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# Polycomb-Group Proteins and *FLOWERING LOCUS T* Maintain Commitment to Flowering in *Arabidopsis thaliana* C W OPEN

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**The switch from vegetative to reproductive growth is extremely stable even if plants are only transiently exposed to environmental stimuli that trigger flowering. In the photoperiodic pathway, a mobile signal, florigen, encoded by *FLOWERING LOCUS T* (*FT*) in *Arabidopsis thaliana*, induces flowering. Because *FT* activity in leaves is not maintained after transient photoperiodic induction, the molecular basis for stable floral commitment is unclear. Here, we show that Polycomb-group (Pc-G) proteins, which mediate epigenetic gene regulation, maintain the identity of inflorescence and floral meristems after floral induction. Thus, plants with reduced Pc-G activity show a remarkable increase of cauline leaves under noninductive conditions and floral reversion when shifted from inductive to noninductive conditions. These phenotypes are almost completely suppressed by loss of *FLOWERING LOCUS C* (*FLC*) and *SHORT VEGETATIVE PHASE*, which both delay flowering and promote vegetative shoot identity. Upregulation of *FLC* in Pc-G mutants leads to a strong decrease of *FT* expression in inflorescences. We find that this activity of *FT* is needed to prevent floral reversion. Collectively, our results reveal that floral meristem identity is at least partially maintained by a daylength-independent role of *FT* whose expression is indirectly sustained by Pc-G activity.**

## INTRODUCTION

The time of flowering in plants is critically important so that flower and fruit production is coordinated with pollinators and seasons. It is regulated by external environmental factors, notably photoperiod and temperature, which provide seasonal cues, and also by internal factors such as age; most plants are not competent to respond to inductive signals unless they have first completed a juvenile phase (reviewed in Kobayashi and Weigel, 2007; Srikanth and Schmid, 2011). Once triggered, the switch from vegetative growth to flowering is usually irreversible. This is likely important in natural environments so that plants do not make sterile flower/shoot intermediates if inductive signals such as temperature fluctuate. Floral commitment is most apparent in annual plants, which usually do not revert to vegetative growth. In perennial plants, which undergo repeated yearly cycles of vegetative and reproductive growth, although not all meristems switch to reproductive growth in any one year, those that do usually show stable floral commitment (Tooke et al., 2005; Wang et al., 2009). However, there are a few examples of plant species and mutants that show distinct types of floral reversion: inflorescence reversion in which the inflorescence reverts to vegetative growth and flower reversion in which the flower itself has features of the inflorescence or even vegetative identity. In these species, floral reversion occurs primarily if inductive signals are not maintained (Tooke et al., 2005). Despite this, the molecular basis for floral commitment is poorly understood, even in plants like *Arabidopsis thaliana* where the molecular mechanisms triggering flowering are well defined.

Aerial parts of plants are formed from shoot apical meristems (SAMs), which undergo several changes in identity before producing flowers. These changes in meristem identity cause modifications in the shoot structure through changes within the basic unit of the metamer, which is composed of an internode and a node. The latter consists of a leaf or bract that harbors a secondary meristem in its axil that give rise to a side shoot (Evans and Grover, 1940; Schultz and Haughn, 1991). In *Arabidopsis*, the SAM sequentially produces three types of metamers: in the vegetative phase, the SAM generates rosette leaves subtending a lateral shoot; after floral induction, the stem elongates (bolting) and cauline leaves with a side shoot, the paraclade, are formed ($I_1$ stage), finally leading to production of flowers without bracts ($I_2$ stage) (Figure 1A; Schultz and Haughn, 1991; Haughn et al., 1995). Detailed analysis of the SAM at the time of floral induction suggests that the $I_1$ stage is a continuation of the vegetative phase and that the SAM directly produces floral primordia on its flanks after induction (Hempel and Feldman, 1994, 1995). Occasionally, e.g., by shifting wild-type plants from short-day (SD) to continuous light, flowers subtended by bracts (cauline leaves) can be observed, indicating that floral bracts can occur in *Arabidopsis* (Hempel et al., 1998). Furthermore, analysis of *SHOOTMERISTEMLESS* (*STM*) and *AINTEGUMENTA* (*ANT*) expression, which are markers for meristems and organs respectively, indicate that primordia are initially leaf-like in character as they are marked by absence of *STM* expression and presence of *ANT* (flower development stages 0 and 1 in the classification of Smyth et al., 1990; Long and Barton, 2000). In early stage 2, the primordium is partitioned into a meristem, marked by *STM* expression, and a "cryptic" bract marked by absence of *STM* (Long et al., 1996;

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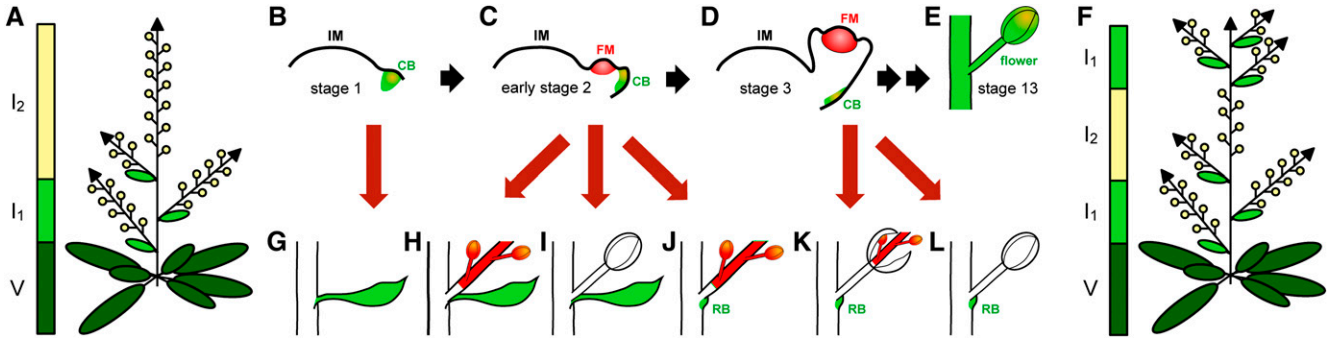


**Figure 1.** Schematic Diagrams of Normal *Arabidopsis* Inflorescence and Flower Development and Floral Reversion.

**(A)** The wild-type SAM produces three metameric types sequentially: rosette leaves are formed at the vegetative state (V), after floral induction, the stem elongates and cauline leaves subtending lateral branches, named paraclades, are generated ($I_1$), followed by production of flowers ($I_2$). After floral induction, flower production is stable even if plants are shifted from LD to SD.

**(B)** to **(E)** Development of the flower metamers (according to Smyth et al. [1990], Chandler [2012], and Long and Barton [2000]): In stage 1 **(B)**, the floral primordium emerges as cryptic bract (CB) and thus has leaf-like identity. In early stage 2 **(C)**, the FM develops between the IM and the cryptic bract. The formation of the sepal primordia at the flanks of the FM marks stage 3 **(D)**. The CB is morphologically not detectable. Stage 13 flower is in **(E)**.

**(F)** Pc-G mutants shifted from LD to SD show floral reversion and, thus, an additional $I_1$ phase after $I_2$.

**(G)** to **(L)** Likely scenario for origin of distinct types of reversion nodes: empty cauline leaf **(G)**, cauline leaf subtending a paraclade **(H)**, cauline leaf subtending a flower **(I)**, paraclade without a cauline leaf but with a rudimentary bract (RB) **(J)**, late reversion of the FM, an inflorescence emerges inside of sepals **(K)**, and flower with RB **(L)**.

Long and Barton, 2000) and briefly morphologically discernable (Kwiatkowska, 2006). During later stages of flower development, cryptic bract outgrowth is suppressed; hence, it is not visible at maturity. Nonetheless, in mutants with compromised floral meristem identity, such as *leafy* (*lfy*) or *apetala1* (*ap1*), the cryptic bract develops more fully and is visible at maturity as a bract subtending flowers or paraclades (Mandel et al., 1992; Weigel et al., 1992). The floral meristem (FM) differs from vegetative or inflorescence SAMs not only in that it makes floral organs, but also in that it is determinate and does not give rise to more meristems or branches.

*Arabidopsis FLOWERING LOCUS T* (*FT*) is the key gene mediating the vegetative to floral transition and is widely conserved in flowering plants (Kardailsky et al., 1999; Kobayashi et al., 1999; Izawa et al., 2002; Kojima et al., 2002). Thus *ft* mutants show delayed flowering but produce normal flowers once the transition occurs. The *FT* product likely corresponds to florigen, the long-sought mobile signal that moves from leaves to the SAM in response to inductive photoperiods and promotes flowering (reviewed in Kobayashi and Weigel, 2007). *FT* is expressed in leaves in inductive photoperiods (long days in *Arabidopsis*) and produces a small protein that moves through the phloem to the shoot apex where it forms a complex with a bZIP transcription factor, FD, to activate targets promoting flower primordium identity including *AP1* and *FRUITFULL* (*FUL*) (Abe et al., 2005; Wigge et al., 2005). A second gene promoting the floral transition is *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*), which encodes a MADS box transcription factor (Samach et al., 2000; Searle et al., 2006). *SOC1*, together with *FUL*, are likely targets needed for *FT* action; thus, *soc1 ful* double mutants largely prevent the early flowering triggered by $35S_{pro}$:*FT* transgenes. In addition, *soc1 ful* double mutants show reversion from inflorescence to vegetative growth, indicating that floral commitment is impaired (Melzer et al., 2008).

