Photoperiod Regulates Flower Meristem Development in *Arabidopsis thaliana*

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ABSTRACT

Photoperiod has been known to regulate flowering time in many plant species. In Arabidopsis, genes in the long day (LD) pathway detect photoperiod and promote flowering under LD. It was previously reported that *clavata2* (*cla2*) mutants grown under short day (SD) conditions showed suppression of the flower meristem defects, namely the accumulation of stem cells and the resulting production of extra floral organs. Detailed analysis of this phenomenon presented here demonstrates that the suppression is a true photoperiodic response mediated by the inactivation of the LD pathway under SD. Inactivation of the LD pathway was sufficient to suppress the *cla2* defects under LD, and activation of the LD pathway under SD conditions restored *cla2* phenotypes. These results reveal a novel role of photoperiod in flower meristem development in Arabidopsis.

Flower meristem defects of *cla1* and *cla2* mutants are also suppressed under SD, and 35S:CO enhanced the defects of *cla3*, indicating that the LD pathway works independently from the CLV genes. A model is proposed to explain the interactions between photoperiod and the CLV genes.

**B**eing sessile, plants have developed mechanisms to detect and respond to a wide range of environmental changes. These changes include photoperiod, which is the length of light and dark periods each day (reviewed in Thomas and Vince-Prue 1997). In many habitats, photoperiod becomes longer from spring to summer and becomes shorter from fall to winter. Photoperiod is therefore a good indicator of impending environmental changes, such as a drop in temperature, and it is not surprising that plants use photoperiod to control their flowering time.

*Arabidopsis thaliana* is a facultative long day (LD) plant whose flowering is delayed under short day (SD) conditions. Molecular genetic analyses using Arabidopsis have identified genes involved in detecting photoperiod (reviewed in Reeves and Coupland 2000). Loss-of-function mutations in *CRYPTOCHROME2* (*CRY2*), *CONSTANS* (*CO*), and *FLOWERING LOCUS T* (*FT*) genes cause almost day-neutral late flowering, indicating that these genes normally promote flowering in response to LD.

*CRY2* is a photoreceptor for blue light, which promotes flowering in Arabidopsis (Guo et al. 1998). *CRY2* is a positive regulator of *CO* activity (Suarez-Lopez et al. 2001; Yanovsky and Kay 2002). The *CO* gene encodes a zinc-finger protein and *CO* mRNA is more abundant in plants under LD than under SD, indicating that *CO* transcript accumulation is promoted by LD signaling (Putterill et al. 1995). Overexpression of *CO* is sufficient to cause day-neutral early flowering (Onouchi et al. 2000). Another red/far-red photoreceptor, PHYTOCHROME B (*PHYB*), inhibits flowering, and *phyB* mutants flower earlier than wild type under both LD and SD (Reed et al. 1993). Investigations into *phyB* mutants indicate that the early flowering of *phyB* is partially dependent on *CO* and that *PHYB* regulates *CO* protein, but not mRNA, levels (Putterill et al. 1995; Blazquez and Weigel 1999; Valverde et al. 2004).

The circadian clock is also involved in measuring photoperiod. Mutations in *GI* and *ELF3* genes affect both circadian rhythms and flowering time (Koornneef et al. 1991; Hicks et al. 1996; Zagotta et al. 1996; Park et al. 1999). *gi* mutants show day-neutral late flowering and *elf3* mutants show day-neutral early flowering. *CO* mRNA level is circadian regulated, and its abundance is decreased in *gi* mutants and increased in *elf3* mutants (Suarez-Lopez et al. 2001). *GI* encodes a nuclear protein of unknown function and *ELF3* encodes a novel nuclear protein that may be a transcription factor (Fowler et al. 1999; Park et al. 1999; Huq et al. 2000; Hicks et al. 2001). Thus, photoreceptors and circadian clock-related genes seem to promote flowering by upregulating *CO* in response to LD conditions (Suarez-Lopez et al. 2001; Valverde et al. 2004).

Recent studies have shown that *CO* directly activates transcription of several genes including *FT* (Samach et al. 2000). *FT* encodes a protein with similarity to a membrane-associated mammalian protein (Kardailsky et al. 1999; Kobayashi et al. 1999). It is not known how *FT* functions to promote flowering.

While flowering time control is the major effect of photoperiod detection in plants, photoperiod seems to influence some of the later stages of flower development

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in many plant species (Thomas and Vince-Prue 1997). After the induction of floral primordia, the flower meristem is established and produces floral organs. Changes in photoperiod are known to cause arrest of floral primordia, male sterility, floral reversion, and changes in sex expression in a variety of plant species (reviewed in Thomas and Vince-Prue 1997). In Arabidopsis, the role of photoperiod in promoting flower meristem identity was revealed in several mutant backgrounds (Okamura et al. 1996, 1997; Mizukami and Ma 1997). However, little is known at the molecular genetic level how photoperiod affects flower meristem development.

