# Flowering-Time Genes Modulate the Response to *LEAFY* Activity

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ABSTRACT

Among the genes that control the transition to flowering in Arabidopsis is a large group whose inactivation causes a delay in flowering. It has been difficult to establish different pathways in which the flowering-time genes might act, because mutants with lesions in these genes have very similar phenotypes. Among the putative targets of the flowering-time genes is another group of genes, which control the identity of individual meristems. Overexpression of one of the meristem-identity genes, *LEAFY*, can cause the precocious generation of flowers and thus early flowering. We have exploited the opposite phenotypes seen in late-flowering mutants and *LEAFY* overexpressers to clarify the genetic interactions between flowering-time genes and *LEAFY*. According to epistatic relationships, we can define one class of flowering-time genes that affects primarily the response to *LEAFY* activity, and another class of genes that affects primarily the transcriptional induction of *LEAFY*. These observations allow us to expand previously proposed models for the genetic control of flowering time.

In contrast to animals, plants continue to generate new organs during their postembryonic phase from pools of undifferentiated cells called meristems. The aerial portion of a plant is produced by the shoot meristem, which generates organs and structures of varying morphology and identity, dependent on the phase of the life cycle. These changes, which are collectively known as phase change (Poethig 1990), can either be gradual, such as the increase in trichome density seen on the lower surface of Arabidopsis leaves, or be precipitous, such as the ones that occur when an Arabidopsis plant makes the transition from vegetative to reproductive growth. Before this transition, the main shoot of Arabidopsis plants produces leaves, in whose axils side shoots (paraclades) later arise. After this transition, the main shoot produces instead flowers that are not subtended by leaves. A large number of genes that control the timing of the transition to flowering has been identified in Arabidopsis by mutant analysis (Peeters and Koornneef 1996). Interestingly, many of these mutants turned out to be also affected in other aspects of phase change, such as changes in leaf shape and trichome density, suggesting that few of the so-called flowering-time genes control only the transition to flowering (Telfer *et al.* 1997).

Unlike flowering-time genes, flower-meristem-identity genes have a more specific role in the initiation of flowers. Inactivation of these genes typically does not affect phase change in general, but causes the replacement of individual flowers with leaves and associated paraclades, or with structures that combine features of flowers and paraclades (reviewed by Yanofsky 1995). The two principal flower-meristem-identity genes in Arabidopsis are *LEAFY* (*LFY*) and *APETALA1* (*AP1*), both of which are expressed in very young flower primordia. However, only *LFY* is expressed also during the vegetative phase, when its RNA is found in young leaf primordia, which arise in positions on the shoot meristem that are homologous to those of the later-arising flowers (Blázquez *et al.* 1997; Hempel *et al.* 1997). *LFY* RNA expression increases upon floral induction, and either increasing the copy number of wild-type *LFY* alleles or constitutive expression of *LFY* in *35S::LFY* plants causes early flowering, indicating a critical role for *LFY* transcription in the transition to flowering (Weigel and Nilsson 1995; Blázquez *et al.* 1997). These observations suggest that flower-meristem-identity genes at least partially mediate the effects of flowering-time genes on the transition to flowering. However, double mutant studies along with the observation of an attenuation of the *35S::LFY* phenotype in short days have also indicated that there are other pathways that act in parallel with, or downstream of, *LFY* transcription (Weigel and Nilsson 1995; Ruiz-García *et al.* 1997). To understand how flowering-time genes are involved in these parallel pathways, we have investigated how late-flowering mutations at 11 different loci affect the response to constitutive *LFY* expression as well as the activity of the *LFY* promoter. Together, these data allow for a new functional classification of flowering-time genes in relation to transcription of *LFY*, an important control point for the transition to flowering.

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## MATERIALS AND METHODS

**Plant material:** *constans* (*co-2*), *gigantea* (*gi-3*), *fca-1*, *fd-1*, *fe-1*, *fha-1*, *ft-1*, *fve-1*, and *fwa-2*, all in the Landsberg *erecta* (L*er*) background, have been described by Koornneef *et al.* (1991). *gibberellin insensitive* (*gai-1*), also in the L*er* background, has been described by Koornneef *et al.* (1985). The naturally occurring *FRIGIDA* allele *FRI-Sf2* has been introgressed six times into the Columbia (Col) background (Lee *et al.* 1993, 1994). *35S::LFY* (Weigel and Nilsson 1995) lines DW151.2.5L and DW151.2.5C are in the L*er* and Col backgrounds, respectively. *LFY::GUS* lines DW150.209 and DW150.214 are in the Col background, and DW150.304 and DW150.307 are in the L*er* background (Blázquez *et al.* 1997, 1998). *AP3::GUS* line pAP3 (Bgl2)-GUS Ler #2 is in the L*er* background (Jack *et al.* 1994; Krizek and Meyerowitz 1996).