Once floral induction has occurred, *LFY* and *AP1* are expressed. Expression of *AP1* is initiated in floral primordia at stage 1 and is thought to mark floral commitment, whereas *LFY* expression initiates in floral anlagen in incipient primordia (stage 0) and is thus expressed before flowers are discernible (Mandel et al., 1992; Weigel et al., 1992). Studies using transient activation of a steroid-dependent *LFY* transgene indicate that persistent *LFY* activity is needed to prevent flower-to-shoot reversion (Wagner and Meyerowitz, 2011), consistent with *lfy* mutants displaying floral reversion under certain conditions (Okamuro et al., 1996). LFY and AP1 proteins bind each other's promoters and upregulate one another's transcription, suggesting that once *LFY* and *AP1* expression are activated in floral primordia they are likely self-perpetuating through positive feedback loops (Wagner et al., 1999; Adrian et al., 2009; Kaufmann et al., 2010).

In addition to flower-promoting factors like *LFY* and *AP1*, the switch to floral meristem identity also requires repression of factors that promote vegetative or inflorescence shoot identity in the early floral primordia. Thus, transcriptional profiling indicates that during the early stages of floral primordium development the majority of genes whose expression significantly changes are downregulated (Wellmer et al., 2006). The downregulated genes include *SHORT VEGETATIVE PHASE* (*SVP*) and *SOC1* which are both direct targets of *AP1* and promote shoot identity (Liu et al., 2009; Kaufmann et al., 2010). Paradoxically, activity of these genes is needed during the earliest stages (stages 0 to 2) of flower development and acts together with *LFY* to activate the floral homeotic genes that specify floral organ identity and determinacy (Liu et al., 2009). Subsequently, *SVP*/*SOC1* need to be switched off for floral meristem identity to be maintained and flower patterning to occur. Thus, expression of *SVP*/*SOC1* transgenes during later stages of flower development causes floral reversion (Liu et al., 2007).

In *Arabidopsis*, as with most plants, flowering persists after transient photoperiodic induction (Corbesier et al., 1996). However, analysis of plants shifted from inductive long-day (LD) to SD conditions shows that both *FT* expression in leaves and *SOC1* expression in the SAM is rapidly lost after the shift (Corbesier et al., 2007; Torti et al., 2012). Floral commitment has similarity with the vernalization response in that both involve a stable memory of a transient environmental stimulus (cold in the case of vernalization), raising the question of whether they share a common mechanistic basis. The memory of vernalization is mediated by Polycomb-group (Pc-G) genes that confer stable epigenetic silencing of a repressor of flowering, *FLOWERING LOCUS C* (*FLC*) (Gendall et al., 2001), which in turn represses expression of *FT* and *SOC1* (Searle et al., 2006). Consistent with their epigenetic role, the Pc-G proteins have biochemical activity toward chromatin; in particular, a complex of four core proteins termed Polycomb Repressive Complex 2 (PRC2) catalyzes the methylation of Lys-27 on histone H3 (H3K27me3), a modification associated with transcriptional repression (reviewed in Margueron and Reinberg, 2011). In *Arabidopsis*, the *MEDEA*, *CURLY LEAF* (*CLF*), and *SWINGER* (*SWN*) genes encode homologs of *Drosophila melanogaster* Enhancer of zeste, the catalytic subunit of the PRC2, while FERTILIZATION INDEPENDENT SEED2, EMBRYONIC FLOWER2 (EMF2), and VERNALIZATION2 (VRN2) show similarity to a second core PRC2 component, *Drosophila* Suppressor of zeste 12 (Chanvivattana et al., 2004). In *clf swn* mutant seedlings, H3K27me3 methylation is completely lost, suggesting that Pc-G activity is eliminated (Lafos et al., 2011). Whereas double mutants for the severe alleles *emf2-3* and *vrn2-1* resemble *clf swn* mutants phenotypically (Schubert et al., 2005), combination of the weaker *emf2-10* allele with *vrn2-1* results in viable, fertile plants, although global H3K27me3 levels are highly reduced (Lafos et al., 2011).

Here, we show that Pc-G proteins are required for floral commitment. Thus, Pc-G mutants show floral reversion such that FMs revert to an earlier inflorescence meristem (IM) identity. Floral reversion in Pc-G mutants requires activity of the two MADS box transcription factors, *FLC* and *SVP*. We show that in plants given a transient photoperiodic induction, *FT* expression does not persist in leaves as previously reported (Corbesier et al., 2007), although it is activated in inflorescences. Furthermore, reduced *FT* expression in inflorescences, both in Pc-G mutants and as a consequence of FT mutation, causes floral reversion. Thus, additional to its known role in triggering the switch to flowering, *FT* has a second role in maintaining flower primordium identity in the inflorescence. Thus, Pc-G proteins maintain commitment to flowering at least partially by promoting *FT* activity in the inflorescence.

## RESULTS

### Generation of Plant Lines with Strongly Depleted Pc-G Activity

After germination, seedlings of mutants lacking Pc-G activity fail to develop leaves or flowers but instead produce embryogenic callus (Figures 2A and 2B; Chanvivattana et al., 2004). Hence, null Pc-G mutants are uninformative for analyzing Pc-G function during vegetative and reproductive development. To reveal whether Pc-G proteins are also required to initiate and maintain reproductive identity, we circumvented this early developmental arrest by constructing a conditional mutant (*iCLF*; see Methods; Figure 2; Supplemental Figure 1) in which a steroid-dependent *CLF* transgene ($CLF_{pro}$*:CLF-GR* described in Schubert et al., 2006) was introduced into the *clf-28 swn-7* background that lacks all Pc-G activity (Lafos et al., 2011). We rescued the early defects by germinating *iCLF* seedlings on steroid-containing media, then withdrew steroid by transplanting to soil. When Pc-G activity was progressively depleted in this way, development continued normally except that leaves were more serrated than normal (Figures 2B and 2C) and most flowers were sterile (Supplemental Figures 1A and 1B). Similar leaf serration is observed in the Pc-G double mutant *emf2-10 vrn2-1* (Figure 2D), which shows a strong global reduction of H3K27me3 levels (Chanvivattana et al., 2004; Lafos et al., 2011). Therefore, these two plant lines, which are deficient in different components of the PRC2, were used to investigate the role of Pc-G in the vegetative and reproductive phase.

### Flower Formation Is Delayed in Plants with Reduced Pc-G Activity

To examine potential defects in flowering time regulation, we grew *emf2-10 vrn2-1* and *iCLF* plants under LD and SD conditions and counted their rosette and cauline leaf number. The *emf2-10 vrn2-1* double mutants flowered appreciably later than the wild type in SD, whereas in LD, their flowering was only slightly delayed (Table 1). The rosette leaf number of *iCLF* plants was also significantly increased in both LD and SD (Supplemental Table 1). Both lines displayed additional developmental defects, including homeotic transformations in organ identity, increased floral organ number, and a lack of floral meristem determinacy evident as a meristematically active fifth whorl (Supplemental Figures 2A and 2B), suggesting misregulation of a similar set of target genes. In addition, when *emf2-10 vrn2-1* mutants were grown in SD, they produced inflorescences with an abnormally high number of cauline leaves (>60) so that flowers were not produced until very late in development (Figure 2E, Table 1). A similar, but less pronounced, phenotype and overall more variability in plant size was observed for *iCLF* plants (Supplemental Figure 1C and Supplemental Table 1). Most of the cauline leaves in *emf2-10 vrn2-1* and *iCLF* in SD did not carry paraclades or any other secondary structure in their axils (73.6%, $n > 750$ for *emf2-10 vrn2-1*; 54.4%, $n > 500$ for *iCLF*). Thus, *emf2-10 vrn2-1* and *iCLF* lines displayed an uncoupling of bolting and flower production under SD conditions, indicating an expanded $I_1$ phase and a requirement for Pc-G proteins to promote the transition from $I_1$ to $I_2$ (Figure 1).

Notably, we observed single flowers in the axils of the last one to five cauline leaves of *emf2-10 vrn2-1* mutants and rudimentary bracts at the base of the pedicels of the flowers in both Pc-G-depleted lines (Figure 1L; Supplemental Figure 2G), indicating a gradual switch from the $I_1$ to $I_2$ phase. Surprisingly, many of the flowers produced in SD in the wild type also showed rudimentary bracts at the base of their pedicels (84.0% for La-0 and 42.5% for Columbia-0 (Col-0), first 20 flowers of 10 plants) and occasionally also stipules at their base (Supplemental Figures 2E and 2F). Thus, even in *Arabidopsis* wild-type, SD conditions induce leaf-like characteristics on flower metamers.