Studies have shown that flower and shoot meristems carry undifferentiated cells that function in a way similar to that of stem cells in animals by continuously replenishing themselves while providing cells for organ formation (reviewed in Clark 2001). Several genes are known to regulate meristem development in Arabidopsis. In plants homozygous for mutations in clavata1 (clv1), clv2, or clv3, stem cells continuously accumulate at the shoot apical meristem, making its size larger as the plants grow (Clark et al. 1993, 1995; Kayes and Clark 1998). Lateral and flower meristems are similarly affected, and increased flower meristem size leads to more floral organs, especially the fourth whorl carpels. Genetic analysis suggested that the three CLV genes may function in the same pathway to regulate stem cells in the meristem. CLV1 encodes a transmembrane receptor kinase that is expressed specifically at the center of the meristem and CLV2 encodes a receptor-like protein with a predicted short cytoplasmic tail (Clark et al. 1997; Jeong et al. 1999). Both CLV1 and CLV2 have extracellular domains with leucine-rich repeats (LRRs) that are protein-protein interaction motifs. CLV3 encodes a 96-amino-acid protein with putative signal sequence, which functions as a diffusible signal in the meristem (Fletcher et al. 1999; Rojo et al. 2002; Lenhard and Laux 2003). Most clv1 alleles are dominant negative and likely interfere with the activity of other receptors with functional overlap (Dievart et al. 2003).

Interestingly, the clv2 flower meristem phenotype is strongly suppressed (returned to normal) under SD (Kayes and Clark 1998). Because clv2 null alleles are suppressed (Jeong et al. 1999), the mechanism of suppression must act not on CLV2, but on other genes regulating flower meristem development. This unique phenomenon seems to indicate a hidden role of photoperiod in flower meristem development because there is no apparent difference in wild-type flowers under LD or SD. In addition, because it occurs in the clv2 mutant background, genes involved in the suppression may interact with the CLV signaling pathway. We have conducted a detailed analysis of this phenomenon and reported here that the clv2 SD suppression is regulated by genes in the LD-dependent flowering pathway that may target proteins downstream of the CLV genes.

**MATERIALS AND METHODS**

**Plant growth:** Plants under LD conditions were grown under continuous light as described by Jeong et al. (1999). Plants under SD conditions were grown at 22°C in a Conviron chamber (Controlled Environments, Pembian, ND) under ~900 ft·c of constant cool white fluorescent light for 8 hr each day. For night-break treatment, plants were first grown under SD without night break for 5 weeks. Plants were then switched to SD plus night break in which they were illuminated by four low fluorescence incandescent red lights for 30 min. in the exact middle of the night period each day until the end of the experiment.

**Plant material:** The co-6, gi-6, fca-6, fha-1, ft-1, phyB-5, and spy-1 alleles were obtained from the Arabidopsis Stock Center (http://www.arabidopsis.org/). elf-3-1 was kindly provided by D. Ry Wagner. 35S:CO, 35S:CO ft-1, and 35S:FT were kindly provided by George Coupland and 35S:CLV3 was kindly provided by Jennifer Fletcher. All clv mutants have been previously described. The backgrounds of the mutations used in genetic studies with clv-1 were all Landsberg er (Ler), except elf-3-1 (which was twice backcrossed from Columbia to Ler) and spy-1 (Columbia).

**Construction of double mutants:** In general, reciprocal crosses were performed, if possible, between the various mutants and clv-2-1. Each single mutant was identified in the F2 population and lines segregating double mutants were found from the selfed progeny in F3. Double mutants were confirmed among their selfed progeny. For each clv2 double mutant, more than three independent lines were obtained and studied, unless specified. For spy-1, which is partially male sterile, clv-2-1 was used as a pollen donor. Because the clv2-1 spy-1 plants were almost completely sterile, seeds from multiple lines of the double mutants were bulked together and used for analysis. For ft-1, which is closely linked to clv-2-1, ~20,000 F2 seeds were sown; flowering seedlings were removed until only late-flowering plants were left; and then the plants were screened for Cv22 flower phenotypes. Two independent lines were obtained and confirmed by PCR analysis. The FT PCR marker consisted of 5′CTA CAA CTG GAA CAA CCT TTG 3′ and 5′ATC ATC ACC GTT CGT TAC TC 3′. After amplification, the DNA was digested with NdeI. This produced fragment sizes of 328, 135, and 17 bp in wild type and fragment sizes of 328 and 152 bp in ft-1. The CLV2 PCR marker consisted of 5′ GTG TGG AGA GTG GTG TCT CCT TTG 3′ and 5′ CTC TGG ATG TGA AAG CTT TGT CAT CAT 3′. After amplification, 119-bp products are obtained. Digestion with NdeI produced fragment sizes of 88 and 31 bp in clv-2-1.