**Growth conditions:** Seeds were stratified for 2–3 days at 4° before sowing. *AP3::GUS* and *LFY::GUS* plants were sown directly on soil. *35S::LFY* plants were grown on plates containing half-strength Murashige and Skoog medium (Murashige and Skoog 1962), with or without 35 μg/ml kanamycin, for 10–12 days before transplanting to soil. Plants were grown at 23° in long days (16 hr of light) or short days (9 hr of light) under a mixture of 3:1 Cool White and Gro-Lux fluorescent lights (Osram Sylvania, Danvers, MA). All studies were carried out in long days, except for *gai-1* and *FRI-Sf2*, which were analyzed in short days, because they show a much stronger phenotype in short days.

**Crosses:** The late-flowering strains containing mutations induced in the L*er* background were crossed to a *35S::LFY* line generated in the same background (DW151.2.5L). The naturally occurring dominant *FRIGIDA* allele *FRI-Sf2*, which had been introgressed into the Col background (Lee *et al.* 1993, 1994), was crossed to a different *35S::LFY* line generated in the Col background (DW151.2.5C). Except for *35S::LFY* transgenics in the Nossen background, typical homozygous *35S::LFY* plants in the Col and L*er* backgrounds self-fertilize only poorly, because pollen from these plants is less vigorous than that of hemizygous plants, probably because of a sporophytic effect in the homozygous genotype, and because ovules of strong *35S::LFY* plants tend to become carpelloid (O. Nilsson and D. Weigel, unpublished results). Because of the fertility problems, we could not construct lines that were doubly homozygous for the *35S::LFY* transgene and late-flowering mutations, most of which are recessive or weakly semidominant. Instead, we generated homozygous late-flowering mutants that carried a single copy of the transgene by backcrossing late mutant × *35S::LFY* $F_1$ hybrids with the late mutant. Hemizygous *35S::LFY* plants were used as pollen donors in the crosses to the various late-flowering mutants. $F_1$ plants carrying the *35S::LFY* transgene were identified by their ability to grow on kanamycin-containing medium. These plants were backcrossed to the respective late-flowering mutants and the success of this cross was again tested by growing plants on kanamycin-containing medium. In the case of the recessive mutants, we screened the nontransgenic backcross progeny for normal segregation of late-flowering and normal-flowering plants. The presence of the latter class, which should be heterozygous for the late-flowering mutation, indicated that the late-flowering locus was not linked to the transgene, and that therefore transgenic, homozygous mutant plants could be expected to segregate at normal frequency. For all the recessive mutations, late-flowering and normal-flowering plants segregated in the nontransgenic backcross population at the expected ratio of approximately 1:1. Because *FRI-Sf2* is almost completely dominant, we analyzed only $F_1$ progeny derived from a cross of *35S::LFY* to the homozygous *FR-Sf2* line.

To introduce *LFY::GUS* into late-flowering mutants, plants homozygous for the transgene were crossed to homozygous late-flowering mutants. Transgenic late-flowering plants were selected in the $F_2$, and doubly homozygous lines were selected in the $F_3$. Analysis of LFY::GUS activity in the dominant *fwa-2* mutant was performed on $F_1$ progeny from the cross between a homozygous *fwa-2* plant and line DW150-307. The controls in this case were $F_1$ plants from the cross between L*er* wild type and DW150-307.

**Analysis of LFY::GUS activity:** GUS activity was measured in individual shoot apices using 4-methylumbelliferyl-β-d-glucuronide as substrate, as previously described by Blázquez *et al.* (1997). To analyze *LFY::GUS* expression in the *35S::LFY* background, $F_1$ progeny derived from a cross between a hemizygous *35S::LFY* plant and homozygous *LFY::GUS* plants was grown in both short-day and long-day conditions until flowering, and monitored at various stages for GUS expression by X-gluc staining as described (Blázquez *et al.* 1997).

**Analysis of flowering time:** Flowering time was determined by counting the total number of leaves on the main shoot before either the first bractless flower was produced or a plant formed a terminal flower, in cases where no bractless flowers were produced. In an otherwise wild-type background, the Landsberg *erecta* line DW151.2.5L and the Columbia line DW151.2.5C produced no bractless flowers in addition to a terminal flower and flowers in the axils of leaves, when grown in long days.