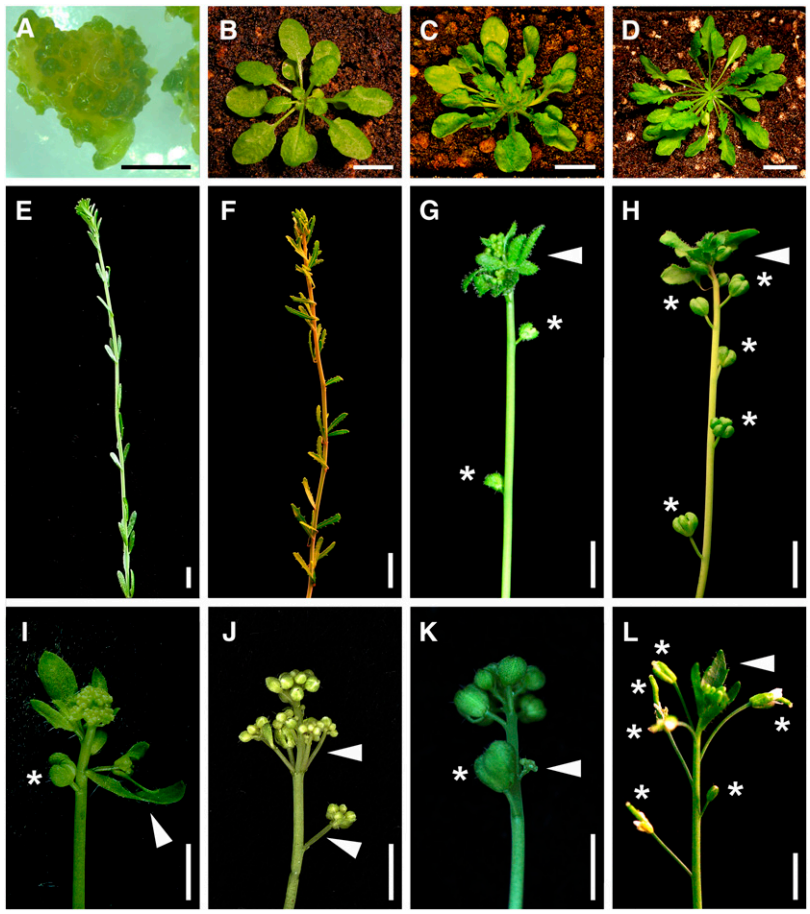


**Figure 2.** Floral Reversion and Vegetative Phenotype of Mutants or Transgenic Lines with Reduced Pc-G Activity.

**(A)** *clf-50 swn-3* callus tissue, 4 months after germination.
**(B)** Wild-type rosette.
**(C)** *iCLF* plant 14 d after withdrawal from dexamethasone.
**(D)** *emf2-10 vrn2-1* double mutants. Plants in **(B)** to **(D)** were grown in SD, 28 DAG.
**(E)** *emf2-10 vrn2-1* stem grown continuously in SD, 85 DAG.
**(F)** *emf2-10 vrn2-1 ft-13* stem shows a similar phenotype under LD (85 DAG) as **(E)** in SD.
**(G)** to **(K)** Stems of plants 35 DAS from LD to SD. *iCLF* **(G)** and *emf2-10 vrn2-1* **(H)**. Arrowhead indicates reversion nodes. *emf2-10 vrn2-1 flc-5* stem **(I)** carries fewer reversion nodes than **(H)**. Arrowhead indicates reversion node with cauline leaf. *emf2-10 vrn2-1 svp-32* **(J)** and *emf2-10 vrn2-1 flc-5 svp-32* **(K)** stem with single reversion node. Arrowheads in **(J)** and **(K)** indicate paraclades without leaf.
**(L)** Floral reversion in *ft-10* under LD, 4 months after germination. Arrowhead indicates leaf at a reversion node.
Asterisks in **(G)** to **(L)** indicate prereversion flowers. Bars = 10 mm **(A)** to **(C)** and **(E)** to **(L)** and 10 mm in **(D)**. See Tables 1, 2, and 4 and Supplemental Table 2 for detailed organ counts.

The leafy shoot phenotype of *emf2-10 vrn2-1* in SD strongly resembled that described for *ft lfy* or *fd lfy* double mutants (Ruiz-Garcia et al., 1997; Abe et al., 2005; Wigge et al., 2005) but *LFY* expression was only moderately reduced (Supplemental Figures 3A, 3B, and 4). Consistent with the extensive uncoupling between bolting and flower production, expression of *AP1*, which is a marker for floral meristem identity, was largely eliminated during the early stages of *emf2-10 vrn2-1* inflorescence development in SD but increased as flowers were produced (Supplemental Figures 3C, 3D, and 4). In addition, expression of *FT* followed a similar trend as *AP1*, whereas *SOC1* and *FUL*, two other key regulators of flowering, showed wild-type expression levels in all stages analyzed (Supplemental Figure 4). Importantly, the floral repressor *FLC* but not *SVP* was upregulated in leafy *emf2-10 vrn2-1* inflorescence apices (Supplemental Figure 4). Using a $FLC_{pro}$:*FLC-GUS* (β-glucuronidase) reporter, we confirmed the higher promoter activity of *FLC* in *emf2-10 vrn2-1* inflorescences compared with the wild type (Supplemental Figures 5A and 5B). Furthermore, the reporter indicated an upregulation of *FLC* in *emf2-10 vrn2-1* rosette leaves, which may explain the delayed flowering of *emf2-10 vrn2-1* in SD (Supplemental Figures 5C and 5D).

Collectively, our analyses of leafy *emf2-10 vrn2-1* inflorescences revealed changes in gene expression for *FLC*, *AP1*, and *FT*, but not for *LFY*, *SOC1*, *FUL*, and *SVP*.

**Table 1.** Flowering Time of Mutants with Reduced Pc-G Activity Measured by Leaf Number

| | LD | | | | SD | | | | Ratio SD/LD | |
|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | *n* | RL | CL | TL | N | RL | CL | TL | RL | CL |
| La-0 | 40 | 6.8 ± 0.2 | 2.7 ± 0.1 | 9.5 ± 0.5 | 35 | 20.3 ± 1.5 | 8.6 ± 0.8 | 28.9 ± 2.1 | 3.0 | 3.2 |
| *ev* | 50 | 12.3 ± 0.3 | 4.5 ± 0.2 | 16.8 ± 0.6 | 50 | 45.7 ± 1.5 | 61.7 ± 4.5 | 107.5 ± 5.2 | 3.7 | 13.7 |
| *ev flc* | 45 | 7.9 ± 0.2 | 4.0 ± 0.2 | 11.9 ± 0.5 | 40 | 23.4 ± 1.2 | 49.0 ± 2.9 | 72.4 ± 3.5 | 3.0 | 12.4 |
| *ev svp* | 20 | 8.9 ± 0.4 | 4.8 ± 0.3[a] | 13.7 ± 0.6 | 20 | 44.4 ± 1.7[a] | 15.3 ± 0.8 | 59.6 ± 1.8 | 5.0 | 3.2 |
| *ev flc svp* | 50 | 5.8 ± 0.3 | 4.0 ± 0.3 | 9.8 ± 0.6[b] | 40 | 15.5 ± 1.0 | 10.6 ± 0.6 | 26.0 ± 1.3[b] | 2.7 | 2.6 |
| *ev ft* | 30 | 42.1 ± 1.2 | >360 | >400 | 10 | 43.7 ± 1.3[a] | >360 | >400 | 1.0 | – |

$P > 0.05$. Data are ± SE. RL, rosette leaves; CL, cauline leaves; TL, total leaves; La-0, Landsberg-0; *ev*, *emf2-10 vrn2-1*; *ev flc*, *emf2-10 vrn2-1 flc-5*; *ev svp*, *emf2-10 vrn2-1 svp-32*; *ev flc svp*, *emf2-10 vrn2-1 flc-5 svp-32*; *ev ft-13*, *emf2-10 vrn2-1 ft-13*.
[a]Not significantly different from *ev*.
[b]Not significantly different from La-0.

### Loss of *FLC*, *SVP*, and *FT* Modulate the *emf2-10 vrn2-1* Leafy Shoot Phenotype

To investigate whether the upregulation of *FLC* is required for the leafy shoot phenotype of *emf2-10 vrn2-1* plants in continuous SD, we created *emf2-10 vrn2-1 flc-5* triple mutants. Because FLC can act in a repressor complex with SVP (Li et al., 2008), we also created *emf2-10 vrn2-1 svp-32* triple and *emf2-10 vrn2-1 flc-5 svp-32* quadruple mutants. Surprisingly, removing *FLC* or *SVP* activity had clearly distinct effects (Table 1). In SD, lack of *FLC* strongly reduced the number of rosette leaves but had little effect on cauline leaf number. By contrast, removing *SVP* activity did not significantly affect rosette leaf number ($P = 0.44$ Student's *t* test), whereas cauline leaf number was reduced to a quarter of that in *emf2-10 vrn2-1*. Importantly, the combined loss of both MADS box transcription factors in *emf2-10 vrn2-1 flc-5 svp-32* quadruple mutants restored cauline leaf number to near wild-type and rosette leaf number was reduced to slightly fewer than the wild type. These results suggested that the prolonged V phase of *emf2-10 vrn2-1* in SD was largely caused by increased *FLC*, whereas the delay in the $I_1$-to-$I_2$ transition required SVP and to lesser extent FLC activity.

The repressor complex of FLC and SVP delays flowering by binding to the promoter of *FT* (Li et al., 2008; Deng et al., 2011). To determine the role of *FT* in delayed flower production in *emf2-10 vrn2-1* mutants, we characterized *emf2-10 vrn2-1 ft-13* triple mutants (Figure 2F, Table 1). In SD, the rosette leaf number of *emf2-10 vrn2-1* double (45.7) and *emf2-10 vrn2-1 ft-13* triple (43.7) mutants were not significantly different ($P = 0.31$, Student's *t* test), indicating that similar to the wild type, *FT* is not active during vegetative development in *emf2-10 vrn2-1* in SD. Unexpectedly, the *emf2-10 vrn2-1 ft-13* plants never produced flowers, but continuously generated cauline leaves after bolting independently of the daylength (>360 after 4 months; Table 1, Figure 2F). Like *emf2-10 vrn2-1* in SD, branching in *emf2-10 vrn2-1 ft-13* was strongly suppressed in both SD and LD (Figures 2E and 2F).

Thus, *FLC* and *SVP* have overlapping and distinct functions in the promotion of bolting and the transition from $I_1$ to $I_2$ in *emf2-10 vrn2-1* mutant plants. In addition, based on genetic analyses of *emf2-10 vrn2-1* and *emf2-10 vrn2-1 ft-13* mutants, *FT* is active in promoting the $I_1$-to-$I_2$ transition in *emf2-10 vrn2-1* inflorescences in SD.

