

Loss of *FLOWERING LOCUS C* Activity Eliminates the Late-Flowering Phenotype of *FRIGIDA* and Autonomous Pathway Mutations but Not Responsiveness to Vernalization

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The MADS domain-containing transcription factor *FLOWERING LOCUS C* (*FLC*) acts as an inhibitor of flowering and is a convergence point for several pathways that regulate flowering time in *Arabidopsis*. In naturally occurring late-flowering ecotypes, the *FRIGIDA* (*FRI*) gene acts to increase *FLC* levels, whereas the autonomous floral promotion pathway and vernalization act to reduce *FLC* expression. Previous work has shown that the Landsberg *erecta* allele of *FLC*, which is not a null allele, is able to partially suppress the late-flowering phenotype of *FRIGIDA* and mutations in the autonomous pathway. In this study, using a null allele of *FLC*, we show that the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutants are eliminated in the absence of *FLC* activity. In addition, we have found that the downregulation of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* by *FRI* and autonomous pathway mutants also is mediated by *FLC*. Complete loss of *FLC* function, however, does not eliminate the effect of vernalization. Thus, *FRI* and the autonomous pathway may act solely to regulate *FLC* expression, whereas vernalization is able to promote flowering via *FLC*-dependent and *FLC*-independent mechanisms.

INTRODUCTION

The promotion of flowering in response to prolonged exposure to cold temperatures is an adaptation to prevent plants from flowering in the fall, prior to winter, and to enable them to flower in spring. This promotion, known as vernalization, does not initiate flowering directly but renders the meristem competent to respond to other environmental and developmental flowering signals (reviewed in Chouard, 1960; Michaels and Amasino, 2000). Although the physiology of vernalization has been studied extensively in many species, the molecular mechanism of vernalization remains largely unknown. In *Arabidopsis*, many naturally occurring ecotypes are relatively late flowering unless they are vernalized (Napp-Zinn, 1979; Karlsson et al., 1993), and thus they behave as winter annuals. Summer annual strains, in contrast, do not require vernalization for early flowering. Crosses between winter and summer annual strains have shown that the winter annual habit is caused by the interaction of two dominant genes: *FLOWERING LOCUS C* (*FLC*) (Koornneef et al., 1994; Lee et al., 1994) and *FRIGIDA* (*FRI*) (Burn et al., 1993; Lee et al., 1993; Clarke and Dean, 1994). *FLC* encodes a MADS domain-containing transcription factor that acts as an inhibitor of flowering, and *FRI* is required for *FLC* expression (Michaels and Amasino, 1999; Sheldon et al., 1999). Most summer annual ecotypes of *Arabidopsis* contain nonfunctional alleles of

FRI (Johanson et al., 2000) and thus have low levels of *FLC* expression (Michaels and Amasino, 1999; Sheldon et al., 1999). Vernalization is able to eliminate the late-flowering phenotype caused by *FLC* and *FRI* (Lee and Amasino, 1995) and results in a downregulation of *FLC* levels (Michaels and Amasino, 1999; Sheldon et al., 1999). This suppression of *FLC* expression by vernalization is stable for the remainder of the plant life cycle, but *FLC* expression returns to high levels in the next generation. Transgenic plants containing *FLC* under the control of the constitutive 35S cauliflower mosaic virus promoter remain late flowering after cold treatment, indicating that *FLC* levels must be reduced for vernalization to be effective (Michaels and Amasino, 1999; Sheldon et al., 1999). Thus, the epigenetic downregulation of *FLC* appears to be a major target of the vernalization pathway, and this downregulation is a component of the acquisition of the competence to flower by the meristem.