## RESULTS

***LFY* promoter activity in *35S::LFY* plants:** Since it has been suggested that the *LFY* ortholog of snapdragon, *FLORICAULA* (*FLO*), positively regulates its own expression (Carpenter *et al.* 1995), we first determined whether activity of the natural *LFY* promoter was affected in *35S::LFY* plants. We crossed a hemizygous *35S::LFY* plant (line DW151.2.5C) to two different homozygous *LFY::GUS* reporter strains (DW150.209 and DW150.214) and monitored GUS expression by X-gluc staining. In both cases, approximately one half of the $F_1$ progeny showed the expected pattern of GUS activity in emerging leaf primordia, and eventually in young flower primordia, as reported before for otherwise wild-type plants (Blázquez *et al.* 1997). In the other half of the progeny, inferred to represent *35S::LFY* plants, GUS activity was never detected in leaf primordia (Figure 1). However, GUS activity could be detected in young flower primordia, both in those arising in the axils of leaves and those arising directly on the flanks of the apical meristem. This observation suggests that at least during the vegetative phase of development there is negative feedback regulation of the endogenous *LFY* locus in *35S::LFY* transgenic plants and, more importantly, that endogenous *LFY* expression does not complicate the interpretation of the phenotype seen in late-flowering mutants carrying the *35S::LFY* transgene.

Depending on the amount of *LFY* overexpression in either a *35S::LFY* line or in plants with supernumerary copies of *LFY* wild-type alleles, various degrees of early flowering can be achieved (Weigel and Nilsson 1995; Blázquez *et al.* 1997; Nilsson and Weigel 1997). To avoid having to decide whether to evaluate differences

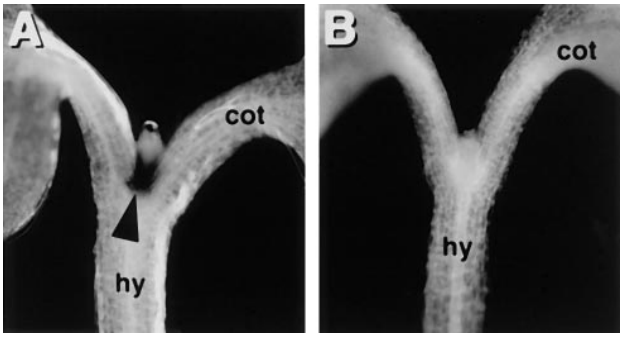


Figure 1.—Suppression of *LFY* promoter activity by *35S::LFY*. (A) Hemizygous *LFY::GUS* plant (DW150.214) in a wild-type background. GUS activity is detected in leaf primordia (arrowhead). (B) Lack of *LFY::GUS* expression in a plant doubly hemizygous for *LFY::GUS* (DW150.214) and *35S::LFY* (DW151.2.5C). Plants were grown in short days for six days. hy, hypocotyl; cot, cotyledon.

in leaf number on an absolute or relative basis, we selected a *35S::LFY* Landsberg line that produced the same total number of leaves (rosette and cauline leaves) as the nontransgenic parent in long days [10.8 ± 1.2 leaves (mean ± standard deviation, $n = 32$)]. Because this line did not reduce the number of leaves compared to wild type, and the *LFY* promoter was not activated (see above), it is likely to produce functional LFY constitutively at a level that is similar to that of wild type at the transition to flowering. We reasoned that saturation of the system with excessive amounts of *LFY* was in this case less likely than with stronger lines.

**Effect of late-flowering mutations on *35S::LFY* phenotype:** Previous studies have shown that Arabidopsis flowers with wild-type morphology can arise in two different ways. Normally, flowers are formed by direct specification of lateral primordia that form on a shoot meristem (Smyth *et al.* 1990; Hempel and Feldman 1994). An alternative way is the conversion of a newly arising or preexisting shoot meristem into a flower. The latter mechanism is occasionally found in wild-type inflorescences at the transition between leaves with associated paraclades and bractless flowers (Hempel and Feldman 1995). It is more common in mutants such as *terminal flower 1* (Shannon and Meeks-Wagner 1991; Alvarez *et al.* 1992), or in transgenic plants, such as those that overexpress *LFY* or *AP1* (Mandel and Yanofsky 1995; Weigel and Nilsson 1995). Thus, late-flowering mutations might affect two aspects of the *35S::LFY* phenotype. First, the number of leaves produced on the main shoot could be changed; second, the paraclade-to-flower conversions could be affected.

None of the late-flowering mutations significantly attenuated the conversion of paraclades to solitary flowers caused by *35S::LFY*. In all backcross populations, the typical *35S::LFY* phenotype, with solitary flowers in the

