The Plant Cell, Vol. 11, 1007–1018, June 1999, www.plantcell.org © 1999 American Society of Plant Physiologists

RESEARCH ARTICLE

Interactions among *APETALA1*, *LEAFY*, and *TERMINAL FLOWER1* Specify Meristem Fate

Sarah J. Liljegren, Cindy Gustafson-Brown, Anusak Pinyopich, Gary S. Ditta, and Martin F. Yanofsky[1]

Department of Biology and Center for Molecular Genetics, University of California at San Diego, La Jolla, California 92093

Upon floral induction, the primary shoot meristem of an Arabidopsis plant begins to produce flower meristems rather than leaf primordia on its flanks. Assignment of floral fate to lateral meristems is primarily due to the cooperative activity of the flower meristem identity genes *LEAFY* (*LFY*), *APETALA1* (*AP1*), and *CAULIFLOWER.* We present evidence here that *AP1* expression in lateral meristems is activated by at least two independent pathways, one of which is regulated by *LFY.* In *lfy* mutants, the onset of *AP1* expression is delayed, indicating that *LFY* is formally a positive regulator of *AP1.* We have found that *AP1*, in turn, can positively regulate *LFY*, because *LFY* is expressed prematurely in the converted floral meristems of plants constitutively expressing *AP1.* Shoot meristems maintain an identity distinct from that of flower meristems, in part through the action of genes such as *TERMINAL FLOWER1* (*TFL1*), which bars *AP1* and *LFY* expression from the inflorescence shoot meristem. We show here that this negative regulation can be mutual because *TFL1* expression is downregulated in plants constitutively expressing *AP1.* Therefore, the normally sharp phase transition between the production of leaves with associated shoots and formation of the flowers, which occurs upon floral induction, is promoted by positive feedback interactions between *LFY* and *AP1*, together with negative interactions of these two genes with *TFL1.*

INTRODUCTION

Like most organisms, plants have a multiphased life cycle, allowing resource accumulation before reproductive development (Poethig, 1990). During the vegetative phase, the primary shoot meristem of Arabidopsis produces a rosette of closely spaced leaves. Transition to the reproductive phase, which is tightly controlled by a complex network of flowering-time genes, is influenced by environmental signals, such as day length, light quality, and temperature, as well as internal cues, such as age (reviewed in Bernier, 1988; Martínez-Zapater et al., 1994; Levy and Dean, 1998). After this transition, the primary shoot meristem of Arabidopsis begins to produce flower meristems rather than leaf primordia on its flanks (Hempel and Feldman, 1994). The last few leaves, called cauline leaves or bracts, eventually become separated by longer stem internodes and can be considered a subset of the vegetative phase (V_2), with production of rosette leaves being the first (V_1). Secondary shoot meristems formed in the axils of cauline and rosette leaf primordia reiterate the V_2 and reproductive phases of the primary shoot in most Arabidopsis ecotypes (Koornneef et al., 1994; Grbic and Bleecker, 1996). Primary and secondary shoot meristems remain indeterminate during the reproductive phase, producing many flowers before senescence.

Specification of Arabidopsis flower meristems is primarily controlled by the meristem identity genes *LEAFY* (*LFY*), *APETALA1* (*AP1*), and *CAULIFLOWER* (*CAL*) (Schultz and Haughn, 1991; Mandel et al., 1992; Weigel et al., 1992; Bowman et al., 1993; Kempin et al., 1995; Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995; Savidge, 1996). Plants homozygous for null alleles of *LFY* exhibit a lengthening of the V_2 phase as several additional cauline leaves with associated secondary shoots are produced (Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992). Furthermore, the transition between the V_2 and reproductive phases, which is precipitous in wild-type plants, becomes more gradual in *lfy* mutants: abnormal flowers produced can have shootlike characteristics and are often subtended by reduced bracts. Mutations in *AP1* affect the transition between the V_2 and reproductive phases to a lesser extent than do mutations in *LFY.* Strong *ap1* mutants often have an additional secondary shoot, and flowers are shootlike, with additional flower meristems produced in the axils of first-whorl organs (Irish and Sussex, 1990; Bowman et al., 1993).

