadian output pathways that control flowering. One of these pathways contains the CO and FT genes, and there is genetic evidence for a second pathway based on the incomplete suppression of 3SS:GI by the co-2 or ft-1 mutations. Other phenotypic effects of the gi mutation, such as in red light signaling and starch accumulation, may represent further independent functions of the protein. Describing the biochemical function(s) of nuclear GI protein will be necessary to more clearly understand its roles in diverse processes.

METHODS

Plant Material and Growth Conditions

The Arabidopsis thaliana Ler ecotype was the wild type. The co-2, gi-3, and ft-1 mutants were kindly provided by M. Koornneef. The lhy-1 (Schaffer et al., 1998) and lhy-11 cca1-1 (Mizoguchi et al., 2002) mutants and 3SS:CO transgenic plants (Onouchi et al., 2000) were described previously. The CCR2:LUC transgenic plants were generated by introduction of the CCR2:LUC transgene (described previously in Doyle et al., 2002 and kindly provided by S. Davis) into different genotypes by Agrobacterium tumefaciens–mediated transformation. The 3SS:GI, 3SS:GI:GFP, and 3SS:GI:C transgenic plants in the gi-3 and Ler genotypes were generated by Agrobacterium-mediated transformation of constructs containing the GI cDNA (Fowler et al., 1999) linked to the 3SS promoter, with or without translational fusions to the GFP coding region from the pAVA393 vector (von Arnim et al., 1998). Two independent transgenic lines, 3SS:GI-A and/or 3SS:GI-B, that were homozygous for single copy insertions of the transgene T-DNAs in Ler were used for the experiments presented here. Plants were grown on soil in controlled environment rooms at 22°C under either LDs (10 h light/14 h dark) or SDs (10 h light/14 h dark) as described (Mizoguchi et al., 2002), unless specified otherwise.

Measurement of Flowering Time

Flowering time was scored by growing plants on soil in LD and SD and counting the number of rosette and cauline leaves on the main stem. Data are presented as mean ± se (n = 8 to 18). Measurement of flowering time was done at least twice with similar results.

Construction and Analysis of Double and Triple Mutants

Double and triple mutants were usually made by crossing lines homozygous for each mutation. Further information on the construction of double and triple mutants can be obtained from the authors.

RT-PCR and RNA Gel Blot Analysis of Gene Expression

For LL and DD experiments, plants were grown on GM agar plates with sucrose at 22°C under LDs (16 h light/8 h dark) for 8 d, then transferred to LL or DD at dawn, and whole plants were used for RNA preparation as described (Mizoguchi et al., 2002). For SD experiments, plants were grown on soil for 10 d, and aerial parts were used for RNA preparation. RNA gel blot analysis was performed as described by Schaffer et al. (1998). RT-PCR was performed with 1 μg of total RNA using a SuperScript first-strand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA). cDNAs were diluted to 100 μl with TE buffer, and 1 μl of diluted cDNA was used for PCR amplification by TaKaRa Ex Taq (TaKaRa, Shiga, Japan). For RT-PCR expression studies, the following primers were used: GI, 5'-TACCATGCTGAACTGGAACAACCTTTGGCAATG-3' and 5'-AAATGTATGCGTTATGGTTAATGG-3', CO, 5'-AGGCACATCGAGGTTCC-3' and 5'-AAATGTATGCGTTATGGTTAATGG-3' (Suarez-Lopez et al., 2001); FT, 5'-ACAACCTGGAACAAACTTTGGCAATG-3' and 5'-ACTATA-TAGGGACTCATCACGCGTTGTTGCG-3' (Blazquez and Weigel, 1999); CCR2, 5'-CTCTTTGAGTCTGGATTTG-3' and 5'-AGAACCTTTGGTAAATCC-3' (Staiger et al., 2003); FKF1, 5'-GTGGTTACGGTGGTGAATTTG-3' and 5'-CTGTCTTTCTCCGGTTTACTG-3' (this work); TUB, 5'-TATGCAAAGCTTCTCTTTCAAGATG-3' and 5'-TACCATGCTGAACTGGAACAACCTTTGGCAATG-3' (this work); TUB, 5'-CTCAAGGAGGTTCCATGTTGCG-3' and 5'-CTCATGCTGAACTGGAACAACCTTTGGCAATG-3' (Kobayashi et al., 1999). Numbers of PCR cycles were as follows: 20 cycles for GI and FKF1, 25 cycles for CO and FT, 15 cycles for CCR2, and 18 cycles for TUB. Annealing temperature was 55°C for GI, 57°C for FT and CCR2, 60°C for CO and TUB, and 62°C for FKF1. Primer specificity was verified by sequencing the PCR products. The PCR
products were separated on 1.5% agarose gels and transferred to Biodyne B membranes (Nippon Genetics, Tokyo, Japan). The RT-PCR products were cloned by pGEM-T Easy Vector System I (Promega, Madison, WI), and plasmids were extracted to be templates for PCR to amplify probe DNA. The membranes were hybridized with radioactive probe DNAs in hybridization solution that contained 5 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate), 0.1% SDS, 0.1% sarcosyl, 0.75% Blocking reagent (Boehringer Mannheim, Mannheim, Germany), and 5% dextran sulfate sodium salt at 65°C for 16 h. The blot was washed with 2 × SSC and 0.1% SDS for 20 min, then 0.5 × SSC and 0.1% SDS for 10 min at 65°C, and then the hybridization signal was visualized using the BioImaging Analyzer (BAS 5000; Fuji Photo Film, Tokyo, Japan); signal intensity was quantified with Science Lab 98 Image Gauge software (version 3.1; Fuji Photo Film). Values were represented relative to the highest value of the samples after standardization to the TBUB control. All the RT-PCR analysis was performed at least twice and usually with independent RNA samples.