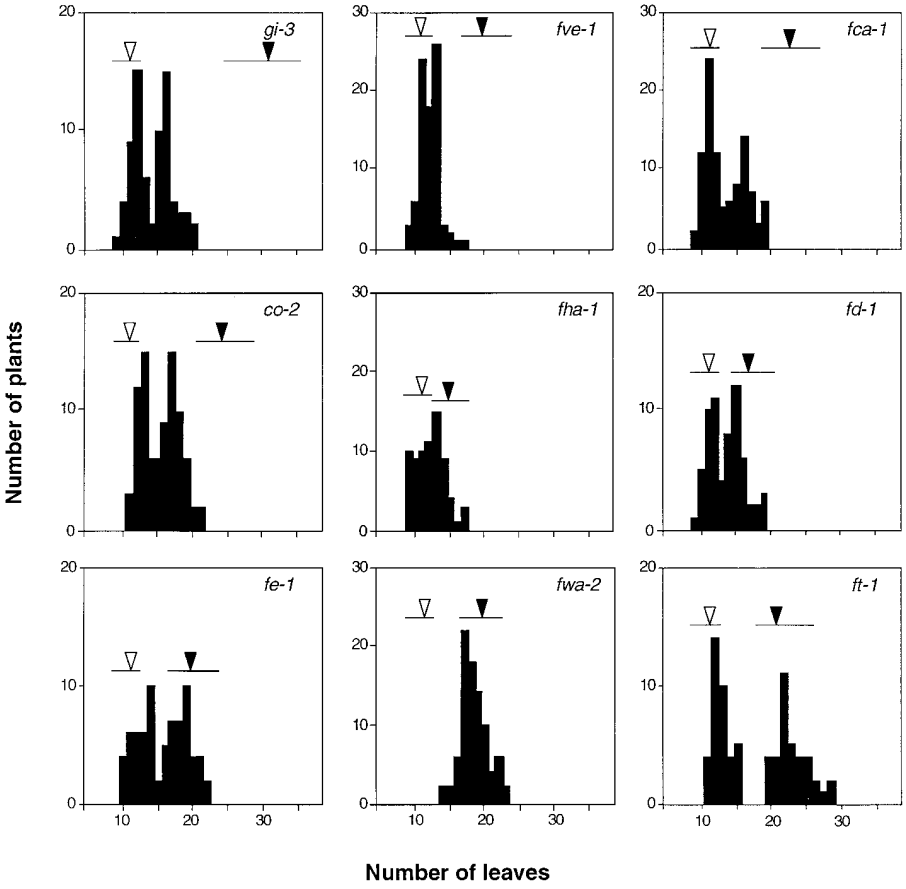


Figure 2.—Flowering time of *35S::LFY* in homozygous and heterozygous late-flowering mutant backgrounds, grown in long days. Frequency distributions for leaf number of plants with *35S::LFY* phenotype in backcross populations (segregating 1:1 homozygous to heterozygous mutant plants) are shown. Open and closed arrowheads indicate the average values for a *35S::LFY* population (segregating 1:2 homozygous to hemizygous transgenic plants) and the parental late-flowering mutant, respectively. Both populations contained 30 plants. The horizontal lines represent the associated ranges of leaf number.

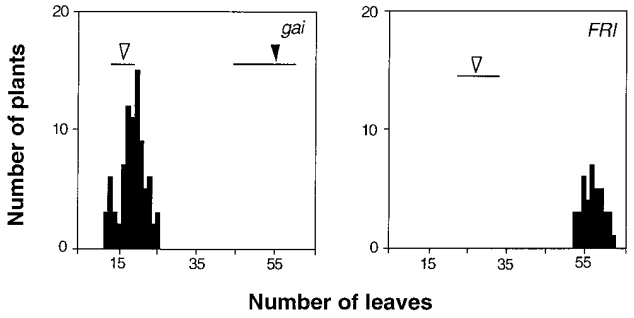


Figure 3.—Flowering time of *35S::LFY* in homozygous and heterozygous late-flowering mutant backgrounds, grown in short days. Frequency distributions for leaf number of plants with *35S::LFY* phenotype, in the case of *gai* in a backcross population (segregating 1:1 homozygous to heterozygous mutant plants) and in the case of *FRI* in an $F_1$ population (all plants heterozygous for *FRI*) are shown. Open and closed arrowheads indicate the average values for a segregating *35S::LFY* population (homozygous and hemizygous plants segregating 1:2) and the parental late-flowering mutant, respectively. Both populations contained 30 plants. The horizontal lines represent the associated ranges of leaf number. *FRI* plants did not flower under our short-day conditions. Note that *gai* is almost completely dominant.

axils of all leaves, was seen in all *35S::LFY* plants, which were identified either by kanamycin selection or by polymerase chain reaction. The strongest deviation from wild-type floral morphology was seen in *35S::LFY ft-1* and *35S::LFY fwa-2* plants, in which the first-whorl organs of the axillary flowers were occasionally leaf-like, and some internode elongation occurred within these flowers (results not shown). However, we never observed secondary flowers, which indicate a more shoot-like quality of the axillary flowers and which, for example, are seen in *35S::LFY ap1-1* plants (Weigel and Nilsson 1995).