### Photoperiod Shifts Induce Floral Reversion in Plants with Reduced Pc-G Activity

When flowering in continuous LDs, a few (<1% of plants) *iCLF* inflorescences reverted to an earlier developmental stage, so that after producing a series of flowers as normal they went back to producing a single paraclade, suggesting that floral commitment was impaired (Supplemental Figure 1B). To test this further, we provided a transient photoperiodic flowering induction by shifting *iCLF* or *emf2-10 vrn2-1* seedlings from inductive LD to SD 21 to 25 d after germination (DAG). Several weeks later, *iCLF* and *emf2-10 vrn2-1* plants made some flowers but then reverted to cauline leaves and paraclade generation before eventually resuming making flowers (Figures 1A, 1F, 2G, and 2H; Supplemental Figure 1D). By contrast, wild-type plants began flowering in LD and did not show obvious floral reversion of IM and FM when they were shifted to SD, except the presence of rudimentary bracts (Table 2; Supplemental Table 2 and Supplemental Figure 2). These findings indicate that Pc-G function is essential for maintenance of floral meristem identity. We observed a reduction of reversion nodes in both *emf2-10 vrn2-1* and *iCLF* if we delayed the shift to SD, suggesting that plants became less susceptible to reversion as time in LD increased.

To resolve spatial and temporal origin of inflorescence and flower reversion in *emf2-10 vrn2-1* and *iCLF*, we carefully analyzed the floral reversion nodes. Four main classes were revealed, which were the result of either flower reversion or inflorescence reversion or combined flower and inflorescence reversion and usually occurred progressively on the bolting stem (Table 2; Supplemental Table 2): (1) paraclades without cauline leaves but often subtended by a rudimentary bract (Figure 1J; Supplemental Figure 2I); (2) "empty" cauline leaves, i.e., not subtending any secondary structure in their axils (Figure 1G; Supplemental Figures 2I and 2K); (3) cauline leaves subtending a paraclade (Figure 1H; Supplemental Figure 2J); and (4) cauline leaves subtending a normal flower (Figure 1I; Supplemental Figure 2K). The different categories likely result from a loss of floral identity at different stages in primordium

**Table 2.** Strong Floral Reversion Nodes at Main Shoot of Strong Hypomorphic Pc-G Mutants

| Genotype | *n* | PC without CL | CL | CL with PC | CL with F | ∑ |
|---|---|---|---|---|---|---|
| La-0 | 42 | 0 # | 0 # | 0 # | 0 # | 0 # |
| *ev* | 80 | 2.3 ± 0.3 | 2.7 ± 0.6 | 18.5 ± 1.4 | 3.6 ± 0.3 | 27.2 ± 1.6 |
| *ev f* | 82 | 2.9 ± 0.2 | 1.6 ± 0.2 | 5.4 ± 0.4 # | 2.6 ± 0.2 # | 12.6 ± 0.5 # |
| *ev s* | 91 | 10.1 ± 0.6 ‡ | 0.3 ± 0.1 # | 0.1 ± 0.0 # | 0.9 ± 0.1 # | 11.4 ± 0.7 # |
| *ev fs* | 68 | 1.0 ± 0.2 # | 0 # | 0 # | 0.04 ± 0.03 # | 1.0 ± 0.2 # |

Data are ± SE. Significantly fewer reversion nodes than *ev* (Student's *t* test): #P < 0.001. Significantly more reversion nodes than *ev* (Student's *t* test): ‡P < 0.001. CL, cauline leaves; PC, paraclades; F, flowers; La-0, Landsberg-0; *ev*, *emf2-10 vrn2-1*; *ev flc*, *emf2-10 vrn2-1 flc-5*; *ev svp*, *emf2-10 vrn2-1 svp-32*; *ev flc svp*, *emf2-10 vrn2-1 flc-5 svp-32*.

development, as shown in Figures 1B to 1E and 1G to 1K. Interestingly, flower reversion also occurred after sepals were initiated (<5% of strong reversion nodes; Figure 1K; Supplemental Figure 2D) or even from the fifth whorl after carpels had formed so that inflorescences emerged from within the fourth whorl siliques (<1% of strong reversion nodes; Supplemental Figures 2B and 2C). Notably, although the different reversion nodes usually appeared in the order (1) to (4) (see above), we observed fluctuations in the order of successive reversion nodes in *emf2-10 vrn2-1* and *iCLF* (e.g., leaf/flower nodes appeared frequently between nodes with empty cauline leaves; Supplemental Figure 2L), suggesting that meristem identity was unstable and fluctuating around a threshold between the vegetative and reproductive state.

To investigate the morphological changes in the IM and in young primordia in the early stage of floral reversion, we analyzed the distribution of *ANT* and *STM* RNA in tissue sections of shifted *emf2-10 vrn2-1* apices 6 d after shift (DAS) as these two genes provide indirect markers for the cryptic bracts that subtend early stage floral primordia (Long and Barton, 2000). The cryptic bracts were more prominent in shifted *emf2-10 vrn2-1* plants than in the wild type; notably, some primordia at stage 2 did not express *STM* and therefore likely lacked meristematic activity (Supplemental Figures 6A to 6D), suggesting that reversions occur mostly in very early primordium development.

Thus, our detailed analyses of floral reversion in Pc-G mutants indicate that Pc-G proteins are not only required for a timely transition to flower production after bolting in SD but are also needed to maintain floral formation when plants are shifted from inducing to noninducing photoperiods.

### Floral Reversion in Pc-G Mutants Depends on *FLC* and *SVP*

We next sought to determine whether the Pc-G target genes *FLC* and *SVP* are not only required for a delayed bolting and transition from $I_1$ to $I_2$ in Pc-G mutants, but also responsible for floral reversion. Analysis of *FLC* expression by RT-PCR and an $FLC_{pro}$:*FLC-GUS* reporter in inflorescence apices revealed an increase in *emf2-10 vrn2-1* and *iCLF* plants relative to the wild type, most notably when plants were shifted to SD (Figures 3A and 4A; Supplemental Figures 5E to 5H and 7A). For *SVP*, RT-PCR revealed a significant upregulation after the shift for *emf2-10 vrn2-1* but not for *iCLF* inflorescence apices (Figure 4A; Supplemental Figure 7B). Analysis of the spatial expression pattern by in situ hybridization showed stronger accumulation of *SVP* mRNA in inflorescence meristems and in the vasculature beneath the meristem of *emf2-10 vrn2-1* plants shifted to SD compared with *emf2-10 vrn2-1* plants continuously grown in LD (Figure 3B). However, the overall expression pattern of *SVP* was not changed in shifted stage 2 *emf2-10 vrn2-1* floral primordia and was excluded from the cryptic bract as in the wild type (Supplemental Figures 6E and 6F). Chromatin immunoprecipitation (ChIP) assays using wild-type inflorescence apices revealed H3K27me3 enrichment at *FLC* and *SVP* (Figure 4B; Supplemental Figure 7D), confirming that Pc-G proteins directly regulate *FLC* and *SVP* in the inflorescence as well as in seedlings. While H3K27me3 at *FLC* was strongly reduced in *emf2-10 vrn2-1* and *iCLF* in LD and SD, no decrease was detected at *SVP* (Figure 4B; Supplemental Figure 7D).

Collectively, these results indicate that *FLC* and *SVP* are direct targets of Pc-G repression during early floral meristem development, but only alterations in *FLC* H3K27me3 and expression levels are correlated with floral reversion in *emf2-10 vrn2-1* and *iCLF*. To test for a genetic requirement for *FLC* and *SVP*, we analyzed floral reversion in *emf2-10 vrn2-1 flc-5* triple, *emf2-10 vrn2-1 svp-32* triple, and *emf2-10 vrn2-1 flc-5 svp-32* quadruple mutants. Floral reversion was strongly reduced in *emf2-10 vrn2-1 flc-5* and *emf2-10 vrn2-1 svp-32* triple mutants and almost completely abolished in *emf2-10 vrn2-1 flc-5 svp-32* quadruple mutants (Figures 2I to 2K and 4C, Table 2). However, the effects of *flc* and *svp* mutation on the different classes of floral reversion were distinct. In *emf2-10 vrn2-1 flc-5*, reversion nodes consisting of cauline leaves with or without paraclades were similarly reduced (~40% compared with *emf2-10 vrn2-1*), whereas in *emf2-10 vrn2-1 svp-32*, reversion nodes lost their cauline leaves (reduced to 5% compared with *emf2-10 vrn2-1*) and therefore showed an increase in paraclades lacking cauline leaves. However, also in *emf2-10 vrn2-1 svp-32*, the total number of floral reversion nodes was reduced (Table 2).

We concluded that floral reversion in Pc-G mutants depends on additive and synergistic function of *FLC* and *SVP* and that Pc-G proteins are needed to keep *FLC* repressed and H3K27me3 decorated in wild-type inflorescences when plants are shifted from LD to SD. As the analyzed Pc-G mutants represent only partial loss-of-Pc-G-function, *SVP* H3K27me3 may be maintained by the residual Pc-G activity present in the mutants.