In addition to studying naturally occurring variation, mutational analysis has been used to identify genes that control flowering in *Arabidopsis*. From mutagenized summer annual backgrounds, mutants have been identified in which the transition to flowering is delayed (Koornneef et al., 1991). These mutations identify genes that act as promoters of flowering, because a loss of function results in later flowering. On the basis of genetic and physiological characterization, these mutants are thought to operate in two pathways (reviewed in Koornneef et al., 1998; Simpson et al., 1999). Genes such as *CONSTANS* (*CO*) and *GIGANTEA* (*GI*) appear

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to be involved in promoting flowering in response to photoperiod. Arabidopsis is a facultative long-day (LD) plant, and the flowering of plants containing mutations in either of these genes is not promoted by inductive photoperiods. Thus, these mutants are day neutral or "blind" to photoperiod. Plants containing mutations in *FCA*, *FPA*, *FVE*, *LUMINIDEPENDENS (LD)*, and *FLOWERING LOCUS D* are delayed in flowering but retain a photoperiod response and therefore are thought to promote flowering in a photoperiod-independent pathway often referred to as the autonomous pathway. Unlike photoperiod pathway mutants, the late-flowering phenotype of autonomous pathway mutants is strongly suppressed by vernalization. Thus, the late-flowering, vernalization-responsive phenotype of autonomous pathway mutants is similar to the winter annual habit of *FRI*-containing late-flowering ecotypes. Furthermore, like *FRI*, mutations in the autonomous pathway result in increased *FLC* expression that is eliminated by vernalization (Michaels and Amasino, 1999; Sheldon et al., 1999). These results indicate that the autonomous pathway promotes flowering, at least in part, by repressing *FLC* expression.

FLC was first identified genetically because the late-flowering phenotype of *FRI* and the autonomous pathway mutant *ld* is partially suppressed in a recessive manner by the Landsberg *erecta* (*Ler*) *FLC* locus (Koornneef et al., 1994; Lee et al., 1994). Subsequent experiments have shown that other autonomous pathway mutants, but not photoperiod pathway mutants, also are partially suppressed by the *Ler FLC* locus (Sanda and Amasino, 1996b). Since the cloning of *FLC*, the *Ler* allele of *FLC* has been examined, but the recessive nature of this allele is unclear; there are no missense or nonsense mutations, and *FLC* mRNA levels and regulation are similar to those seen in other ecotypes (Sheldon et al., 2000; our unpublished results). Because the *Ler* allele of *FLC* is not a clear loss-of-function allele, we have reexamined the *FLC* dependence of the late-flowering phenotype of *FRI* and late-flowering autonomous and photoperiod pathway mutants by using a null allele of *FLC*. We also have used the null allele of *FLC* to determine if vernalization acts exclusively by downregulating *FLC* expression or if it can also promote flowering independent of *FLC*.

RESULTS

The Null Allele of *FLC* Is Able to Completely Suppress the Late-Flowering Phenotype of *FRI*

Previous studies have shown that the extreme late-flowering phenotype of lines containing dominant alleles of *FRI* and *FLC* is suppressed by the *Ler FLC* locus (Koornneef et al., 1994; Lee et al., 1994; Michaels and Amasino, 1999). However, the suppression mediated by the *Ler FLC* locus is incomplete; in the *Ler* background, *FRI* approximately doubles the number of vegetative nodes formed at the shoot