In contrast to the paraclade-to-flower conversions, flowering time as measured by the number of leaves on the main shoot of *35S::LFY* plants was affected by several of the late-flowering mutations (Figures 2 and 3). In this study, we analyzed backcross populations of plants that were all hemizygous for the *35S::LFY* transgene and that were expected to segregate 1:1 for plants that were heterozygous and homozygous for late-flowering mutations, respectively (see materials and methods). The transgenic backcross progeny exhibited in all cases, except for *fha-1*, *fwa-2*, and *gai-1*, a bimodal distribution of flowering time, with early- and late-flowering populations being of approximately equal size. The two populations were interpreted as representing heterozygous and homozygous mutant plants, respectively. In the case of *fwa-2* and *gai-1*, the absence of a bimodal distribution could be attributed to the late-flowering mutations being dominant, while in the case of *fha-1*, the difference between wild type and late-flowering mutants is quite small.

*A priori*, we can envision two extreme scenarios for the interaction of *35S::LFY* with late-flowering mutations. If *35S::LFY* is completely epistatic, a late-flowering mutant carrying the *35S::LFY* transgene should produce the same number of leaves as *35S::LFY* plants in an otherwise wild-type background, indicating that the delay in flowering caused by such a late-flowering mutation is mainly caused by down-regulation of *LFY* transcriptional activity. Alternatively, if the late-flowering mutation is completely epistatic, a late-flowering mutant carrying the *35S::LFY* transgene should produce the same number of leaves as late-flowering siblings that are nontransgenic. This would in turn suggest that such a late-flowering mutation is affecting a pathway that is important for the response to *LFY* transcriptional activation, and that this pathway is acting in parallel with or downstream of *LFY*.

Importantly, there was no direct correlation between the number of leaves produced by the different late-flowering mutants in a nontransgenic background compared to the number of leaves produced in a *35S::LFY* background. However, in three cases did we observe one of the extreme possibilities discussed above. *35S::LFY fe-1*, *35S::LFY ft-1*, and *35S::LFY fwa-2* plants produced the same number of leaves as nontransgenic *fe-1*, *ft-1*, and *fwa-2* plants. The other combinations were either closer to that of the *35S::LFY* parent, which was the case for *gi-3*, *fve-1*, and *gai-1*, or closer to the late-flowering parent, which was the case for *fha-1* and *fd-1*. Two mutants, *fca-1* and *co-2*, did not fit clearly into either group, as *35S::LFY fca-1* and *35S::LFY co-2* plants segregated with leaf numbers that were intermediate between those of *35S::LFY* plants and those of nontransgenic *fca-1* and *co-2* mutants. The overall ranking of the mutants with respect to their effect on *35S::LFY* thus is approximately *ft-1* = *fe-1* = *fwa-2* > *fha-1* = *fd-1* > *fca-1* = *co-2* > *gi-3* = *fve-1* = *gai-1*.

Under our short-day conditions, plants expressing the *FRI-Sf2* allele never flowered, and this aspect of the *FRI* phenotype was rescued by *35S::LFY*. However, leaf number in *35S::LFY FRI-Sf2* plants was substantially increased compared to that of *35S::LFY* plants that did not carry the *FRI-Sf2* allele (Figure 3).

**Effect of late-flowering mutations on *LFY* promoter activity:** That the phenotype of several late-flowering mutations can be largely corrected by constitutive expression of *LFY* suggests that the delay in flowering in these plants is caused to a large extent by a reduction in *LFY* RNA levels. On the other hand, an opposite prediction cannot be made for mutants that seem to be insensitive to constitutive *LFY* expression, because the competence defect could mask the effects of a reduction in *LFY* expression.

To monitor the effects of late-flowering mutations on *LFY* promoter activity, we crossed them to a line carrying a *LFY::GUS* transgene, which closely mimics the expression of endogenous *LFY* RNA during both vegetative and reproductive development (Blázquez *et al.* 1997). To compare the effects of late-flowering mutations on *LFY* promoter activity, we focused on the time point at