**Carpel counting:** Mature siliques were individually observed under a dissection microscope. Both ends of the siliques were looked at to determine the carpel number and a partially formed carpel was counted as one.

**RESULTS**

The suppression of clv2 phenotypes is a true photoperiod response: To test if the suppression of clv2 under SD was a true photoperiod response or the consequence of reduced light energy, we used a night-break treatment in which the long night period is broken with a brief light treatment. Night break has been used to mimic the effect of LD, without significantly increasing light energy (Reed et al. 1994). Night-break treatment added to SD photoperiod promotes flowering in plants that normally flower in response to LD (Thomas and Vince-Prue 1997).
Wild-type and clv2-1 plants were first grown under SD for 5 weeks. The 16-hr night periods were then interrupted by low-fluence red light treatment (night break). As expected, the treatment induced flowering. While wild-type flowers were unaffected by the night-break treatment, the suppression of the clv2-1 flower phenotype was clearly disrupted by the treatment (Table 1). The clv2-1 flowers in SD plus night break showed mutant phenotypes similar to LD-grown clv2. These results demonstrate that it is photoperiod conditions and not other aspects of growth under SD that are responsible for the clv2 phenotypic suppression.

The SD suppression of clv2 phenotypes is dependent on ELF3 and PHYB: Next we sought to identify genes involved in the suppression of clv2 phenotypes under SD. Obvious candidates were the genes in the LD pathway for induction of flowering as they are involved in detecting LD photoperiod. Mutations in these genes do not delay flowering under SD, indicating that the LD pathway is not active under SD (Reeves and Coupland 2000). We hypothesized that the inactivation of the LD pathway is responsible for the suppression of clv2 flower meristem defects under SD.

ELF3 is known to repress the LD pathway. elf3 mutants exhibit constitutive activation of CO and photoperiod-insensitive early flowering (Zagotta et al. 1996; Suarez-Lopez et al. 2001). If clv2 phenotypes require the LD pathway, then clv2-1 elf3-1 double mutants would be expected to exhibit clv2 flower meristem phenotypes in SD. clv2-1 phyB-5 double mutants were also assessed, because PHYB may be involved in repression of CO activity under SD (Putterill et al. 1995). As a control for early flowering per se, we used a mutation that is known to cause early flowering through different mechanisms. Mutations in the SPINDLY (SPY) gene display phenotypes related to constitutive GA signaling, including early flowering (Jacobsen and Olszewski 1993). The spy-1 allele was chosen because it has the strongest flowering phenotype. However, it was later reported that this allele carries a mutation in a linked gene LONG HYPOCOTYL 2 (HY2; Jacobsen et al. 1996). HY2 gene encodes phytochromobilin synthase, which makes the chromophore for phytochromes (Kohchi et al. 2001). Because hy2 mutants flower early and spy-1 hy2 flowers earlier than spy null alleles, synergistic effect between hy2 and spy is likely to be responsible for the strong early flowering phenotype of spy-1 hy2 double mutants (Koornneef et al. 1980; Jacobsen et al. 1996). This actually makes the spy-1 allele a better control for our analysis because spy-1 hy2 flowers nearly as early as elf3-1. Under our growth conditions, clv2-1 elf3-1 and clv2-1 spy-1 hy2 flowered with 2.13 ± 0.135 (n = 69) and 2.83 ± 0.238 (n = 65; mean ± 95% C.I.) leaves under SD, respectively.

When these genetic combinations were analyzed under SD, clv2-1 elf3-1 flowers showed dramatic restoration of clv2 flower meristem defects (Table 2). clv2-1 phyB-5 flowers displayed a weaker restoration of clv2 phenotypes, which is consistent with the observation that phyB plants still show some response to LD, indicating the LD pathway is still partially active phyb mutants (Reed et al. 1994). clv2-1 spy-1 hy2 plants showed no reversion of the suppression despite flowering as early as clv2-1 elf3-1 plants, demonstrating that it is not early flowering per se but activation of the LD pathway that is responsible for the reversion of clv2 phenotypes. Therefore, the repression of the LD pathway by the ELF3 and PHYB genes is critical for the suppression of clv2 flower meristem defects under SD.