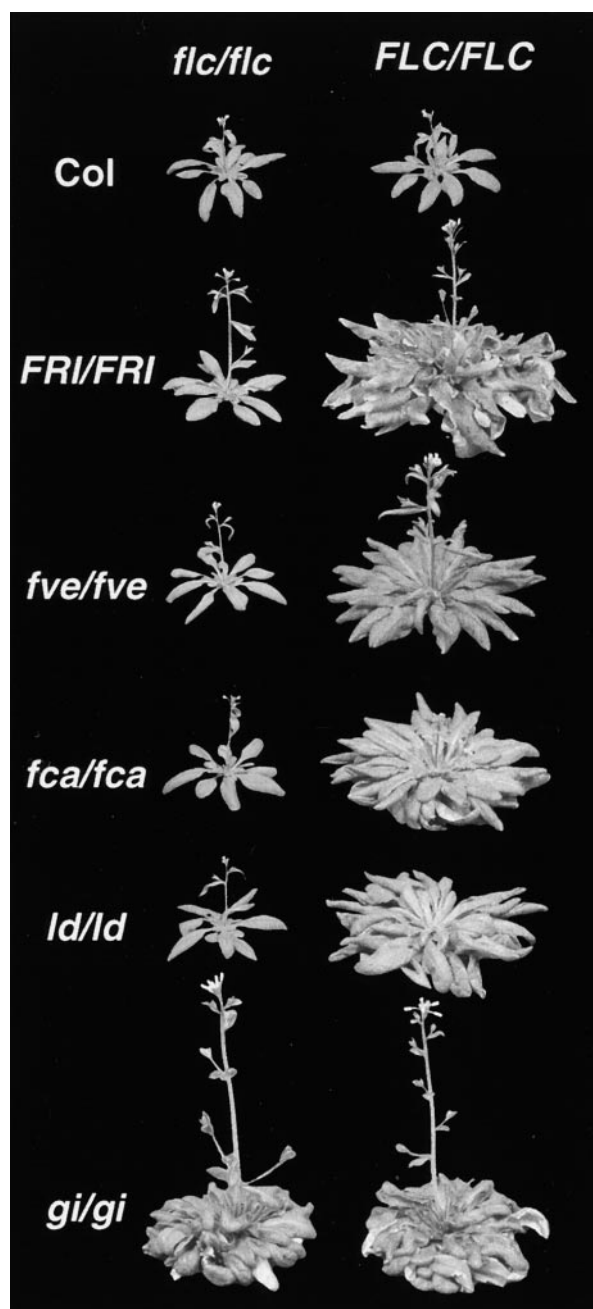


Figure 1. Dependence of the Late-Flowering Phenotype of *FRI* and Certain Late-Flowering Mutants on *FLC*.

Plants at left are homozygous for an *flc* null allele. Plants at right contain wild-type *FLC* alleles and are shown as controls. Plants were grown under LD conditions.

apical meristem before flowering occurs (Koornneef et al., 1994; Lee et al., 1994). One model that accounts for this incomplete suppression is that the *Ler* allele of *FLC* has some low level of activity and the late-flowering phenotype of *FRI* in the *Ler* background is the result of increased *FLC* activity. Another model is that *Ler* contains an inactive allele of *FLC* and *FRI* acts to regulate flowering through both *FLC*-dependent and *FLC*-independent mechanisms.

To distinguish between these models, we analyzed the flowering behavior of isogenic lines containing dominant or recessive *FRI* alleles in an *flc* mutant background. If *FRI* acts solely through *FLC*, then the *FRI* genotype should have no effect in the absence of *FLC* activity, whereas if *FRI* can delay flowering independent of *FLC*, *FRI*-containing lines should remain late flowering in the absence of *FLC* activity. The results are shown in Figures 1 to 3. In the wild-type Columbia (Col) background, *FRI* strongly delays flowering, as has been shown previously (Lee et al., 1994). In an *flc-3* mutant background, however, the effect of *FRI* is eliminated under both LD (Figures 1 and 2) and short-day (SD) conditions (Figure 3). Thus, the late-flowering phenotype of *FRI* is likely to result entirely from an upregulation of *FLC* activity, and the *Ler* allele of *FLC* is unlikely to be a null allele.

FLC Affects Flowering in SD Conditions

In summer annual strains of *Arabidopsis* lacking *FRI* activity, *FLC* expression is not detected by RNA gel blot analysis from plants grown under LD (Michaels and Amasino, 1999; Sheldon et al., 1999) or SD (our unpublished results) conditions. This suggests that the expression, and subsequent effect on flowering time, of *FLC* may be dependent on *FRI*. To determine if a loss of *FLC* function affects flowering time in the absence of *FRI*, Col and *flc-3* were grown under LD and SD. Under LD, the flowering time of *flc-3* is indistinguishable from that of wild-type Col (Figures 1 and 2). Under SD, however, *flc-3* flowers ~12 leaves earlier than does the wild type (Figure 3). Thus, *FLC* affects flowering time in summer annual strains lacking *FRI* activity in SD conditions. Although the *FLC* transcript is not detected in the absence of *FRI*, the early-flowering phenotype of *flc-3* in SD indicates that *FLC* must be expressed at some level and that this low level of expression can affect flowering in the absence of inductive photoperiods.