[1] To whom correspondence should be addressed. E-mail marty@biomail.ucsd.edu; fax 619-822-1772.

The phenotype of *ap1* mutants is enhanced further by mutations in *CAL* such that a more complete conversion of flower meristems into inflorescence meristems occurs in *ap1 cal* double mutants (Bowman et al., 1993). Because flower meristems are not produced by *ap1 cal* primary shoots under standard growth conditions, these shoots never make a complete transition between the V_2 and reproductive phases (Bowman et al., 1993). Interestingly, *cal* single mutants are indistinguishable from wild-type plants, indicating that all of the functions of *CAL* are encompassed by those of *AP1.* In *ap1 cal lfy* triple mutants, which are phenotypically identical to *ap1 lfy* double mutants, a more complete transformation of flowers into shoots has been observed, although some flowerlike traits are present in the most apical structures (Weigel et al., 1992; Bowman et al., 1993; Schultz and Haughn, 1993). Thus, *LFY*, *AP1*, and *CAL* act together to promote a coordinated phase transition between leaf and shoot production (V_2) and flower meristem formation (reproductive phase).

Studies of gain-of-function transgenic plants constitutively expressing *LFY*, *AP1*, or *CAL* under control of the cauliflower mosaic virus 35S promoter reinforce the conclusion based on the loss-of-function studies described above and suggest that *AP1* activity is downstream of and regulated by *LFY.* 35S::*LFY*, 35S::*AP1*, and 35S::*CAL* plants flower early and show a transformation of both primary and secondary shoot meristems into flower meristems, although the 35S::*CAL*–conferred phenotype is notably weaker than that of 35S::*LFY* or 35S::*AP1* (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995; Savidge, 1996). Whereas mutations in *LFY* do not have a significant effect on the 35S::*AP1*–conferred phenotype, the shoot-to-flower conversions of 35S::*LFY* plants are notably suppressed by mutations in *AP1* (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995). Furthermore, *LFY* precedes *AP1* expression in wild-type lateral meristems upon floral induction (Gustafson-Brown, 1996; Simon et al., 1996; Hempel et al., 1997). Taken together, these results strongly implicate *LFY* as a positive regulator of *AP1* activity.

AP1 expression is spatially restricted to flower meristems by action of the inflorescence meristem identity gene *TERMINAL FLOWER1* (*TFL1*), because *AP1* is ectopically expressed in the transformed flowers and primary apex of *tfl1* mutants (Bowman et al., 1993; Gustafson-Brown et al., 1994). In wild-type plants, *AP1* and *TFL1* are expressed in nonoverlapping patterns, with *TFL1* expressed in a subapical region of shoot meristems, whereas *AP1* expression is limited to developing flowers (Mandel et al., 1992; Bradley et al., 1997). Besides playing an influential role in regulating phase change, *TFL1* has been proposed to be an antagonistic partner of *AP1* in the establishment of meristem identity, because *TFL1* promotes the identity of an indeterminate shoot meristem and *AP1* that of a determinate floral meristem (Shannon and Meeks-Wagner, 1993; Gustafson-Brown et al., 1994; Ratcliffe et al., 1998). The phenotype of plants constitutively expressing *AP1* mirrors the phenotype of plants with loss-of-function mutations in *TFL1.* 35S::*AP1* plants and *tfl1* mutants show a shortening of all growth phases and transformation of shoots into flowers, suggesting that *TFL1* activity may be compromised in 35S::*AP1* plants (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992; Mandel and Yanofsky, 1995).

Here, we present our investigations of *AP1* regulation by *LFY* and *LFY* regulation by *AP1.* In addition, we provide evidence for negative regulation of *TFL1* by *AP1.* Our studies provide new insights into the coordinated process of specifying meristem identity.