Under LD, all of the early flowering mutations showed additive interactions with clv2 except spy-1 hy2. Flowers of clv2-1 spy-1 hy2 are more sterile than spy-1 hy2, and the carpels are severely malformed (data not shown).

The LD pathway is required for clv2 defects under LD: If inactivation of the LD pathway is responsible for the suppression of clv2 flower meristem defects under SD, then genetically inactivating the LD pathway in a clv2 background should suppress the clv2 flower phenotypes. To test this idea, we made double mutants between clv2-1 and mutations in the LD pathway, co-6, fr-6, ft-1, and ft-1. Our hypothesis predicts that clv2 flowers would be suppressed even under LD in these mutant backgrounds. While these mutations cause late flowering only under LD, mutations in the FCA gene delay flowering under both LD and SD (Koornneef et al. 1991; Macknight et al. 1997). Because fca mutations are still responsive to LD, we used fca-6 to distinguish between general flowering delay and photoperiod effect.

When the double mutants were assessed under LD photoperiod, all of the mutations in the LD pathway strongly suppressed clv2 flower phenotypes (Table 3). The observation that co-6 strongly, but not completely.
suppressed clv2 phenotypes may be due to the fact that co-6 is not a null allele (Robson et al. 2001). The clv2-1 ft-1 double mutant displayed a phenotype most similar to the wild type, with nearly all flowers composed of two carpels and with the absence of a valveless phenotype (see below). In contrast, the fca-6 mutation only weakly modified the clv2 phenotype (Table 3). The weak suppression by fca-6 could be explained by recent data that FT mRNA level is reduced in fca mutants, which indicate cross-talk between FCA and the LD pathway (Samach et al. 2000). These results show that the inactivation of the LD pathway is sufficient to cause suppression of clv2 flower meristem defects.

One factor to consider when looking at the mean carpel number is the valveless phenotype of clv2. This phenotype is variably expressive and can be enhanced in certain genetic backgrounds (Kayes et al. 1998). Because valves are scored to determine the numbers of carpels present within a gynoecium, flowers with one or zero valves could affect the mean carpel number. For example, both clv2-1 gi-6 and clv2-1 fha-1 flowers exhibited a low, but significant level of valvelessness (Table 3). This slightly exaggerates the level of clv2 suppression caused by these two mutations. If only clv2-1 gi-6 flowers with two or more carpels were included, the mean would be 2.13 carpels per flower. Moreover, the presence of valveless flowers in these double mutants reveals that not all clv2 defects are suppressed by inactivation of GI or FHA.

**clv1 and clv3 flower meristem defects are suppressed under SD:** On the basis of analysis of clv1-6 and clv3-1 alleles, it had been previously proposed that the SD suppression was specific to clv2 (Kayes and Clark 1998). In light of the recent determination of the dominant-negative character of most clv1 alleles (Dievart et al. 2003), we repeated these experiments on a larger scale. A variety of clv1 and clv3 alleles were assessed in detail under LD and SD growth conditions (Table 4). The clv1-7 allele, which encodes a protein without the kinase domain, and three T-DNA insertion null alleles of clv1 were all suppressed under SD (Table 4). Flowers of clv3-1 and clv3-3 plants also showed suppression under SD (Table 4). These results clearly show that the SD suppression occurs in clv1 and clv3 plants as well as clv2 plants.

**35S:CO affects flower meristem development independent of CLV3:** It has been reported that the 35S:CO plants develop flowers that exhibit clv phenotypes (Onouchi et al. 2000). To investigate this in detail, we examined the mean carpel number of the 35S:CO flowers under LD and SD, revealing a significant in-

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**TABLE 2**

ELF3 and PHYB are required for clv2 phenotypic suppression

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Short day</th>
<th>Extra (%)</th>
<th>Long day</th>
</tr>
</thead>
<tbody>
<tr>
<td>clv2-1</td>
<td>2.36 ± 0.051 (540)</td>
<td>29.6</td>
<td>3.74 ± 0.074 (290)</td>
</tr>
<tr>
<td>phyB-5 clv2-1</td>
<td>2.89 ± 0.088 (316)</td>
<td>62.7</td>
<td>3.87 ± 0.011 (550)</td>
</tr>
<tr>
<td>elf3-1 clv2-1</td>
<td>3.40 ± 0.077 (358)</td>
<td>83.0</td>
<td>3.61 ± 0.095 (116)</td>
</tr>
<tr>
<td>spy-1 hy2 clv2-1</td>
<td>2.06 ± 0.025 (425)</td>
<td>5.18</td>
<td>ND</td>
</tr>
</tbody>
</table>

Notes: ND, not determined.