### Reduced *FT* Expression in Pc-G Mutant Inflorescences Contributes to Floral Reversion

Next, we aimed to reveal the downstream targets of *FLC* and *SVP* that are required for stable floral commitment. FLC and

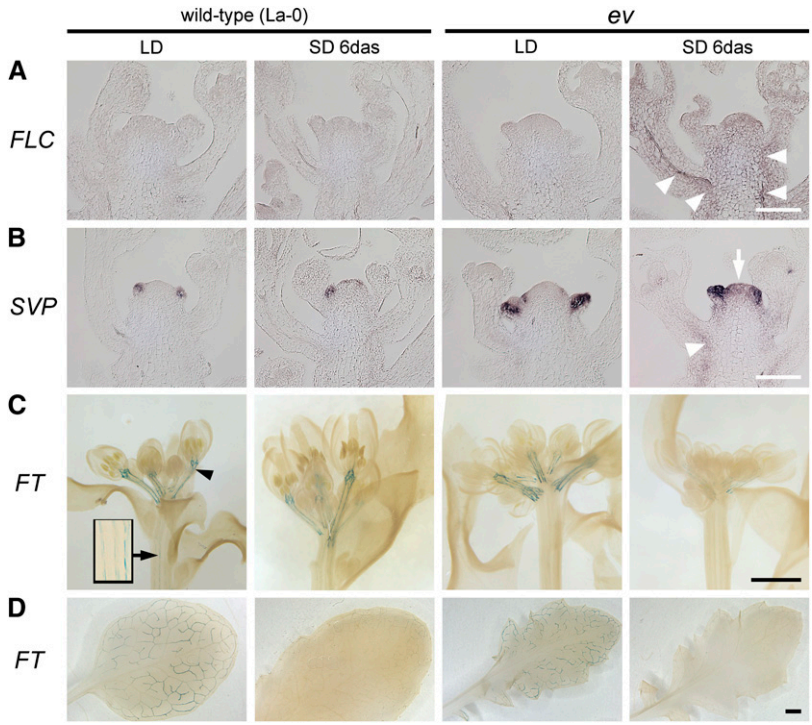


**Figure 3.** Misexpression of *FLC* and *SVP* and Downregulation of Their Target Gene *FT* in Pc-G Mutants.

**(A)** and **(B)** In situ RNA hybridization: a shift from LD to SD leads to *FLC* **(A)** and *SVP* **(B)** upregulation in *emf2-10 vrn2-1* (*ev*), but not in wild-type inflorescence apices (27 DAG). Expression is detected in the vasculature of the stem (arrowheads) and in the inflorescence meristem (arrow). A control experiment using *STM* RNA probes verified that these differences did not reflect variation in sample preservation (Supplemental Figure 6). Bars = 100 μm.

**(C)** and **(D)** $FT_{pro}$*:GUS* expression in inflorescence apices (**[C]**; bars = 400 μm) and rosette leaves (**[D]**; bars = 1 mm). Arrowhead in **(C)** indicates strong staining in the vasculature of pedicels; arrow/inset in **(C)** show weak staining in the vasculature of stem. Plants are 27 d old and were continuously grown in LD or 21 d in LD, shifted to SD, and grown for a further 6 d (SD 6 DAS).

SVP, as part of a repressor complex, delay flowering by binding to the promoters of *SOC1* and *FT* in leaves (Hepworth et al., 2002; Helliwell et al., 2006; Searle et al., 2006; Fujiwara et al., 2008; Li et al., 2008; Jang et al., 2009; Deng et al., 2011). Both genes were expressed in both LD, SD, and after LD to SD shift in wild-type inflorescence apices (Figures 3C and 5A**;** Supplemental Figures 4 and 8A). Therefore, regulation of *FT* expression is apparently different in leaves and inflorescences as its expression in leaves was strongly reduced when plants were shifted to SD as previously reported (Corbesier et al., 2007; Hiraoka et al., 2013) (Figures 3D and 5B). We confirmed that *FT* is active in wild-type inflorescences after the shift to SD using $FT_{pro}$*:LUCIFERASE* and $FT_{pro}$*:GUS* reporters, which revealed strongest expression in the vasculature of the stem and of pedicels (Figures 3 and 5), consistent with recent studies (Adrian et al., 2010; Hiraoka et al., 2013). Plants grown in continuous SDs showed weaker but detectable *FT* expression in the inflorescence (Figure 5E), suggesting that high *FT* expression in the inflorescence under noninductive conditions may require a transient photoperiodic induction. We then analyzed flowering-promoting genes in Pc-G mutant backgrounds. Neither the expression of the *FLC/SVP* target gene *SOC1* nor of *FUL*, which is needed with *SOC1* to prevent floral reversion (Melzer et al., 2008), was significantly decreased in shifted *emf2-10 vrn2-1* mutants compared with shifted wild-type plants (Supplemental Figure 8A). Similarly, the expression of *AP1* was also not altered, whereas *LFY* expression was increased in *emf2-10 vrn2-1* inflorescences compared with wild-type plants both in LD and SD (Supplemental Figure 8B). By contrast, *FT* expression in *emf2-10 vrn2-1* and *iCLF* inflorescences was strongly reduced in LD relative to the wild type and further reduced when plants were shifted to SD (Figures 3C and 5A; Supplemental Figure 7C). It was restored to near wild-type levels in *emf2-10 vrn2-1 flc-5* and *emf2-10 vrn2-1 flc-5 svp-32* inflorescences but not in *emf2-10 vrn2-1 svp-32*, indicating that *FLC* but not *SVP* activity caused the *FT* downregulation (Figure 5A). Thus, only the activity of the flowering-promoting gene *FT* was reduced in *emf2-10 vrn2-1* after transient photoperiodic induction, whereas the key meristem identity genes *SOC1*, *FUL*, and *LFY* were unchanged or even elevated.

The correlation between *FT* level and floral reversion together with our genetic analysis showing that the leafy inflorescence phenotype of *emf2-10 vrn2-1* plants grown in continuous SD is largely due to reduced *FT* activity (Figures 2E and 2F; Supplemental Figure 4) suggested that reduced *FT* expression might be the basis for floral reversion in shifted *emf2-10 vrn2-1* plants. To further test this, we used an estradiol-inducible *FT* construct in transgenic *emf2-10 vrn2-1* plants (*iFT emf2-10 vrn2-1*) to activate *FT* in specific tissues and at distinct time points. When *FT* was induced by

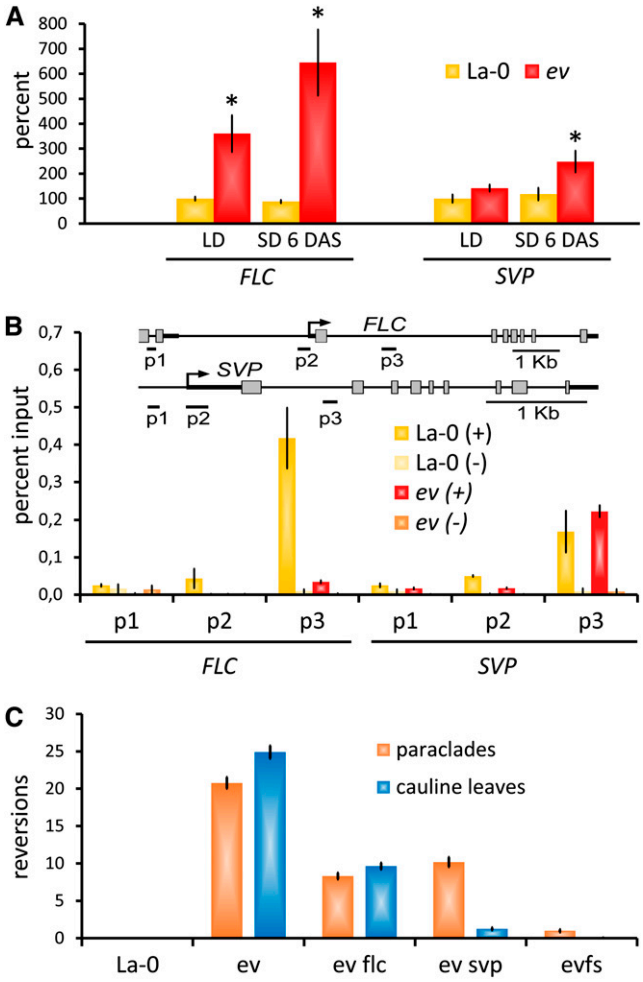


**Figure 4.** *FLC* and *SVP* Are Required for Floral Reversion in *emf2-10 vrn2-1* Mutants.

**(A)** Quantitative RT-PCR analyses of *FLC* and *SVP* mRNA expression in *emf2-10 vrn2-1* (*ev*) inflorescence apices (harvested 8 h after lights on) normalized to *eIF4*, relative to expression in La-0 (LD). Each bar represents the mean of five biological replicates ± SE. Asterisks indicate significant change of expression (Student's *t* test: $P \leq 0.05$) compared with the equally treated wild-type control (La-0).

**(B)** ChIP assay: *FLC* and *SVP* chromatin are enriched for H3K27me3 in inflorescence apices of the wild type (La-0). H3K27me3 is strongly reduced at the *FLC* locus in *emf2-10 vrn2-1* but not at *SVP*. (+) H3K27me3 antibody; (−) no antibody control. Data represent mean of two biological replicates ± SE.

**(C)** Loss of both *FLC* and *SVP* suppress floral reversion in *emf2-10 vrn2-1* plants; reversion nodes per plant, reversions of IM to tSAM (indicated by cauline leaves), and of FM to IM (indicated by paraclades) are distinguished ± SE ($n \geq 40$). See Table 2 for detailed counts of the different reversion node types. *evfs*, *emf2-10 vrn2-1 flc-5 svp-32*.

[See online article for color version of this figure.]

treating whole plants or apices with estradiol after shifting to SD, floral reversion was partially suppressed (Figures 5F to 5I, Table 3; Supplemental Table 3). Together, these results suggest that *FT* activity in *emf2-10 vrn2-1* inflorescences is required to prevent floral reversion in short days, when *FT* activity is lacking in leaves.