RESULTS

AP1 Expression Is Delayed in *lfy* Mutants

Because previous genetic studies suggest that *AP1* acts downstream of *LFY* to promote flower meristem identity, we investigated the molecular basis of this interaction by analyzing the onset of *AP1* RNA accumulation in *lfy* null (*lfy-12*; Huala and Sussex, 1992) mutants. Compared with wild-type plants grown under continuous light (CL), *lfy-12* mutants produce approximately four additional cauline leaves with associated shoots followed by ~10 shoots without visible bracts before more flowerlike nodes are observed (Table 1). As shown in Figure 1, whereas *AP1* expression was apparent in the lateral meristems of wild-type plants by day 11 or 12, in *lfy* mutants, appreciable *AP1* expression was not detected until approximately day 15. When *AP1* RNA begins to accumulate in *lfy* mutants, it appears patchy and at reduced levels relative to the wild type. At later time points, *AP1* expression levels increase, which likely correlates with the graded transition along the inflorescence axis to flowerlike nodes seen in *lfy* mutants.

AP1 Activity Is Largely Downstream of *LFY*

If *AP1* acts primarily downstream of *LFY* to specify flower meristem identity, then we anticipate that mutations in *LFY* should have little or no effect on the early-flowering and shoot-to-flower transformations of plants constitutively expressing *AP1.* Therefore, we examined the effects of a null allele of *LFY* (*lfy-12*) on the CL and short day (SD; 8 hr of light and 16 hr of dark) phenotypes of 35S::*AP1* plants. Phenotypes of plants constitutively expressing *AP1* are depicted in Figures 2 and 3. As previously described (Mandel and Yanofsky, 1995) and demonstrated in Table 1, 35S::*AP1* plants flower significantly earlier than do wild-type plants under both CL and SD conditions. After producing five to eight total leaves, the primary shoot meristem of a CL-grown 35S::*AP1* plant is transformed into a compound terminal flower (Figures 2A and 2D). Secondary shoot meristems produced in the axils of cauline and rosette leaves are usually converted into solitary flowers (Figures 2B and 2D), although

Table 1. Effect of *lfy* and *tfl1* on Leaf Number and Shoot Morphology of 35S::*AP1* Plants in CL and SD conditions[a]

Genotype	Rosette Leaves	Cauline Leaves	Total Leaves[b]	Lateral Shoots[c]	Floral Nodes[c]	Total Nodes
CL						
Wild type	8.0 ± 0	2.5 ± 0.7	10.5 ± 0.7	2.5 ± 0.7	ND[d]	ND
lfy[e]	8.5 ± 0.8	7.0 ± 0.6	15.5 ± 0.8	ND	ND	ND
35S::*AP1*	3.3 ± 0.7	2.1 ± 0.3	5.4 ± 0.7	0.2 ± 0.4	1.9 ± 0.6	5.4 ± 0.7
35S::*AP1 lfy*	3.3 ± 0.8	3.5 ± 0.5	6.8 ± 0.8	1.2 ± 1.0	2.3 ± 0.8	6.8 ± 0.8
Wild type	10.5 ± 0.6	2.9 ± 0.5	13.4 ± 0.8	2.9 ± 0.5	ND	ND
tfl1	7.2 ± 0.7	1.5 ± 0.5	8.7 ± 0.7	0	3.0 ± 0.8	10.2 ± 1.2
35S::*AP1*	5.2 ± 0.6	2.7 ± 0.8	7.9 ± 1.3	1.0 ± 0.9	1.8 ± 1.2	8.0 ± 1.3
35S::*AP1 tfl1*	3.6 ± 0.5	1.7 ± 0.4	5.3 ± 0.5	0	1.7 ± 0.4	5.3 ± 0.5
SD						
Wild type	59.8 ± 2.7	9.6 ± 0.9	69.4 ± 2.1	9.6 ± 0.9	28.2 ± 5.7	97.6 ± 7.5
lfy	60.8 ± 7.0	56.8 ± 1.9	117.6 ± 6.8	55.2 ± 3.5	1.6 ± 2.1	117.6 ± 6.8
35S::*AP1*	10.3 ± 1.4	2.7 ± 0.7	13.0 ± 1.8	2.7 ± 0.7	16.1 ± 10.5	29.1 ± 11.5
35S::*AP1 lfy*	11.2 ± 1.7	4.8 ± 0.8	16.0 ± 2.4	4.7 ± 0.8	0.2 ± 0.4	16.0 ± 2.4
Wild type	58.5 ± 4.3	11.6 ± 0.9	70.1 ± 4.1	11.6 ± 0.9	40.7 ± 3.1	110.7 ± 4.2
tfl1	50.4 ± 3.2	8.4 ± 0.9	58.8 ± 4.1	7.7 ± 1.0	27.1 ± 2.6	85.3 ± 4.6
35S::*AP1*	18.5 ± 2.8	5.9 ± 1.5	24.4 ± 4.1	5.9 ± 1.5	31.2 ± 1.9	55.6 ± 4.9
35S::*AP1 tfl1*	11.4 ± 1.6	3.9 ± 1.0	15.3 ± 2.5	0.9 ± 1.4	7.0 ± 1.9	19.3 ± 4.4