Autonomous Pathway Mutations Are Dependent on *FLC* for Their Late-Flowering Phenotype

The late-flowering phenotype of many autonomous pathway mutants is reduced by the *Ler FLC* locus, whereas photoperiod pathway mutants (*co* and *gi*) are relatively unaffected (Sanda and Amasino, 1996a, 1996b). Like *FRI*, mutations in autonomous pathway genes also result in increased *FLC* expression. Thus, as discussed above for the role of *FRI*, we

determined the dependence of the late-flowering phenotype of these mutations on *FLC*. Accordingly, the flowering behavior of double mutants between the *flc-3* null allele and *fca*, *fpa*, *fve*, *ld*, *co*, *gi*, and *suppressor of overexpression of constans1 (soc1)* was determined. The results are shown in Figures 1 to 3. Under LD conditions, the late-flowering phenotype of autonomous pathway mutations (*fca*, *fpa*, *fve*, and *ld*) was completely eliminated in the presence of a null allele of *flc*, whereas the flowering time of *co*, *gi*, and *soc1* was unaffected by the absence of *FLC*. Under SD conditions, the late-flowering phenotype of *ld*, *fca*, and *fve* was eliminated in the *flc-3* background, whereas *fpa* was slightly later flowering (Figure 3). The flowering time of non-autonomous pathway mutations was unaffected by *FLC* (*co*), or it showed an additive effect (*gi* and *soc1*) under SD (Figure 3). Thus, *FCA*, *FPA*, *FVE*, and *LD* are likely to act upstream of *FLC* and promote flowering by inhibiting *FLC* expression, whereas *CO*, *GI*, and *SOC1* are likely to act in pathways that are downstream or independent of *FLC*.

The *FLC* Null Mutant Is Responsive to Vernalization

As described above, the late-flowering phenotype of *FRI* and autonomous pathway mutants can be eliminated by either vernalization, which permanently downregulates *FLC* expression,

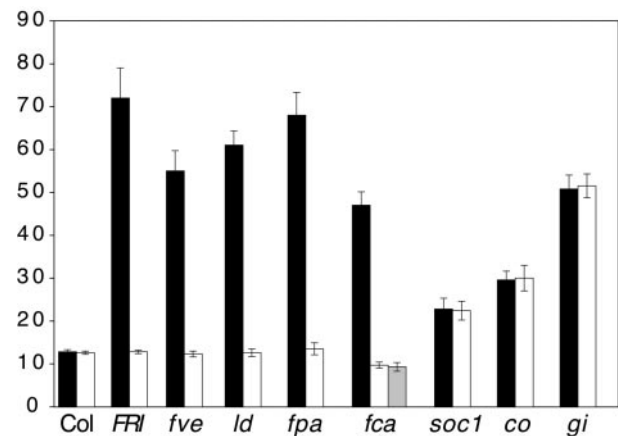


Figure 2. Effect of *FLC* on Flowering Time under LD Conditions.

Black bars represent plants homozygous for wild-type *FLC*, and white bars represent plants homozygous for an *flc* null allele. All analyses were performed in the Col background except for those involving *fca*. For *fca*, an F_2 population was generated from a cross between *flc-3* in Col and an *fca* allele in Wassilewskija. Because of the different backgrounds, a minimum of 10 plants of each of the following genotypes was isolated in the F_2 generation, and their leaf numbers were averaged: black bar, *fca/fca FLC/FLC*; white bar, *fca/fca flc/flc*; gray bar, *FCA/FCA flc/flc*. The white and gray bars show the effect of the *FCA* in the *flc* null background. Error bars indicate \pm SD. The y axis indicates the number of rosette leaves formed prior to flowering.

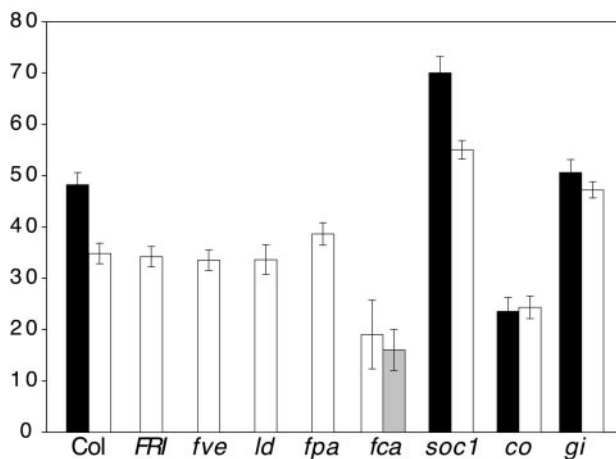


Figure 3. Effect of *FLC* on Flowering Time under SD Conditions.