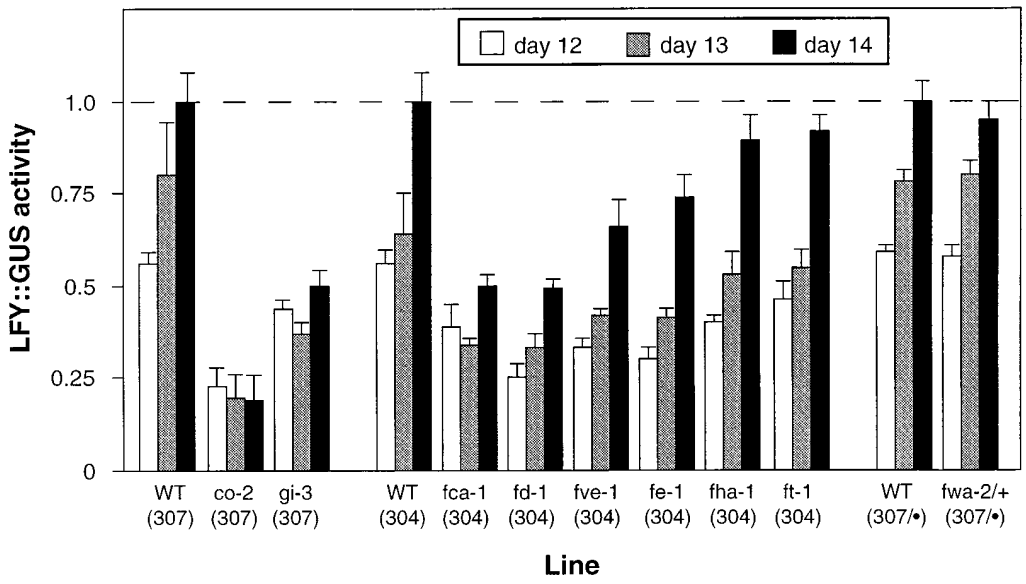


Figure 4.—Relative *LFY* promoter activity in late-flowering backgrounds. GUS activity was measured during the period of flower initiation in wild type. Values are the average of at least 20 samples and were normalized to the GUS activity of the control lines DW150.304 (304) and DW 150.307 (307) at day 14. The error bars represent a 95% confidence interval. 307/· indicates that the *LFY::GUS* line is hemizygous.

which wild-type plants switch to flowering in long days. We have previously shown that levels of *LFY* activity are critical for determining the time point of the switch to flowering, and that the *LFY* promoter is significantly upregulated at this time (Blázquez *et al.* 1997). The exact time point of floral induction in wild type under our long-day conditions was established by growing a population of transgenic L*er* plants which express the GUS reporter under the control of the *APETALA3* (*AP3*) promoter in flowers from stage 3 onward (Jack *et al.* 1994). We analyzed cohorts of 20 plants, and found that no plants showed *AP3::GUS* activity on day 13 after germination, while 13 (65%) were GUS-positive on day 14, and all were GUS-positive on day 15. Since floral stages 1 through 2 last approximately 2 days (Smyth *et al.* 1990), we deduced that wild-type plants formed the first flower around day 12. Therefore, we measured *LFY::GUS* activity in late-flowering backgrounds on days 12, 13, and 14. In an otherwise wild-type background, GUS activity in *LFY::GUS* plants increased almost twofold from day 12 to day 14 (Figure 4), in agreement with previously published results (Blázquez *et al.* 1997).

We found that *LFY::GUS* levels were always lower in the late-flowering backgrounds, with initial levels on day 12 being approximately 60% (*co-2*) to 10% (*fwa-2*) below those of wild type (Figure 4). However, the changes in *LFY::GUS* levels across the 3-day interval were strikingly different in individual mutants. In *co-2*, *gi-3*, and *fca-1* mutant backgrounds, induction of *LFY::GUS* levels was either absent or substantially weakened, and levels on day 14 were reduced by approximately 80% (*co-2*) to 50% (*gi-3*, *fca-1*), when compared to wild-type levels. In the other mutants, *LFY::GUS* activity increased at least twofold from day 12 to day 14. Compared to wild type, the most normal profiles were seen in *fha-1*, *fwa-2*, and *ft-1* mutants.

## DISCUSSION

Previous attempts to organize flowering-time genes into functional groups or pathways have mostly been based on the response of the mutants to environmental conditions, and on the phenotype of late-flowering double mutants (Koornneef *et al.* 1991; Martínez-Zapater *et al.* 1994). Epistatic interactions between late-flowering mutations are, overall, consistent with the classification along physiological lines (Koornneef *et al.* 1998).

More recently, double mutants in which loss-of-function mutations of the meristem-identity genes *LFY* and *AP1* were combined with late-flowering mutations have revealed distinct functional groups among flowering-time genes (Ruiz-García *et al.* 1997). We have taken yet another, complementary, approach by analyzing late-flowering mutations in a transgenic background in which expression of *LFY* is independent of environmental or genetic factors. Based on our results, we can arrange the genes defective in the late-flowering mutants into one group that is primarily important for *LFY* transcriptional activation, and into another group that is acting mainly in parallel with or downstream of *LFY*, controlling the response to *LFY* activity.

**Late-flowering mutations affect the response to *LFY* activity:** One of the major consequences of floral induction in Arabidopsis is the transcriptional upregulation of several flower-meristem-identity genes, including *LFY* and *AP1* (Simon *et al.* 1996; Blázquez *et al.* 1997; Hempel *et al.* 1997). Thus, one can expect that late-flowering mutations exert their effect through a failure in the proper upregulation of flower-meristem-identity genes. Formally, however, it is also possible that a mutant is late flowering because it is not competent to respond to the activity of flower-meristem-identity genes.