[a] Results from four independent experiments are presented (all plants are of the Columbia ecotype); each value represents the mean ±SD.
[b] Measurement of flowering time.
[c] With or without subtending bract; nodes are classified as lateral shoots or as flowers based on the presence or absence of tertiary structures, respectively.
[d] ND, not determined.
[e] *lfy* mutants grown in an independent CL experiment produced 18.7 ± 1.7 lateral shoots and 32.8 ± 4.9 floral nodes compared with 3.0 ± 0 lateral shoots and 27.3 ± 2.6 floral nodes produced by wild-type plants.

partial shoot-to-flower conversions can be seen in some basal positions (Figures 2C and 3). In numerous CL experiments with homozygous 35S::*AP1* plants, complete secondary shoot-to-flower conversions were observed at ~60% of all leaf nodes, with a range of 30 to 100% per plant (data not shown). Converted flowers of 35S::*AP1* plants (Figure 2B) usually have one extra sepal (total of 4.9 ± 0.4 sepals) and one extra petal (total of 4.6 ± 0.5 petals) compared with wild-type flowers; one extra sepal and petal also have been observed in *tfl1*-transformed flowers (Alvarez et al., 1992).

As previously described by Mandel and Yanofsky (1995), the early-flowering and shoot architecture of CL-grown 35S::*AP1* plants are generally unaffected by mutations in *LFY*, although some attenuation has been observed. 35S::*AP1 lfy* plants produce approximately one extra cauline leaf before the primary shoot meristem is converted into a leafy terminal flower (Table 1 and Figure 2D). Additional floral meristems, which develop in the axils of the outer leaflike organs of the terminal flower, give it the appearance of the "leafy starbursts" described for *tfl1 lfy* mutants (Shannon and Meeks-Wagner, 1993; Figures 2E and 3). Secondary shoot-to-flower conversions of 35S::*AP1 lfy* plants are largely unaffected, although internode elongation between some of the outer leaflike floral organs of the converted flowers can be observed at some of the basal nodes (Figure 2F).