Black bars represent plants homozygous for wild-type *FLC*, and white bars represent plants homozygous for an *ftc* null allele. All analyses were performed in the *Col* background except for those involving *fca*. For *fca*, F_3 lines were grown from each of the F_2 plants described in Figure 2, and their leaf numbers were averaged: white bar, *fca/fca ftc/ftc*; gray bar, *FCA/FCA ftc/ftc*. The white and gray bars show the effect of the *FCA* in the *ftc* null background. Lines containing *FRI*, *fve*, *ld*, and *fpa* with wild-type *FLC* alleles did not flower after forming >80 leaves (data not shown). Error bars indicate \pm SD. The y axis indicates the number of rosette leaves formed prior to flowering.

or loss-of-function mutations in *FLC*. These observations, coupled with the result that transgenic plants containing *FLC* under the control of the constitutive 35S promoter are late flowering and insensitive to vernalization (Michaels and Amasino, 1999; Sheldon et al., 1999), indicate that downregulation of *FLC* is a major function of the vernalization pathway. This raises the question of whether vernalization acts exclusively to downregulate *FLC* or whether it can also promote flowering via *FLC*-independent mechanisms. To address this question, an *ftc* null allele and the wild type were cold treated and grown under SD, that is, under conditions in which the flowering of the *ftc* mutant is delayed. The results are shown in Figure 4. After vernalization, both *Col* and *ftc-3* flowered after forming only half the number of leaves as non-cold-treated plants. Thus, the loss of *FLC* function does not eliminate the response to vernalization, and vernalization must promote flowering through *FLC*-independent pathways as well as through inactivation of *FLC*.

Effect of *FRI* and the Autonomous Pathway on *SOC1* Expression Is Mediated by *FLC*

SOC1 is a MADS domain-containing transcription factor that acts as a promoter of flowering (Borner et al., 2000; Lee et al., 2000; Samach et al., 2000). *SOC1* transcript levels are

reduced in late-flowering backgrounds containing *FRI* or autonomous pathway mutations, but they are restored to wild-type levels after vernalization (Lee et al., 2000; Samach et al., 2000). The fact that *SOC1* levels are regulated in a manner opposite that of *FLC* levels suggests that *FLC* may act as an inhibitor of *SOC1* expression and that the effects of *FRI*, the autonomous pathway, and vernalization on *SOC1* expression may be mediated by *FLC* (Lee et al., 2000; Samach et al., 2000). To test this model, *SOC1* transcript levels were determined in lines containing *FRI* or autonomous pathway mutants with or without a functional allele of *FLC*. The results of RNA gel blot analyses are shown in Figure 5. In lines containing *FRI*, *ld*, or *fpa* with a wild-type *FLC* allele, *SOC1* levels were suppressed. In the corresponding lines containing an *ftc* null allele, however, *SOC1* levels were similar to those in the wild type. Thus, the downregulation of *SOC1* by *FRI*, *ld*, and *fpa* is blocked in the absence of *FLC*.

As a direct test of the ability of *FLC* to suppress *SOC1* expression, *SOC1* transcript levels were determined in a transgenic line containing *FLC* under the control of the constitutive 35S cauliflower mosaic virus promoter. As shown in Figure 5, *SOC1* levels are suppressed in the *FLC* overexpression line, despite the absence of *FRI* or the presence of an autonomous pathway mutation. Thus, *FLC* expression is sufficient to suppress *SOC1* levels.

DISCUSSION

Previous studies have shown that *FLC* acts as a repressor of flowering and is an integration point of several pathways

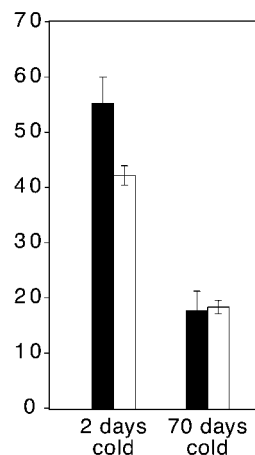


Figure 4. Effect of Vernalization in an *ftc* Null Mutant.