† Values represent the mean number of carpels per flower ±95% C.I. Total number of flowers analyzed is given in parentheses. Only the first 10 flowers of any given plant were analyzed. Results from several different experiments were combined.

† Percentage of flowers with more than two carpels.

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**TABLE 3**

Inactivation of the LD pathway suppresses clv2 phenotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Long day</th>
<th>Extra (%)</th>
<th>One carpel (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ler</td>
<td>2.01 ± 0.020 (140)</td>
<td>1.43</td>
<td>0</td>
</tr>
<tr>
<td>clv2-1</td>
<td>3.76 ± 0.065 (410)</td>
<td>95.9</td>
<td>0</td>
</tr>
<tr>
<td>fha-1 clv2-1</td>
<td>2.13 ± 0.055 (299)</td>
<td>13.7</td>
<td>3.01</td>
</tr>
<tr>
<td>gi-6 clv2-1</td>
<td>2.04 ± 0.047 (380)</td>
<td>11.1</td>
<td>7.63</td>
</tr>
<tr>
<td>co-6 clv2-1</td>
<td>2.34 ± 0.081 (190)</td>
<td>28.4</td>
<td>0</td>
</tr>
<tr>
<td>ft-1 clv2-1</td>
<td>2.03 ± 0.013 (700)</td>
<td>2.71</td>
<td>0.14</td>
</tr>
<tr>
<td>fca-6 clv2-1</td>
<td>3.17 ± 0.066 (570)</td>
<td>76.3</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Notes: † Values represent the mean number of carpels per flower ±95% C.I. Total number of flowers analyzed is given in parentheses. Only the first 10 flowers of any given plant were analyzed. Results from several different experiments were combined.

† Percentage of flowers with more than two carpels.

† Percentage of flowers with only one carpel.
increase in mean carpel number (Table 5). This CO gain-of-function phenotype is consistent with our data that co loss-of-function mutations suppressed clv2 flower meristem defects (Table 3). The 35S:CO flowers did not show obvious suppression under SD, consistent with our model that the LD pathway needs to be inactive for the SD suppression (Table 5). CO is known to directly activate transcription of FT and other genes (Samach et al. 2000). Interestingly, the ft-1 mutation suppressed the Clv− phenotype of 35S:CO plants (Table 5), indicating that FT is largely necessary for CO to regulate flower meristem development. Consistent with this, we found that 35S:FT plants also display weak Clv− flowers (Table 5).

To determine if 35S:CO was acting to inhibit the CLV pathway or acting independently, we generated 35S:CO clv3-2 double mutants. If 35S:CO affects components of the CLV pathway to cause a clv phenotype, it should not enhance the defects of the clv3-2 allele, which is thought to represent the null phenotype for the CLV pathway. However, 35S:CO significantly enhanced clv3-2 (Table 5), demonstrating that, at least when overexpressed, CO can function independently from the CLV genes to regulate flower meristem development.

**Dominant-negative clv alleles are largely unaffected by SD:** All clv1 alleles with strong or intermediate phenotypes are dominant negative and likely interfere with functionally related receptor kinases (Dievart et al. 2003). In contrast to the clv1 null alleles, these dominant-negative alleles showed reduced degree of suppression under SD (Table 4). While the clv1-6 allele is only slightly stronger than clv1 null alleles, clv1-6 flowers showed much less suppression than the null alleles. clv1-1, which is stronger than clv1-6, showed very little suppression. This is not due to the fact that their defects are more severe than those of the null alleles because the clv3-1 allele that is stronger than clv1-1 is well suppressed (Table 4). These results indicate that the clv1-6 and clv1-1 proteins are interfering with protein(s) required for the SD suppression.

This idea is further supported by analysis of the clv1-10 allele. clv1-10 was isolated as an intragenic enhancer of clv1-1 and has an even stronger dominant-negative effect as a result of a second missense mutation in the LRR domain (Dievart et al. 2003). clv1-10 flowers were found to have 7.57 carpels per flower under LD, making it the clv1 allele with the strongest flower phenotype (Table 4). Because clv1-10 is stronger than clv3-2 single mutants and all previously characterized clv1 clv2, clv1 clv3, and clv2 clv3 double mutants, clv1-10 protein should be interfering with factors outside the CLV pathway. These results suggest that the clv1-10 protein and the LD pathway may be acting on the same target. In this regard, it is interesting that the average carpel number of clv1-10 mutants closely matches with that of 35S:CO clv3-2 double mutants (Table 4).