Most converted flowers of 35S::*AP1 lfy* plants appear identical to flowers produced by *lfy* mutants, with leaflike organs in a spiral arrangement comprising the outer whorls and a few carpelloid organs in the center (Figure 2G). However, flowers in apical positions of 35S::*AP1 lfy* plants, such as those that develop in the axils of the terminal flower's outer whorl organs, can display characteristics more typical of weak *lfy* flowers. Approximately one flower per 35S::*AP1 lfy* plant clearly resembles flowers of intermediate or weak *lfy* alleles (Weigel et al., 1992; Schultz and Haughn, 1993), with concentrically arranged floral organs and a few petals and/or stamens (Figure 2H). Because petals and stamens are normally never observed in flowers of plants with this null allele of *LFY* (Huala and Sussex, 1992), their appearance in 35S::*AP1 lfy* plants supports the hypothesis that *AP1* can activate downstream genes responsible for petal and stamen development independently of *LFY* (Weigel and Meyerowitz, 1993). Interestingly, similar flowers with more wild-type characteristics are also observed in apical positions of *tfl1 lfy* plants (Shannon and Meeks-Wagner, 1993).

Under noninducing growth conditions, such as at lower temperatures or under shorter photoperiods, the floral meristem identity defects of *lfy* mutants are further enhanced (Huala and Sussex, 1992; Weigel et al., 1992; Schultz and Haughn, 1993). Besides a dramatic increase in the number

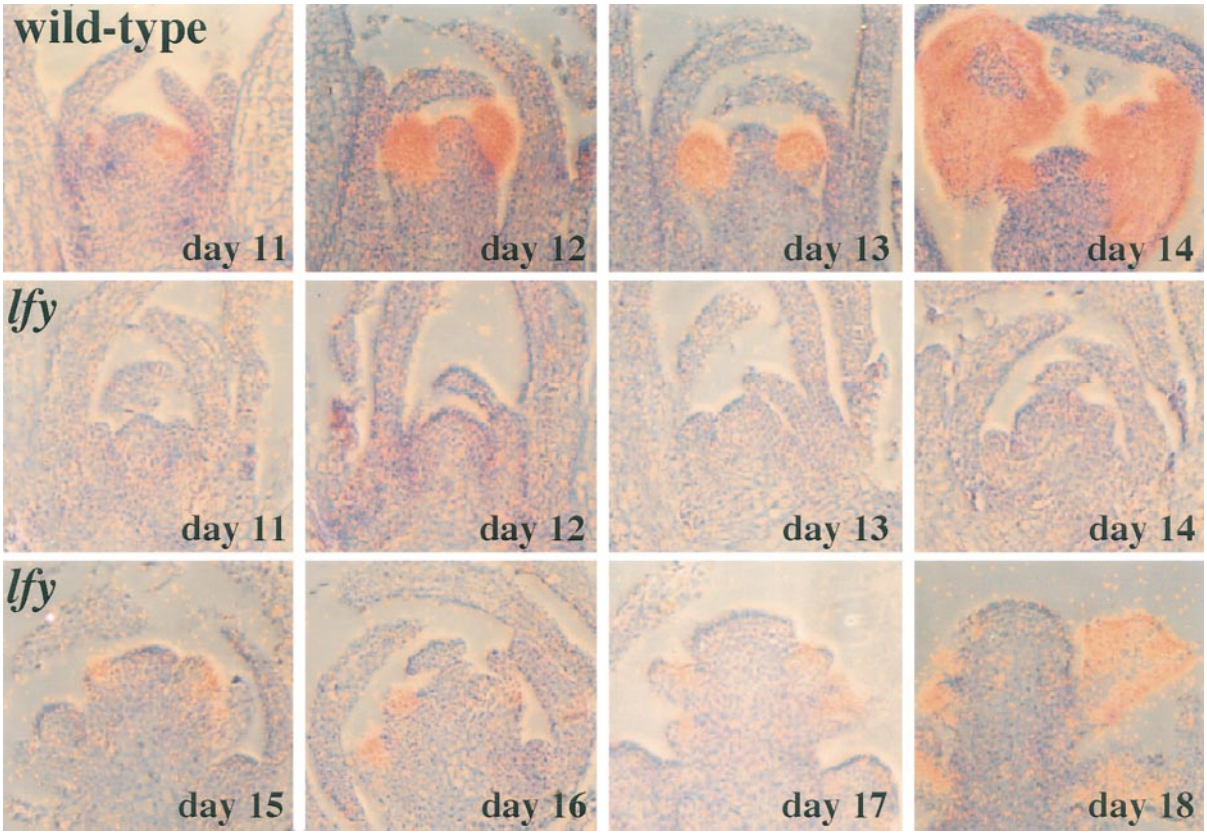

Figure 1. Expression of *AP1* in Wild-Type and *lfy* Plants.