### *FT* Maintains Inflorescence and Floral Meristem Identity

Our results raised the question of whether *FT* activity also has a role in maintaining floral commitment in wild-type inflorescences. *FT* acts redundantly with *LFY* to activate *AP1* in floral meristems (Ruiz-García et al., 1997; Abe et al., 2005; Wigge et al., 2005), so that *ft lfy* double mutants bolt and generate cauline leaves but never produce flowers, suggesting a role for *FT* in specifying floral meristem identity. To reveal a role for *ft* single mutants in maintaining floral commitment, we performed a careful analysis of metamers in the *ft-10* (Col) null allele. In LD, these *ft* mutant plants resemble SD-grown wild-type plants in lacking *FT* activity in leaves, but differ in that they also lack *FT* activity in inflorescences. As previously reported, *ft-10* mutants flowered late in LD and SD and showed an increase in cauline leaf number, particularly in SD where *ft-10* resembled *iCLF* (Supplemental Table 1). In addition, we observed inflorescence and flower reversion in *ft-10* in both LD and SD, but never in the corresponding wild type (Figure 2L, Table 4). About 70% of the pedicels of *ft-10* mutant flowers carried rudimentary bracts under LD (Supplemental Figure 2H; first 10 flowers of 18 plants) phenocopying the rudimentary bracts in the wild type in SD (Supplemental Figures 2E and 2F). Importantly, the *ft-10* mutants initiated flower production, but then showed an oscillation of leaf and flower emergence in the following nodes suggesting that *FT* activity in inflorescences is required for the maintenance of IM and FM identity.

## DISCUSSION

### Pc-G Proteins Are Needed for Floral Commitment

Pc-G proteins have been shown to regulate numerous traits in plants, and a common underlying theme is that they maintain changes in developmental phase by repressing genes that promote an earlier phase. For example, after seed germination, the Pc-G permit transition from embryonic to seedling identity by repressing regulatory genes, such as *FUSCA3*, that are normally expressed earlier during embryonic development and specify embryonic identity (Chanvivattana et al., 2004; Makarevich et al., 2006). Here, by genetically manipulating Pc-G activity, we reveal roles for Pc-G in maintaining phase transitions as they promote commitment to flowering after transient photoperiodic induction and induce the switch from cauline leaf to flower production in noninductive conditions. These roles are consistent with a function of Pc-G in sustaining developmental transitions as they involve repression of genes such as *FLC* that are normally expressed during the earlier vegetative phase and repress the floral transition (Michaels and Amasino, 1999; De Lucia et al., 2008; Pien et al., 2008). Our genetic analyses indicate that activities of both *FLC* and *SVP* are critical for reversion and the switch to flower production in *emf2-10 vrn2-1* mutants (Figure 6). Both genes are direct targets of Pc-G, as they are enriched for H3K27me3 in inflorescence tissue. This is necessary for *FLC* regulation: *FLC* loses H3K27me3 and is upregulated in *iCLF* and *emf2-10 vrn2-1* mutants. For *SVP*, the role of Pc-G is less clear; we did not see consistent decreases in H3K27me3 enrichment at *SVP* in Pc-G mutants and observed only minor differences in expression in inflorescences. It is therefore possible that the role of *SVP* in floral reversion is indirect (at least for the Pc-G mutants

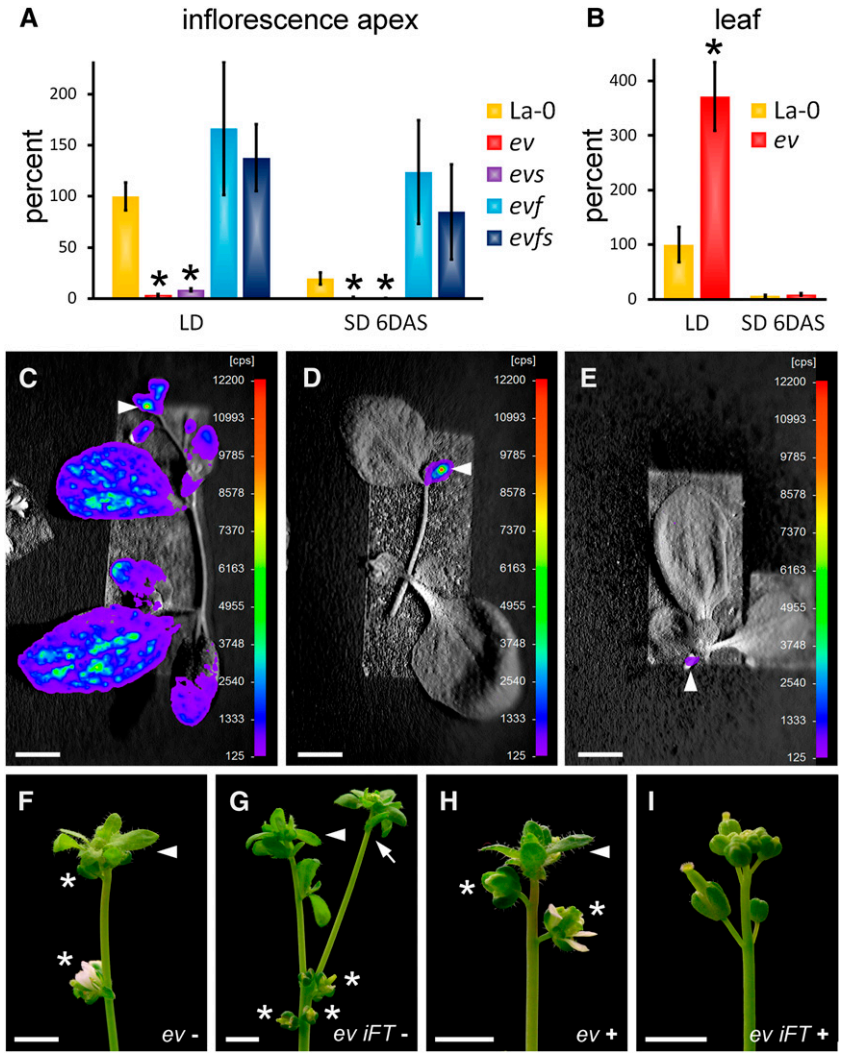


**Figure 5.** Downregulation of *FT* Causes Floral Reversion in PcG Mutants.

**(A)** and **(B)** Quantitative RT-PCR analyses of *FT* expression in inflorescence apices **(A)** and rosette leaves **(B)** normalized by *eIF4*, relative to expression in La-0 (LD). Asterisks indicate significant change of expression (Student's *t* test: $P \leq 0.05$) compared with the equally treated La-0. *FT* expression is reduced when plants are shifted from LD to SD (**[A]** and **[B]**), but less in inflorescence apices compared with rosette leaves. Lack of *FLC* but not *SVP* in *emf2-10 vrn2-1* (*ev*) rescues *FT* expression in apices. Average of three **(B)** to six **(A)** (except for *evs*, where $n = 3$) biological replicates per bar is shown ± SE. *ev*, *emf2-10 vrn2-1*; *evs*, *emf2-10 vrn2-1 svp-32*; *evf*, *emf2-10 vrn2-1 flc-5*; *evfs*, *emf2-10 vrn2-1 flc-5 svp-32*; plants were 27 DAG and inflorescence apices were harvested 8 h after lights on.

**(C)** to **(E)** $FT_{pro}$*:LUCIFERASE* activity in wild-type shoots 50 DAG in LD **(C)**, 11 DAS from LD to SD **(D)**, and in continuous SD **(E)**. Note that after LD to SD shift, no expression in leaves is seen, whereas expression is readily detected in inflorescence apices (arrowhead). Color coding represents relative counts per second (cps).

**(F)** to **(I)** Suppression of floral reversion by high *FT* expression. *emf2-10 vrn2-1* (**[F]** and **[H]**) and *emf2-10 vrn2-1 iFT* (**[G]** and **[I]**) plants at 62 DAG, shifted from LD to SD at 21 DAG, without estradiol (**[F]** and **[G]**) or with estradiol (**[H]** and **[I]**). A solution of 10 μM estradiol was supplied for 7 DAS by spraying plants once each day. Bars = 10 mm.

analyzed in this study), i.e., it is required as a cofactor for another, unknown factor ("X") that is misexpressed in Pc-G mutants or that chromatin of *SVP* target genes promoting reversion are more accessible in Pc-G mutants and activated (Figure 6). As the Pc-G mutants analyzed here have residual EMF2 (in *emf2-10 vrn2-1* mutants) or CLF activity (in *iCLF*), *SVP* H3K27me3 may be conferred by the EMF-PRC2, whereas *FLC* H3K27me3 and repression may be mediated by both EMF-PRC2 and VRN-PRC2 (Chanvivattana et al., 2004; Figure 6). Nonetheless, it seems likely that the role of Pc-G, *FLC*, and *SVP* in floral commitment is conserved in flowering plants. The Pc-G proteins are present in all plants, and recent research suggests that *FLC* is much more widely conserved in angiosperms than previously thought, having been identified in monocots (Reeves et al., 2007; Ruelens et al., 2013). *FLC* plays

**Table 3.** Suppression of Floral Reversion in *ev* by High *FT*

| Genotype/Treatment | PC without CL | CL | CL with PC | CL with F | ∑ |
|---|---|---|---|---|---|
| *ev* − estradiol | 1.3 ± 0.3 | 0.6 ± 0.3 | 8.8 ± 1.5 | 2.3 ± 0.5 | 13.1 ± 1.5 |
| *ev* + estradiol | 1.5 ± 0.4 | 0.3 ± 0.1 | 8.3 ± 1.6 | 2.2 ± 0.3 | 12.2 ± 1.8 |
| *ev iFT* − estradiol | 1.6 ± 0.5 | 0.6 ± 0.2 | 8.8 ± 1.4 | 1.9 ± 0.3 | 12.8 ± 1.6 |
| *ev iFT* + estradiol | 0.1 ± 0.1* | 0 | 0** | 0* | 0.1 ± 0.1** |

Data are ± SE. *n* = 12 plants. CL, cauline leaves; PC, paraclades; F, flowers; *ev*, *emf2-10 vrn2-1*; *ev iFT*, *emf2-10 vrn2-1 iFT*. Significantly fewer reversion nodes than *ev* − estradiol (Student's *t* test): *P ≤0.01 and **P < 0.001.

a role in floral reversion in natural environments as the ecotype Skye-0 shows *FLC*-dependent floral reversion (Poduska et al., 2003). In addition, *SVP* is widely conserved, and in barley (*Hordeum vulgare*), a monocot that is distantly related to *Arabidopsis*, *SVP* orthologs are also expressed during vegetative development and repressed during FM development (Trevaskis et al., 2007). Furthermore, experiments using transgenics show that misexpression of *SVP* orthologs in flowers causes floral reversion, particularly when plants were grown in noninductive short days (Trevaskis et al., 2007). It is therefore plausible that the Pc-G/*FLC*/*SVP* module provides a general mechanism for floral commitment.