Luminescence Measurement and Analysis of Circadian Period Length Using CCR2::LUC Transgenic Plants

T2 generation plants from independent CCR2::LUC transformant lines were used for the analysis of luminescence and period length. Seven-day-old plants grown on agar in SD and LD were transferred into agar-filled wells of 96-well opaque microtiter plates and treated with 20 μL 5 mM d-luciferin per plant (n = 24). From the next day, the luminescence of individual seedlings was measured by counting in a Packard Topcount (Packard, Meriden, CT). The average luminescence for each genotype at each time point was calculated from the luminescence normalized for each emitting individual. The period length of free running cycles was estimated from at least 96 h of luminescence measurements starting 24 h after transfer into LL or 12 h into DD using FFT-NLLS software (Plautz et al., 1997).

Analysis of Hypocotyl Length

In the red light fluence response experiments, seeds were placed on GM agar plates without sucrose, kept 5 d in the dark at 4°C, exposed to 6 h of white light then 18 h of darkness, and then seedlings were grown either in DD or in E30-LD cabinets (Perceval Scientific, Perry, Iowa) with red LED light (670 nm) filtered through layers of neutral density filters (Filter 299; Lee Filters, Andover, UK) to obtain different red light fluence levels. The hypocotyl length of each red light–grown seedling was divided by the mean hypocotyl length of the seedlings grown in DD, to avoid difficulties with uneven germination, as described (Fankhauser and Casal, 2004). The data presented are the mean ± SE of the pooled standardized measurements (n = 60) from three independent experiments. In the SD experiments, seeds were placed on agar plates in SD conditions (8 h light/16 h dark). Light was provided by Philips TL741 tubes (Eindhoven, The Netherlands) giving photosynthetically active radiation of 48 μmol m−2 s−1. Hypocotyl length was measured after 10 d, and the mean value ± SE was calculated (n = 80 for each genotype).

Effects of light intensity and genotype were analyzed using two-way analysis of variance as part of the Sigmastat 3.0 software (SPSS ASC, Erkrath, Germany).

Confocal Microscopy of GFP Fluorescence

The hypocotyls of 8-d-old transgenic 35S::GI/GFP gi-3 seedlings grown on GM agar plates under LD conditions were analyzed using a Zeiss LSM 510 Meta confocal laser scanning microscope (Jena, Germany). Confocal images were collected using the 63× oil-immersion lens. Excitation of fluorescence was at 488 nm with an argon laser, and the GFP fluorescence was detected at 500 to 530 nm and the autofluorescence of mainly chlorophyll from 602 to 709 nm. Simultaneously, transmission images were taken using the 543-nm HeNe laser. For emission spectral fingerprinting at 488 nm excitation (argon laser), lambda stacks were recorded with the Meta detector (Zeiss) between 494.7 and 591 nm in 10.7-nm steps. Emission spectra of GFP, of the green fluorescent chloroplast component(s), and of the background were measured in respective regions of the lambda stacks and selected for the linear unmixing calculation.

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REFERENCES


Distinct Roles of GIGANTEA in Promoting Flowering and Regulating Circadian Rhythms in Arabidopsis
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