The clearest cases that we have observed are those of

*FT* and *FWA*. The *ft-1* and *fwa-2* mutations are epistatic to *35S::LFY* not only in the sense that they delay the cessation of leaf production to the same extent in a nontransgenic and in a *35S::LFY* background, but also in that they are the only late-flowering mutations that have at least a small effect on the ectopic flowers that are produced in the axils of leaves of *35S::LFY* plants. Coincidentally, *ft-1* and *fwa-2* have also the smallest effects on *LFY* promoter activity, when compared to the other late-flowering mutants. Although both mutants flower more than a week later than wild type, upregulation of *LFY* promoter activity very closely follows that of wild type during the 3-day period when flowers are initiated in wild type. *FT* and *FWA* thus control primarily competence to respond to *LFY* expression.

A case that is essentially the opposite of *FT* and *FWA* is seen with genes that are involved in gibberellin (GA) biosynthesis or signal transduction. *35S::LFY* is almost completely epistatic to the severe delay in flowering or even failure to flower seen when *gai-1* or *ga1-3* mutants are grown in short days (Blázquez *et al.* 1998; this article). This observation is consistent with the finding that transcriptional activation of the *LFY* promoter is very much reduced in *ga1-3* mutants (Blázquez *et al.* 1998).

Among the other genes that are involved in *LFY* regulation is *CO*. The strong late-flowering phenotype of *co-2* mutants can be corrected to a large extent by constitutive expression of *LFY*, suggesting that a major defect of *co-2* mutants is in the induction of *LFY* transcription. This assumption is confirmed by analysis of *LFY* promoter activity in *co-2* mutants, which is much lower than in wild type. An important observation is that there is not merely a failure in induction of *LFY* promoter activity, but that *LFY* promoter activity is significantly reduced from the very beginning (day 12), when compared to wild type. These observations are consistent with the known properties of *CO*, which likely encodes a transcription factor and whose induction in vegetatively growing plants leads to rapid accumulation of *LFY* transcripts (Putterill *et al.* 1995; Simon *et al.* 1996).

Other late-flowering mutants, including *gi-3*, *fve-1*, and *fca-1*, are complemented by *35S::LFY* to an even higher degree than *co-2*. In all these mutants, the induction of *LFY::GUS* activity is weak during the 3-day period when floral initiation occurs in wild type, confirming that the corresponding genes are involved in *LFY* transcriptional regulation.

**Functional classifications of flowering-time genes:** In their initial physiological analysis of 11 late-flowering mutants, Koornneef *et al.* (1991) assigned these mutants to three groups based on their responses to vernalization and differences in day length. Vernalization largely corrects the late-flowering phenotype of the first group of mutants, *fca*, *fpa*, *fve*, and *fy*. In addition, these mutants flower much later in short days than in long days. The genes defective in these mutants have therefore been proposed to act in a constitutive floral promotion pathway that acts independently of the environment (Martínez-Zapater *et al.* 1994). Our results suggest that at least two genes from this group, *FCA* and *FVE*, fall into a group of genes that is proposed to be mainly involved in *LFY* transcriptional regulation. Both genes have been suggested to promote flowering and the production of abaxial leaf trichomes by increasing the level of, or response to, GA (reviewed by Martínez-Zapater *et al.* 1994; Telfer *et al.* 1997). It is interesting to note that two genes involved in GA synthesis and sensitivity, *GA1* and *GAI*, which are also thought to act in the constitutive floral promotion pathway (Martínez-Zapater *et al.* 1994), belong to the "transcriptional" group as well (Blázquez *et al.* 1998). It is therefore possible that the portion of the delay in flowering of *fca* and *fve* mutants that can be attributed to reduced *LFY* expression is mediated by the GA pathway.

The second group of mutants identified by Koornneef *et al.* (1991) consists of *fd*, *fe*, *ft*, and *fwa*. Flowering of these mutants is delayed by short days, but less so than for the first group, and these mutants show only a small or no response to vernalization. In our analysis, the genes defective in these four mutants were the clearest examples of "competence" genes, most likely to act in parallel with *LFY*.

The third and last group of mutants identified by Koornneef *et al.* (1991) includes *co* and *gi*, both of which respond neither to vernalization nor to short days, and which therefore identify a day-length-sensitive pathway. In these mutants, *LFY* promoter activity is severely reduced, and the delay in flowering can be largely corrected by *35S::LFY*. Therefore, these genes fall primarily into the transcriptional group of genes.