### Differing Roles of *FLC* and *SVP* in Reversion

Unexpectedly, our genetic analysis revealed that *FLC* and *SVP* have distinct roles in floral reversion. First, the *emf2-10 vrn2-1 flc-5 svp-32* quadruple mutant shows less floral reversion than either the *emf2-10 vrn2-1 flc-5* or the *emf2-10 vrn2-1 svp-32* triple mutant, suggesting that *FLC* and *SVP* act in parallel. Second, removing *FLC* activity in the *emf2-10 vrn2-1* background increases *FT* expression, whereas removing *SVP* activity does not, suggesting that the role of SVP in promoting reversion is independent of *FT* (Figure 6). Third, the phenotypes of the two triple mutants differ, with *SVP* activity being more specifically required for the production of cauline leaves. *Arabidopsis* is unusual in that its flowers are not subtended by a leaf-like organ termed a bract. However, detailed analysis has suggested that after the floral transition, the youngest primordia to arise on the flanks of the inflorescence meristem are leaf-like organs, which usually do not grow out during later flower stages and hence are termed cryptic bracts (Figure 1) (Long and Barton, 2000; Kwiatkowska, 2006; Chandler, 2012). The different types of reversion nodes observed in the Pc-G mutants likely reflect the time at which floral primordium identity is compromised and reversion occurs. For example, cauline leaves with "empty" axils may arise if floral primordium identity is inhibited extremely early, likely before or around the time the FM is specified on the adaxial side of cryptic bract primordia. Inhibition at later stages may result in cauline leaves subtending paraclades, paraclades only, or in weaker cases flowers subtended by cauline leaves or rudimentary bracts (Figure 1). *SVP* therefore seems to inhibit early stages in floral primordium development, whereas *FLC* has more continuous effects, perhaps consistent with the more general misexpression of *FLC* in *emf2-10 vrn2-1* inflorescences.

### *FT* Is Needed for Floral Commitment

The *FT* gene was originally identified based on its requirement to accelerate time to flowering in LD in *Arabidopsis*. Subsequently, *FT* or its orthologs have been found to act more generally and to control other traits, including bud dormancy, potato tuberization, leaf dissection, and lateral bud outgrowth (Böhlenius et al., 2006; Shalit et al., 2009; Navarro et al., 2011; Hiraoka et al., 2013). Our analyses reveal a daylength-independent role for *FT* in the maintenance of IM and FM identity.

We and others have detected $FT_{pro}$*:GUS* reporter expression in the vasculature of the pedicels of older flowers in inflorescences in LD and SD (Figures 3C and 5C) (Adrian et al., 2010; Hiraoka et al., 2013). We believe this reflects native *FT* expression as RT-PCR detects *FT* mRNA in inflorescence tips of shifted wild-type plants but much less so in shifted *emf2-10 vrn2-1* plants. Additionally, the reporters that contain 8.1 kb of sequences upstream of *FT* reliably reflect the expression of *FT* in vasculature in leaves in LD but not SD and the same promoter fragment driving *FT* cDNA complements null *ft-10* mutations (Adrian et al., 2010). Importantly, our genetic analysis shows that the *FT* expression we detect in the inflorescence is functionally relevant. In particular, we show that *ft* mutants grown in LD or SD conditions show floral reversion after bolting (Table 4). Thus, although FT plays a negligible role in triggering flowering in SD, as it is not expressed in leaves (Kobayashi and Weigel, 2007), once bolting and floral induction has occurred it is transcriptionally activated in the inflorescence and here has a role in maintaining floral commitment. Our results further suggest that

**Table 4.** Strong Floral Reversion Nodes in *ft-10* Mutants

| | LD | | | | | | SD | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | PC | CL | CL + PC | CL + F | ∑ | N | PC | CL | CL + PC | CL + F | ∑ |
| Col-0 | 12 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 0 |
| *ft-10* | 18 | 0.3 ± 0.1* | 0 | 2.3 ± 0.5* | 0.4 ± 0.2 | 3.0 ± 0.6* | 18 | 0.1 ± 0.1 | 0.1 ± 0.1 | 3.2 ± 0.5* | 0.2 ± 0.1 | 3.6 ± 0.4* |

Data are ± SE. Significantly more reversion nodes than Col-0: *P ≤ 0.01 (Student's *t* test). CL, cauline leaves; PC, paraclades; F, flowers.

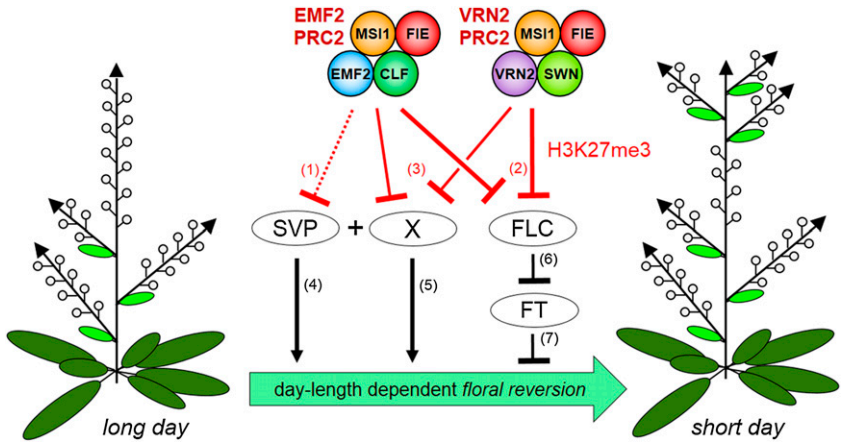


**Figure 6.** Model: Prevention of Daylength-Dependent Floral Reversion by Pc-G Proteins and *FT*.

At least two differently composed PRC2 complexes, the EMF2-PRC2 and the VRN2-PRC2, prevent daylength-dependent floral reversion. (1) *SVP* is a target of H3K27me3 in inflorescences that is likely mediated by the EMF2-PRC2 as loss of VRN2 (in *emf2-10 vrn2-1*) or SWN (in *iCLF*) do not reduce H3K27me3 levels at *SVP*. (2) In both lines with reduced Pc-G activity, H3K27me3 is strongly reduced at the *FLC* locus and *FLC* mRNA accumulates. (3) At least one other H3K27me3 target (X) promotes daylength-dependent floral reversion (5), since lack of SVP (4) and FLC (6) only incompletely suppress daylength-dependent floral reversion in *emf2-10 vrn2-1* mutants. Factor X is likely misexpressed and loses H3K27me3 in partially depleted Pc-G mutants and may be a cofactor for SVP. The upregulation of *FLC* (6) but not *SVP* (4) represses *FT* expression in *emf2-10 vrn2-1* mutants. (7) *ft* mutants show photoperiod-independent floral reversion, whereas induced *FT* suppresses daylength-dependent floral reversion in *emf2-10 vrn2-1* mutants. In addition, *FT* is also regulated by pathways acting in parallel to *ev* (not indicated; see Discussion).

the decreased *FT* expression in *emf2-10 vrn2-1* inflorescences is partly responsible for their loss of floral commitment. First, in *emf2-10 vrn2-1 flc svp* quadruple mutants, which show drastically reduced floral reversion relative to *emf2-10 vrn2-1* double mutants, *FT* expression in inflorescences is restored to near wild-type levels. Second, when a *FT* transgene is induced in LD to SD shifted *emf2-10 vrn2-1* plants, floral reversion is reduced. Since removing *SVP* activity in the *emf2-10 vrn2-1* background reduces floral reversion without restoring *FT* activity, it is likely that additional genes besides *FT* are involved. It is also notable that *emf2-10 vrn2-1 ft-13* triple mutants show a more extreme phenotype than do *emf2-10 vrn2-1* double mutants in SD. This reflects the fact that *emf2-10 vrn2-1* reduces but does not eliminate *FT* activity and implies that there are *ev*-independent pathways that act in parallel to regulate *FT*.

### Persistent FT Activity Is Needed for Floral Commitment

How does *FT* activity in the inflorescence maintain floral commitment in plants shifted to noninductive conditions? In transiently induced plants, it is likely that the *LFY/AP1* autoregulatory loop stabilizes FM identity once *LFY/AP1* expression has been initiated in young floral primordia, but this does not explain how flower primordium identity is repeatedly established on the flanks of the SAM. Here, movement of mobile FT protein into the SAM from flower primordia may be important for continued production of flowers. Although the generality of this mechanism is unknown, characterization of *FT* orthologs from legumes has revealed a correlation between floral commitment and *FT* expression in inflorescences (Hecht et al., 2011; Sun et al., 2011), and genetic studies in tomato (*Solanum lycopersicum*) show that *FT* is needed for floral commitment as well as for floral induction (Molinero-Rosales et al., 2004). In addition, floral commitment in *Impatiens balsamina* requires a leaf-derived, mobile signal, which is consistent with florigen being the signal (Tooke and Battey, 2000). Thus, there are other examples in diverse plant species that stable floral commitment does not only rely on signaling events in the meristem but requires continuous signaling from lateral organs producing a mobile signal.