### TABLE 4

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Long day</th>
<th>Short day</th>
<th>95% C.I.</th>
<th>Total number of flowers analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>clv1-7</td>
<td>3.17 ± 0.076 (480)</td>
<td>2.45 ± 0.065 (400)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clv1-12 (Col)</td>
<td>2.69 ± 0.110 (210)</td>
<td>2.31 ± 0.124 (70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clv1-13 (Col)</td>
<td>2.81 ± 0.126 (150)</td>
<td>2.18 ± 0.139 (40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clv1-11</td>
<td>3.66 ± 0.029 (620)</td>
<td>2.64 ± 0.086 (240)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clv3-1</td>
<td>6.03 ± 0.090 (320)</td>
<td>4.65 ± 0.135 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clv3-3</td>
<td>2.60 ± 0.084 (280)</td>
<td>2.02 ± 0.025 (140)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clv1-6</td>
<td>3.84 ± 0.041 (600)</td>
<td>3.45 ± 0.082 (270)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clv1-1</td>
<td>4.46 ± 0.057 (450)</td>
<td>4.31 ± 0.065 (370)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clv1-10</td>
<td>7.57 ± 0.121 (300)</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND, not determined.

* Values represent the mean number of carpels per flower ±95% C.I. Total number of flowers analyzed is given in parentheses. Only the first 10 flowers of any given plant were analyzed. Results from several different experiments were combined.

### TABLE 5

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Long day</th>
<th>Short day</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>35S:CO</td>
<td>2.33 ± 0.060 (389)</td>
<td>2.21 ± 0.052 (443)</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>clv3-2</td>
<td>6.25 ± 0.107 (310)</td>
<td>ND</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>35S:CO clv3-2</td>
<td>7.57 ± 0.205 (130)*</td>
<td>ND</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>35S:CO ft-1</td>
<td>2.08 ± 0.053 (110)**</td>
<td>ND</td>
<td>0.01*; &lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>35S:FT</td>
<td>2.10 ± 0.059 (106)**</td>
<td>ND</td>
<td>0.003*</td>
<td></td>
</tr>
</tbody>
</table>

ND, not determined; NA, not applicable.

* Values represent the mean number of carpels per flower ±95% C.I. Total number of flowers analyzed is given in parentheses. Only the first 10 flowers of any given plant were analyzed. Results from several different experiments were combined.

**Student’s t-test value for mean differing from Col (see Table 3).**

***Student’s t-test value for mean differing from clv3-2.***

****Student’s t-test value for mean differing from 35S:CO.**
studies of clv mutants, it has been a standard procedure to score the first 10 flowers per plant for severity of flower phenotypes. However, during our studies with clv2 plants under SD, we noticed that at the top of the inflorescence, flowers display the clv2 phenotype. To determine the exact pattern of the suppression, all of the flowers along the inflorescences were scored and compared (Table 6). Flowers from position 11 to 20 showed less suppression compared to the first 10 flowers. When the most acropetal 10 flowers, not overlapping with the flowers 11 to 20, were counted, these flowers were similar to LD-grown clv2 flowers. These results clearly show an acropetal gradient of the suppression along the inflorescences of clv2 plants. Similar gradients were seen along the inflorescences of clv1 or clv3 plants under SD and in clv2 double mutants with cof, ft-1, gi-6, cry2, and for-1 mutants under LD (data not shown).

### DISCUSSION

**Photoperiod regulates flower meristem development in Arabidopsis:** In Arabidopsis, photoperiod is known to play a major role in promoting the transition to flowering (KOORNNEEF et al. 1998; LEVY and DEAN 1998; PINIERO and COUPLAND 1998). The results reported here demonstrate that photoperiod has a novel role in flower meristem development in Arabidopsis. The suppression of clv2 flower meristem defects under SD is a true photoperiodic response because the night-break treatment reversed the effect of SD (Table 1). The requirement of an unbroken night period is the hallmark of photoperiodic responses (THOMAS and VINCE-PTRUE 1997). Because the night break did not lead to the full clv2− phenotype, one may argue that the suppression is a function of the age of the plants and not the photoperiod (i.e., the longer clv2 plants grow before flowering, the more suppressed the phenotype will be). However, previous analysis of the suppression by SD to LD transfer does not favor this explanation. When clv2 plants were transferred after flowering under SD to LD, the emerging flowers rapidly reverted back to mutant phenotypes, strongly indicating that the age of the plant does not matter (KAYES and CLARK 1998). Rather, it is likely that the night break led to a weaker Clv− phenotype because of the difference in the intensity of LD signaling between our LD growth conditions (constant light) and the simulated LD growth conditions (SD plus night break).