Sections of wild-type primary apices at days 11 to 14 appear at top; sections of *lfy* primary apices at days 11 to 18 are at center and bottom. *AP1* expression is first apparent in flower meristems formed at the flanks of the primary shoot meristems of day 11 or day 12 wild-type plants. In *lfy* plants, the onset of *AP1* expression is delayed until approximately day 15 and is present as a patchy signal in meristem primordia formed at the flanks of the primary meristem but not in the leaf primordium associated with each secondary meristem (see day 17). *AP1* expression remains patchy in older *lfy* flower meristems, as shown for day 18.

of secondary shoots produced, *LFY* activity appears to be absolutely required for bract suppression under SD conditions, because all nodes are subtended by well-developed bracts in *lfy* null mutants (Table 1 and Figure 3B). Under SD conditions, 35S::*AP1* plants produce ~18 total leaves before beginning to produce flowers, whereas wild-type plants make the transition to flowering after ~70 total leaves (Table 1). The shoot-to-flower transformations of CL-grown 35S::*AP1* plants are largely attenuated by short photoperiods (Figure 3 and Table 1). After producing five to 30 flowers, the primary shoot meristem either senesces or forms a terminal flower. Secondary shoots are abbreviated compared with wild-type shoots and also either senesce or form terminal flowers at their apices.

SD-grown 35S::*AP1 lfy* plants produce approximately two more cauline leaves with associated shoots than do 35S::*AP1* plants before a terminal flower is formed at the primary apex (Table 1 and Figure 3B). However, the total number of nodes produced by 35S::*AP1* plants is not increased by mutations in *LFY*, because 35S::*AP1* plants usually produce several floral nodes before either senescing or forming a terminal flower (Table 1). Flowers produced by SD-grown 35S::*AP1* plants occur at positions normally occupied by leaves with associated shoots in wild-type plants (Figure 3B). Thus, *LFY* activity appears to be responsible for the bract suppression and identity of these 35S::*AP1* floral nodes, implying that *AP1* is able to activate *LFY.* Mutations in *LFY* similarly affect the identity but do not increase the number of nodes produced by secondary shoot meristems of 35S::*AP1* plants: only cauline leaves with associated shoots are produced before meristems are converted to terminal flowers (Figure 3B; data not shown). 35S::*AP1 lfy* terminal flowers at both primary and secondary shoot apices appear as leafy starbursts (Figure 3B). Approximately 40% of the leafy starbursts formed by 35S::*AP1 lfy* secondary shoots show some type of floral reversion, suggesting that these terminal structures may retain more of a shootlike character than those of 35S::*AP1* plants.

The fact that mutations in *LFY* have only small effects on the early flowering and shoot-to-flower transformations of plants constitutively expressing *AP1* suggests that *AP1* acts primarily downstream of *LFY* to specify flower meristem identity. Alternatively, constitutive expression of *AP1* could hyperactivate one of two parallel flower-promoting pathways such that mutations in *LFY*, which also affect an *AP1*-independent pathway, are compensated for by hyperactivation of the *AP1*-dependent pathway.