In conclusion, *FT* activity in inflorescences is required for floral commitment, and the Pc-G genes are needed to permit this. However, an important question remains as to how *FT* transcription is activated or maintained in inflorescences in LD-to-SD-shifted plants. *FT* itself is unlikely to provide the "memory" of transient inductive signals in shifted plants, but rather is needed for maintaining commitment. Thus, shifting experiments show that plants that are only at a very early stage in inflorescence development lacking stage 3 or older flower primordia at the time of shifting (Torti et al., 2012) are fully committed, yet we do not detect *FT* transcription in the IM or very early flower primordia. Genes such as *FUL*, whose expression persists in the IM and which are needed for floral commitment, are strong candidates to provide the initial memory (Melzer et al., 2008; Torti et al., 2012). We detected *FT* transcription in the vasculature of young flowers (Figure 3) and speculate that *FT* may be activated downstream of floral organ identity or other genes active during flower development. Regardless of how this occurs, the activation of FT in young flowers would provide a mobile signal (FT protein) that reinforces flower primordium identity on the flanks of the IM. In this way, a promotive signal from older flowers back to the IM would make flowering self-perpetuating once initiated.

## METHODS

### Plant Materials and Growth Conditions

Plants were grown at 22°C under LD (16 h light/8 h dark) or SD (8 h light/16 h dark) conditions. *emf2-10 vrn2-1* was described previously (Lafos et al., 2011). *ft-10* (GABI_290E08) is in the Col-0 background and was isolated from a T-DNA library generated by GABI-Kat (Yoo et al., 2005; Kleinboelting et al., 2012). C. Dean kindly provided seeds of $FLC_{pro}$*:FLC-GUS* and *flc-5* (Greb et al., 2007). *svp-32* (Lee et al., 2007) (SALK_072930) was obtained from the Nottingham Arabidopsis Stock Centre. Seeds of $FT_{pro}$*:LUC* (Adrian et al., 2010) and $FT_{pro}$*:GUS* (Kotake et al., 2003) were kindly provided by F. Turck and K. Goto, respectively.

### Construction of *iCLF*

We generated the *iCLF* (*clf-28 swn-7* $CLF_{pro}$*:CLF-GR*) line by introducing the previously described $CLF_{pro}$*:CLF-GR* (Schubert et al. 2006) steroid-dependent transgene into the *clf-28 swn-7* background by floral dip transformation of *swn-7 clf-28*/+ plants. In the presence of dexamethasone steroid, *iCLF* plants are near wild-type (Supplemental Figure 1) as *CLF* activity complements both *swn-7* and *clf-28* mutations due to the functional redundancy between the *SWN* and *CLF* genes (Chanvivattana et al., 2004). In the absence of steroid, *iCLF* plants have a weaker phenotype than *clf-28 swn-7* mutants (Supplemental Figure 1), suggesting that the $CLF_{pro}$*:CLF-GR* transgene is slightly leaky. In depletion experiments, *iCLF* plants were grown on tissue culture plates containing 10 μM dexamethasone for 10 or 14 d and then transferred to soil, resulting in a progressive decrease in Pc-G activity from day 10 to 14 onwards. *iCLF* $FLC_{pro}$*:GUS* plants were generated by crossing $FLC_{pro}$*:GUS* into the *clf-50 swn-3* $CLF_{pro}$*:CLF-GR* background.

### Construction of *iFT*

*iFT* was constructed by introducing the *FT* cDNA into the plasmid pMDC7 (Curtis and Grossniklaus, 2003), which is a derivative of pER8 (Zuo et al., 2000). FT cDNA was amplified with primers FT-ATG (5′-ATGTCTATAA-ATATAAGAGACCCTCTT-3′) and FT-TAG (5′-CTAAAGTCTTCTTCCT-CCGC-3′), cloned into pCR8-GW/Topo (Invitrogen), and introduced into pMDC7 via an LR reaction (Gateway; Invitrogen). Transgenic *iFT Arabidopsis* plants were generated using *Agrobacterium tumefaciens*–mediated transformation in *emf2-10 vrn2-1*.

### Shift Experiments

Seeds were sown on soil, grown for 21 d (emf2-10 vrn2-1 and La-0) or 29 d (*iCLF* and Col-0) in LD, then shifted to SD and retained there for at least 4 weeks before examination of the phenotype. Rosette leaves and inflorescence apices, excluding flowers older than stage 13 (Smyth et al., 1990), were harvested 27 DAG (emf2-10 vrn2-1 and La-0) or 34 DAG (*iCLF* and Col-0) for gene expression analyses. Additionally, the length of the initial period in LD was varied from 7 d to 5 weeks to determine whether sensitivity to reversion varied with developmental age. Because *emf2-10 vrn2-1 flc-5* and *emf2-10 vrn2-1 flc-5 svp-32* flowered slightly earlier than *emf2-10 vrn2-1* in LD (Table 1), differences in reversion might reflect that they were at a more advanced developmental stage at the time of shift. The time in LD was therefore varied, between 7 and 28 d, before shifting to SD so that each genotype was shifted at a range of different stages.

### RNA Extraction and Real-Time RT-PCR Analysis

Total RNA of two to six biological replicates of leaves and inflorescence tips, excluding open flowers (see Shift Experiments above), was extracted with TRIZOL (Invitrogen) and cDNA was synthesized using M-MLV reverse transcriptase (Promega). The primers used are listed in Supplemental Table 4.

### In Situ RNA Hybridization

In situ RNA hybridization was performed using digoxigenin-labeled mRNA probes as described previously (Chanvivattana et al., 2004). *STM*, *ANT*, and *LFY antisense* probes were generated using plasmids generously provided by R. Simon. *FLC* (Pien et al. 2008) and *SVP* plasmids for *antisense* probes (Hartmann et al., 2000) were kindly provided by C. Dean and P. Huijser, respectively. *AP1 antisense* probe was generously provided by Coral Vincent and George Coupland (Torti et al., 2012).

### ChIP Assay

ChIP assays were performed as described previously (Schubert et al. 2006). The primers used are listed in Supplemental Table 5.

### Luciferase Imaging

Initially, plants were sprayed with a solution of 20 mM luciferine (Synchem) to degrade accumulated LUCIFERASE and kept for 1 h. Plants were then sprayed again and imaged in a NightOWL II LB 983 (Berthold Technologies). Exposition time was 10 min and resolution 1 × 1 pixels.

### GUS Staining

Detection of GUS activity in tissue preparations were performed as described with minor modifications (Jefferson et al., 1987).

### Imaging

Photoshop adjustment involved only image exposure using adjustment levels and sharpening using unsharp mask.

### Accession Numbers

Sequence data from this article can be found in GenBank/EMBL data libraries under accession numbers At4g02020 (SWN), At2g23380 (CLF), At5g51230 (EMF2), At4g16485 (VRN2), At1g65480 (FT), At2g22450 (SVP), At5g10140 (FLC), At2g45660 (SOC1), At5g61850 (LFY), At5g60910 (FUL), and At1g69120 (AP1).

### Supplemental Data

The following materials are available in the online version of this article.

**Supplemental Figure 1.** Phenotypes of *iCLF* (*clf-28 swn-7* $CLF_{pro}$*:CLF-GR*) Plants.

**Supplemental Figure 2.** Floral Phenotype, Rudimentary Bracts, and Classes of Reversion Nodes.

**Supplemental Figure 3.** *LFY* and *AP1* Expression in Wild-Type Inflorescences and Leafy *emf2-10 vrn2-1* Shoots in SD.

**Supplemental Figure 4.** Gene Expression of Flowering Time and Meristem Identity Genes in Wild-Type (La-0) and *emf2-10 vrn2-1* (*ev*) Inflorescences in SD.

**Supplemental Figure 5.** $FLC_{pro}$*:GUS* Expression in Wild Type, *emf2-10 vrn2-1* Mutants, and *iCLF*.

**Supplemental Figure 6.** Gene Expression in Inflorescences and Cryptic Bracts in Wild-Type and *emf2-10 vrn2-1* Mutant Floral Primordia.

**Supplemental Figure 7.** Expression Analyses of *FLC*, *SVP*, and *FT* and H3K27me3 ChIP for FLC and SVP in *iCLF* versus the Wild Type.

**Supplemental Figure 8.** qRT-PCR Analyses of *LFY*, *SOC1*, *FUL*, and *AP1* in *emf2-10 vrn2-1* (*ev*) and Wild-Type Inflorescence Apices.

**Supplemental Table 1.** Flowering Time of *iCFL* and *ft-10* Mutants Measured by Leaf Number.

**Supplemental Table 2.** Strong Reversion Nodes at the Main Shoot of *iCLF*.

**Supplemental Table 3.** Suppression of Floral Reversion in *ev* by High *FT*.

**Supplemental Table 4.** RT-PCR Primers.

**Supplemental Table 5.** ChIP PCR Primers.

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## AUTHOR CONTRIBUTIONS

R.M.-X., J.G., and D.S. designed and performed the research and wrote the article. O.C. and L.P. performed research.

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## NOTE ADDED IN PROOF

Liu et al. (2014) also revealed a photoperiod-independent function of *FT* in maintaining floral commitment.

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**Polycomb-Group Proteins and *FLOWERING LOCUS T* Maintain Commitment to Flowering in *Arabidopsis thaliana***

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