Black bars represent *Col* (homozygous for wild-type *FLC*), and white bars represent an *ftc* null allele. Plants were cold treated for 2 or 70 days before growth under SD conditions. Error bars indicate \pm SD. The y axis indicates the number of rosette leaves formed prior to flowering.

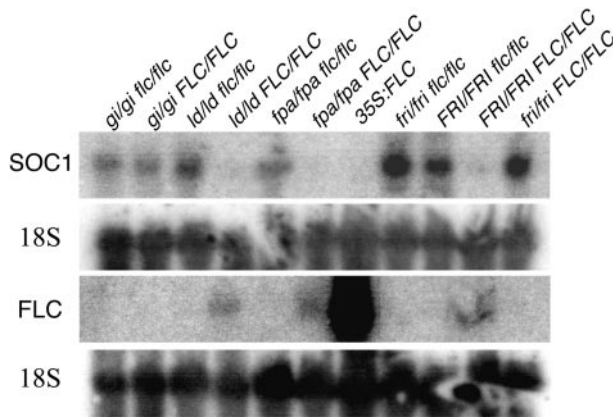


Figure 5. RNA Gel Blot Analysis of *SOC1* Expression in Various Genotypes.

RNA was isolated from 12-day-old plants grown under continuous light. *FLC* expression is shown for comparison. Blots were probed with 18S rDNA as a control for loading.

that control flowering time (Koornneef et al., 1994; Lee et al., 1994; Sanda and Amasino, 1996b; Michaels and Amasino, 1999; Sheldon et al., 1999). The autonomous floral promotion pathway acts to suppress *FLC* expression; thus, mutations in autonomous pathway genes result in increased *FLC* levels and produce a late-flowering phenotype. In winter annual strains, the *FRI* gene acts to increase *FLC* expression and is epistatic to the suppression of *FLC* expression by the autonomous pathway. The late-flowering phenotype of both *FRI* and the autonomous pathway mutants is suppressed by vernalization (Napp-Zinn, 1979; Koornneef et al., 1991; Lee and Amasino, 1995), which causes a stable downregulation of *FLC* expression (Michaels and Amasino, 1999; Sheldon et al., 1999). Genetic analyses have shown that the *Ler* allele of *FLC* is able to partially suppress the late-flowering phenotype of *FRI* and autonomous pathway mutants (Koornneef et al., 1994; Lee et al., 1994; Sanda and Amasino, 1996a, 1996b). The *Ler* allele of *FLC*, however, is not a clear null allele (Sheldon et al., 2000; our unpublished results). Thus, the partial suppression of the late-flowering phenotype of *FRI* and autonomous pathway mutants in *Ler* could support two models: one in which the remaining late-flowering phenotype of *FRI* and autonomous pathway mutants in the *Ler* background is the result of residual *FLC* activity; or another in which the *Ler* allele of *FLC* is inactive, and *FRI* and autonomous pathway mutations delay flowering in part via an *FLC*-independent mechanism. Therefore, to determine the dependence of *FRI* and autonomous pathway mutations on *FLC*, we have reexamined the interactions between *FLC* and the autonomous pathway, *FRI*, and vernalization by using a null allele of *FLC*.

The data presented here demonstrate that in the presence of a null allele of *FLC*, the late-flowering phenotype of *FRI* and the autonomous pathway mutants *ld*, *fca*, and *fve* is

eliminated. Thus, it appears that the late-flowering phenotype of these mutants is entirely the result of increased *FLC* expression (Figure 6). In contrast, we find that the late-flowering phenotype of *soc1* and photoperiod pathway mutants such as *co* and *gi* is unaffected by a loss of *FLC* function. This is predicted from current models in which these genes act either independently (photoperiod pathway) (Koornneef et al., 1998; Simpson et al., 1999) or downstream (*SOC1*) (Lee et al., 2000; Samach et al., 2000) of *FLC*.