A separate attempt to classify flowering-time genes has recently been undertaken by Ruiz-García *et al.* (1997), who combined four different late-flowering mutations with mutations in the meristem-identity genes *LFY* and *AP1*. Despite similar lateness of the corresponding single mutants, *fpa lfy* and *fve lfy* showed a much less extreme phenotype than *ft lfy* and *fwa lfy* double mutants, in which all signs of flower development disappeared. These interactions were taken as indication that *FT* and *FWA* are particularly important for a pathway that acts in parallel with *LFY* and leads to *AP1* activation. While *lfy* mutations only delay *AP1* activation (Gustafson-Brown 1996), *AP1* expression is largely eliminated in *ft lfy* and *fwa lfy* double mutants (Ruiz-García *et al.* 1997). Why, then, are *FT* and *FWA* also important for the *LFY* pathway, as inferred from the attenuation of the *35S::LFY* phenotype in an *ft-1* or *fwa-2* background? The answer is likely that *FT*/*FWA* and *LFY* are not only redundant regulators of *AP1*, but that *FT*/*FWA* and *LFY* also potentiate each other's ability to activate *AP1*.

In contrast to *FT* and *FWA*, *FPA* and *FVE* were interpreted as having redundant roles in the activation of both *LFY* and *AP1* (Ruiz-García *et al.* 1997). Because *co lfy* double mutants are similar to *fpa lfy* and *fve lfy*

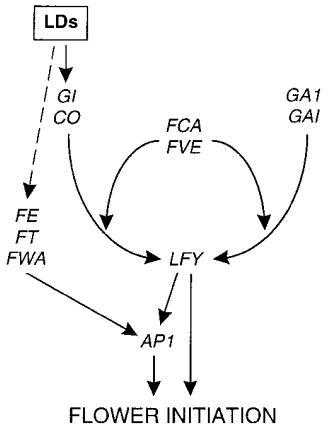


Figure 5.—Classification of flowering-time genes. This model describes the major interactions of flower-meristem identity genes *LFY* and *AP1* with flowering-time genes *CO*, *GI*, *FCA*, *FE*, *FT*, *FVE*, *FWA*, and with *GA1* and *GAI*, which are involved in GA biosynthesis and signal transduction. A dashed line indicates that these genes are largely under the control of the long-day (LD) pathway.

double mutants (Putterill *et al.* 1995), *CO* would by the same criterion belong in the *FPA* and *FVE* group. Furthermore, Roldán *et al.* (1997) have recently shown that the late-flowering phenotypes of *co*, *gi*, *fca*, *fpa*, and *fve* mutants, but not those of *ft* and *fwa*, can be rescued by growing them on vertical plates containing sucrose.

Our functional grouping, with *FE*, *FT*, and *FWA* in the competence group, and *CO*, *GI*, *FCA*, and *FVE* in the transcriptional group, perfectly overlaps with the one proposed by Ruiz-García *et al.* (1997), on the basis of genetic interactions, and the one proposed by Roldán *et al.* (1997), on the basis of physiological responses. From an extensive analysis of the epistatic interactions among 10 late-flowering mutants, Koornneef *et al.* (1998) proposed a model in which *FE*, *FT*, and *FWA* belong to two different subgroups that act upstream of *CO* and *GI* in the long-day pathway. Our analysis supports the distinction between *FE*, *FT*, and *FWA* on the one hand and *CO* and *GI* on the other in the long-day pathway, although we have no evidence for *FE*, *FT*, and *FWA* acting either upstream or downstream of *CO* and *GI*.

**A model for the action of flowering-time genes:** Our data along with those published previously (Koornneef *et al.* 1991, 1998; Putterill *et al.* 1995; Roldán *et al.* 1997; Ruiz-García *et al.* 1997; Blázquez *et al.* 1998) lead us to propose a simple model for the interaction between flowering-time and flower-meristem-identity genes (Figure 5). The model includes two largely redundant pathways, of which the GA-dependent one is active in both long and short days, while the *CO*/*GI* pathway is active only in long days. The genes of the constitutive or autonomous pathway (Martínez-Zapater *et al.* 1994), which includes *FCA* and *FVE*, interact with both of these pathways to stimulate flowering. In contrast, *FE*, *FT*, and *FWA* act in a parallel, again partially redundant pathway, which is more active in long than in short days (indicated by a dotted line in Figure 5). This pathway enhances the ability of *LFY* to induce downstream genes such as *AP1* during the early phase of inflorescence development. By placing *FE*, *FT*, and *FWA* partially downstream of *CO* and *GI*, one can also explain how *co* and *gi* mutations affect both transcription of *LFY* and the response to *LFY* activity. We emphasize, however, that we are taking into account only the major effects of the different genes.

Because the activation of flower-meristem-identity genes and the response to their activity define specific steps in the signal transduction pathways leading to flower initiation, flower-meristem-identity genes can be taken as a useful reference point to assess where flowering-time genes might act. However, since competence genes define a pathway that acts in parallel with *LFY*, genetic analysis is not sufficient to determine where in the plant or when during the life cycle competence genes act. The answer to this question has to come from molecular analyses.

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