**Genes in the LD pathway are critical for the suppression:** Our genetic analyses strongly indicate that the SD suppression occurs because the genes in the LD-dependent flowering promotion pathway are not active under SD. Constitutive activation of the LD pathway by the clv3-1 mutation was sufficient to block the suppression under SD (Table 2), and inactivation of the LD pathway by cry2-1, gi-6, co-6, and ft-1 mutations was sufficient to cause the suppression even under LD (Table 3). These results also indicate that the LD pathway normally maintains clv2 flower phenotypes under LD conditions. Moreover, we found that the SD suppression is not specific to clv2 mutants (Table 4). It is likely that the suppression of clv1 and clv3 flower meristem defects under SD is mediated by the inactivation of the LD pathway as well. In this model, the LD pathway can be thought of as an enhancer of clv mutant phenotypes. The fact that the effect of photoperiod can be seen only in clv mutant backgrounds indicates that the proposed WUS/CLV3 feedback loop (SCHOOF et al. 2000) may normally mask the effect of photoperiod on flower meristem development.

Our results show that photoperiod regulates flower meristem development in Arabidopsis through activities of the LD pathway genes such as FT. However, these genes may respond to other physiological cues in addition to photoperiod. Therefore, it would be expected that anything that affected the activity of FT would influence flower meristem development in Arabidopsis. In this regard, we propose that the weak suppression of clv2 phenotypes by for-6 is due to the partial control of FT expression by the FCA gene (SAMACH et al. 2000).

**Multiple roles of the LD pathway during Arabidopsis flower development:** CO encodes a putative transcription factor that directly activates transcription of FT and other genes (SAMACH et al. 2000). ft mutations partially suppress the early flowering of the 35S:CO plants, indicating that CO promotes flowering through FT. Our studies using both loss-of-function and gain-of-function mutants revealed that CO and FT play an additional role in flower meristem development (Tables 3 and 5). The

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**TABLE 6**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Long day*</th>
<th>Short day*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First 10</td>
<td>Next 10</td>
</tr>
<tr>
<td>clv2-1</td>
<td>3.76 ± 0.065 (410)</td>
<td>2.36 ± 0.051 (540)</td>
</tr>
</tbody>
</table>

* Values represent the mean number of carpels per flower ±95% C.I. Total number of flowers analyzed is given in parentheses. Only the first 10 flowers of any given plant were analyzed. Results from several different experiments were combined.
ft-1 mutation suppressed the Clv− phenotypes of 35S:CO flowers, indicating that CO regulates flower meristem development through FT. However, 35S:FT phenotypes were much weaker than those of 35S:CO (Tables 3 and 5), while 35S:FT plants flower as early as 35S:CO (Kardailsky et al. 1999; Kobayashi et al. 1999). These results suggest that CO and FT may employ different mechanisms to regulate flowering time and flower meristem development or that the threshold of activity for flower induction and meristem regulation may be different.

Another role of CO has been described in previous studies on the agamous (ag) mutants that make indeterminate flowers (Okamura et al. 1996). Under SD, the ag flowers revert to shoots. This floral reversion also occurs in co ag double mutants under LD (Mizukami and Ma 1997), indicating that the CO gene has a role in maintaining flower meristem identity. Although co mutations cause both the floral reversion in ag and the suppression of flower meristem defects in clv2, the suppression of the flower meristem defects is not due to a reduction of flower meristem identity. While spy and hy1 mutations blocked ag floral reversion under SD (Okamura et al. 1996), these mutations did not block the suppression of clv2 under SD (Table 2; data not shown). Thus, CO seems to affect at least three distinct processes in Arabidopsis by promoting flower initiation, flower meristem identity, and flower stem cell identity. Consistent with these roles, overexpression of CO causes day-neutral early flowering, a terminal flower at the shoot apex, and flowers with Clv− phenotypes, respectively (Onouchi et al. 2000). Many LD pathway components seem to be conserved for all three processes, although differences in the terminal targets of the pathway are likely in each case.

**A genetic model for photoperiodic control of flower meristem development:** On the basis of our results, we propose a model to explain how photoperiod regulates flower meristem development in Arabidopsis (Figure 1). SD suppression of mutants of all three clv loci shows that CLV1, CLV2, or CLV3 are not essential for the suppression (Table 4). Available evidence so far strongly suggests that for meristem development CLV1 and CLV2 receptors cannot signal in clv3-2 mutant background (Kayes and Clark 1998; Brand et al. 2000). Therefore, the clear enhancement of clv3-2 phenotypes by 35S:CO suggests that the LD pathway can function in the absence of CLV signaling (Table 5). The CLV genes eventually regulate transcription of the WUS gene, which is required for stem cell specification at the meristem (Brand et al. 2000; Schoof et al. 2000). wus mutants are unable to initiate or maintain meristems and are epistatic to clv mutations (Laux et al. 1996; Schoof et al. 2000). This model would predict that wus mutations should be epistatic to 35S:CO. In this scenario, signals from the LD pathway and the CLV genes may converge to regulate WUS expression and/or function (Figure 1). We have recently observed that WUS expression is lost specifically from the shoot apical meristems of strong clv mutants (K. A. Green and S. E. Clark, unpublished data), but this should not affect the model because the WUS downregulation does not occur in clv flower meristems.