The late-flowering phenotype of *fpa* mutations, like that of *fca*, *fve*, and *ld*, is eliminated by a loss of *FLC* function in LD conditions. *fpa*, however, is distinguished from the other autonomous pathway mutants by a slight late-flowering phenotype under SD conditions in the absence of *FLC*; *fpa/flc* double mutants flower approximately four leaves later than does the *flc* single mutant. We have also noticed that the *fpa* mutant exhibits a smaller rosette diameter in SD conditions, reminiscent of a gibberellin (GA) deficiency. This phenotype was not observed in other autonomous pathway mutants. Meier et al. (2001) have also reported this SD phenotype as well as increased expression of genes involved in GA metabolism in *fpa* mutants. Thus, *FPA* appears to have functions beyond the regulation of *FLC*, and the slight late-flowering phenotype in SD in the absence of *FLC* may be a result of altered GA metabolism. (GA deficiency severely delays flowering in SD [Wilson et al., 1992].)

In the *Col* background, which lacks *FRI* activity, the *flc-3* mutation has no effect on flowering time under inductive LD photoperiods, but it causes slightly earlier flowering than it does in the wild type under SD conditions. *FLC* transcript is undetectable by gel blot analysis of RNA from *Col* (Michaels and Amasino, 1999; Sheldon et al., 1999); thus, the lack of an effect of *flc* mutations on flowering time in LD conditions is not surprising. The early-flowering phenotype of the mutant under SD conditions, however, indicates that *FLC* must be present at some low level even in the absence of *FRI* or autonomous pathway mutations and that this low expression level has a measurable affect on flowering in noninductive photoperiods.

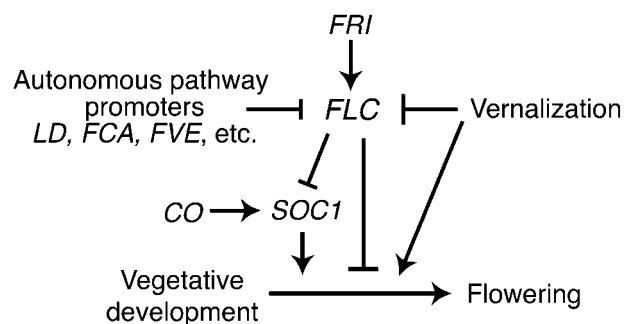


Figure 6. Local Model for the Interaction of *FLC*, *FRI*, the Autonomous Pathway, and Vernalization in the Regulation of Flowering Time.

We also examined the effect of *FLC* on *SOC1* expression. Previous studies have shown that *SOC1* has an expression pattern opposite that of *FLC* (Lee et al., 2000; Samach et al., 2000): *SOC1* is downregulated by *FRI* or mutations in the autonomous pathway and is upregulated by vernalization. The antagonistic expression patterns of *FLC* and *SOC1* are consistent with a model in which *FLC* acts as a negative regulator of *SOC1* and that *FRI*, the autonomous pathway, and vernalization affect *SOC1* levels via changes in *FLC* expression (Lee et al., 2000; Samach et al., 2000).

In this study, we have directly tested the role of *FLC* in regulating *SOC1* expression, and our results support this model. In the presence of *FRI* or autonomous pathway mutations, *SOC1* levels are suppressed. However, if a null allele of *FLC* is combined with *FRI* or autonomous pathway mutations, the suppression is eliminated. Therefore, *FLC* is required for the suppression of *SOC1* by *FRI* or autonomous pathway mutations. Furthermore, overexpression of *FLC* from a constitutive promoter can suppress *SOC1* levels in the absence of *FRI* or autonomous pathway mutations. Thus, *FLC* expression is sufficient to suppress *SOC1* levels. It should be noted, however, that *SOC1* expression is not controlled exclusively via *FLC*, because overexpression of the photoperiod pathway gene *CO*, which does not affect *FLC* levels, is able to increase *SOC1* expression (Samach et al., 2000). Also, the inhibition of flowering by *FLC* in lines containing *FRI* or autonomous pathway mutants is unlikely to be due solely to the downregulation of *SOC1* because the late-flowering phenotypes of these lines are much more severe than are those of the *soc1* null allele. Thus, the downregulation of *SOC1* alone cannot account for the lateness of flowering in lines containing *FRI* or autonomous pathway mutants, and *FLC* likely acts to block flowering via *SOC1* downregulation as well as via *SOC1*-independent mechanisms (Figure 6).