The observations on the behavior of clv1 dominant-negative alleles suggest an interesting hypothesis on how the LD pathway may regulate WUS in the flower meristem. While clv1 null alleles were clearly suppressed, the clv1 dominant-negative alleles showed much reduced or no suppression of their flower meristem defects (Table 4). It was proposed that the products of the clv1 dominant-negative alleles interfere with additional receptor kinase(s) that are functionally redundant with CLV1 within the meristems (Dievart et al. 2003). Absence of the SD suppression may also occur because the clv1 dominant-negative products interfere with receptor kinase(s). In this scenario, a putative flower receptor kinase (FRK) is inhibited by the LD pathway. Under SD (when the LD pathway is inactive) the FRK would be activated and mask the loss of CLV pathway activity. clv1 dominant-negative alleles will not be phenotypically suppressed because their gene product interferes with the putative FRK. This putative FRK may be specific to the flower meristem because the shoot meristem defects are not suppressed under SD (Kayes and Clark 1998; Figure 1). We would hypothesize that clv1-10 protein would interfere with the function of the flower-specific
FRK. Interestingly, while cle1-10 flower defects were more severe than those of cle3-2 plants, the shoot meristem defects of cle1-10 plants were less severe than those of cle3-2 plants (data not shown), indicating that cle1-10 protein likely interacts with a flower-specific factor outside of the CLV pathway. Recent expression profiling has revealed that the expression of many genes is altered by photoperiod induction (SCHMID et al. 2003). Among the repressed genes are two LRR receptor-like kinases, At5g53320 and At4g18640, which could be candidates for the proposed FRK.

Alternatively, because FT encodes a protein with similarity to a Raf kinase inhibitor (KARDAILSKY et al. 1999; KOBAYASHI et al. 1999), the cle1 dominant-negative proteins may interfere with unknown target proteins of FT. Biochemical studies of cle1 dominant-negative proteins will be essential to test the interactions proposed in these models.

The biological significance of this photoperiodic control of flower meristem development is not clear. One possibility concerns the nature of the ecotypes used in this study, Landsberg erecta (Ler) and Columbia (Col), which are summer annual, rapid cyclers. A. thaliana ecotypes that evolved to summer annual habitat lost a vernation requirement through the accumulation of mutations within the FRI gene (JOHANSON et al. 2000; GAZZANI et al. 2003). This allowed rapid flowering without a wintering over. Interestingly, FRI is a repressor of flowering promoting genes such as FT (reviewed in SIMPSON and DEAN 2002). Therefore, Ler and Col represent unique genetic backgrounds in which FT can be highly expressed in response to LD photoperiod. This increased activity of FT may provide another adaptation for rapid cyclers by repressing the putative FRK pathway to promote stem cells in the flower meristem (Figure 1). Flower meristem development is also sensitive to growth conditions, such that cle mutant phenotypes are sensitive to nutrient and light quality, and even wild-type plants can exhibit weak Clv− flower phenotypes when provided with high light and high fertilizer growth conditions. Ecotypes in the wild certainly experience major differences in resource conditions depending on the timing of their flowering, and rapid-cycling summer annuals may have fewer resources and require less rigid control of differentiation compared to overwintering ecotypes.

Acropetal gradient of the SD suppression: We observed that the SD suppression of cle2 flower meristem defects was not maintained in later-arising flowers (Table 6). The acropetal gradient of suppression was present in every genotype we tested, including cle1, cle3, and all of the cle2 double mutants, although to varying degrees (data not shown). Interestingly, acropetal difference in phenotype has been observed for several mutants in flower meristem identity genes in Arabidopsis. For example, in the floral reversion of ag flowers under SD and in ag co double-mutant flowers under LD, only the first 10–15 flowers were reverted (MIZUKAMI and MA 1997). Later-arising flowers exhibit typical ag phenotypes. In leafy mutants, while early arising flowers develop as shoots, later-arising flowers exhibit more and more floral characteristics (SCHULZT and HAUHN 1991; WEIGEL et al. 1992). It is not clear whether this acropetal increase of flower meristem identity is related to the acropetal loss of the SD suppression.

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