Recent experiments have shown that *FLC* expression plays a key role in creating a vernalization requirement in *Arabidopsis* (Michaels and Amasino, 1999, 2000; Sheldon et al., 1999, 2000). Lines containing *FRI* or autonomous pathway mutations have relatively high levels of *FLC* expression and are late flowering unless vernalized, which leads to a mitotically stable inactivation of *FLC* expression (Michaels and Amasino, 1999; Sheldon et al., 1999). The data presented here show that *FLC* inactivation by loss-of-function mutations also is effective at promoting flowering in these vernalization-requiring backgrounds. Conversely, transgenic plants with constitutive *FLC* expression are late flowering and insensitive to vernalization (Michaels and Amasino, 1999; Sheldon et al., 1999). Thus, the downregulation of *FLC* expression by cold appears to be key in the promotion of flowering by vernalization. The results presented in this study, however, demonstrate that the *flc-3* null allele of *FLC* remains responsive to vernalization. Thus, although *FLC* clearly is a major target of the vernalization pathway, vernalization is capable of promoting flowering via *FLC*-independent mechanisms as well (Figure 6).

FLC plays a central role in the regulation of flowering time in *Arabidopsis* by integrating signals from *FRI*, the autonomous floral promotion pathway, and the vernalization pathway. The data presented here show that the effects of *FRI* and autonomous pathway mutants on flowering time and *SOC1* expression are dependent on a functional allele of *FLC*. The vernalization pathway, however, is able to promote flowering in the absence of *FLC* and thus is capable of promoting flowering via *FLC*-independent as well as *FLC*-dependent mechanisms.

METHODS

Plant Materials

The *Arabidopsis thaliana* *FLC* null allele *flc-3*, *FRI/FRI*; *FLC/FLC*, *FRI/FRI*; *flc/flc*, *ld-1*, and *co-1* are in the Columbia (Col) background and have been described previously (Redei, 1962; Lee et al., 1993; Michaels and Amasino, 1999). *fca* was isolated from T-DNA mutagenesis in the Wassilewskija background. *fpa* and *gi* were isolated from T-DNA mutagenesis in the Col background. The mutants *fve* and *soc1* were kindly provided by Jose Martinez-Zapater (Universidad Autonoma, Madrid, Spain) and Marty Yanofsky (University of California–San Diego, La Jolla) and are in the Col background.

Plant Growth Conditions

All plants were grown under $\sim 100 \mu\text{E m}^{-2} \text{sec}^{-1}$ cool-white fluorescent light at 22°C. Long-day (LD) conditions consisted of 16 hr of light followed by 8 hr of darkness; short-day (SD) conditions consisted of 8 hr of light followed by 16 hr of darkness. For experiments involving vernalization, seeds were plated on agar-solidified medium containing 0.65 g/L Peter's Excel 15-5-15 fertilizer (Grace Sierra, Milpitas, CA) and were kept at room temperature overnight to allow them to become metabolically active before being transferred to 2°C for 70 days. During cold treatment, samples were kept under SD conditions. A minimum of 10 plants was averaged for each data point.

RNA Gel Blot Analysis

Total RNA was isolated using RNA Isolator (Genosys Biotechnologies, The Woodlands, TX), according to the manufacturer's instructions. For RNA gel blots, 15 μg of RNA was separated by denaturing formaldehyde agarose gel electrophoresis as described by Sambrook et al. (1989). RNA gel blots were probed with a ^{32}P -ATP-labeled cDNA fragment that did not contain the conserved MADS box domains of *FLC* or *SOC1*. Blots also were probed with an 18S rRNA probe as a control for the quantity of RNA loaded.

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Loss of *FLOWERING LOCUS C* Activity Eliminates the Late-Flowering Phenotype of *FRIGIDA* and Autonomous Pathway Mutations but Not Responsiveness to Vernalization

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