AP1 Is Able to Activate *LFY*

Analysis of SD-grown 35S::*AP1 lfy* plants indicates that *LFY* mediates the bract suppression and identity of floral nodes produced by 35S::*AP1* plants. In addition, converted floral meristems of CL-grown 35S::*AP1 lfy* plants develop characteristics of *lfy* mutant flowers. Taken together, these results strongly suggest that *AP1* is able to activate *LFY.* To test this hypothesis further, we examined the onset of *LFY* expression in 35S::*AP1* plants grown under CL. As shown in Figure 4A, *LFY* is expressed in flower meristems of wild-type plants, which first appear at approximately day 12, and also is observed in leaf primordia (Weigel et al., 1992; Blázquez et al., 1997; Hempel et al., 1997). In 35S::*AP1* plants at day 8, *LFY* expression was observed at high levels in converted flower meristems and primary shoot apices that have assumed a floral fate (Figure 4B).

AP1 and *LFY* Act Cooperatively to Specify Flower Meristem Identity

Converging lines of evidence suggest that the combined activities of *LFY* and *AP1* are more effective at conferring a floral fate onto meristems than is either activity alone. Under flower-inducing conditions, plants carrying mutations in both *AP1* and *LFY* display a nearly complete transformation of all flowers into bracts bearing axillary shoots, whereas only basal floral nodes are transformed to shoots in *lfy* single mutants (Huala and Sussex, 1992; Weigel et al., 1992). Genetic analysis with plants constitutively expressing *LFY* demonstrates that lateral shoots gain a floral identity when *LFY* is constitutively expressed, and these transformations are largely reversed if *AP1* activity is absent (Weigel and Nilsson, 1995). Furthermore, although constitutive *AP1* activity is sufficient to convert lateral shoots into flowers, converted flowers display some shootlike characteristics if *LFY* activity is absent. Under noninductive growth conditions, overlapping requirements for *LFY* and *AP1* to specify floral fate are more evident: meristem identity defects of both *lfy* and *ap1* single mutants are significantly enhanced (Huala and Sussex, 1992; Weigel et al., 1992; Bowman et al., 1993; Schultz and Haughn, 1993), and constitutive *AP1* activity is largely unable to confer floral identity onto lateral shoot meristems.

SD-grown plants constitutively expressing *AP1* and *LFY* provide further evidence for the cooperative nature of *AP1* and *LFY* activities in specifying floral meristem identity (Figure 2). Under SD conditions, 35S::*LFY* and 35S::*AP1* plants flower with a similar number of total leaves; after bolting and producing an inflorescence of flowers, the primary shoots of 35S::*LFY* plants form terminal flowers, whereas those of 35S::*AP1* plants usually senesce (Figure 2J).

SD-grown 35S::*AP1* 35S::*LFY* plants display a dramatically enhanced transformation of the primary shoot meristem relative to 35S::*AP1* and 35S::*LFY* plants (Figures 2I and 2J). Many fewer leaves and flowers are produced by 35S::*AP1* 35S::*LFY* plants, and a terminal flower is formed without bolting. Therefore, *LFY* and *AP1* together more effectively override the lowered reproductive competence of SD-grown shoot meristems than either *LFY* or *AP1* alone.

Constitutive *AP1* Activity Affects *TFL1* Expression

Striking similarities between the early-flowering and shoot-to-flower transformation phenotypes of *tfl1* loss-of-function mutants and 35S::*AP1* gain-of-function plants suggest that *TFL1* function is compromised by constitutive *AP1* activity (Figure 3). At least two models of opposing *TFL1* and *AP1* activities can explain the similar phenotypes observed. Constitutive *AP1* activity could be regulating *TFL1* expression; alternatively, constitutive expression of *AP1* could be bypassing *TFL1* activity without affecting its expression. To investigate these possibilities, we examined *TFL1* expression in 35S::*AP1* and wild-type plants grown under CL.

In wild-type plants, *TFL1* expression appears in subapical regions of primary and secondary shoot meristems (Bradley et al., 1997), as shown in Figure 4. We observed faint expression of *TFL1* at the primary shoot apex of day 6 wild-type plants; by day 12 and at all subsequent time points, high levels of *TFL1* expression were apparent in subapical regions of the primary apex and secondary shoot meristems (Figure 4C). In 35S::*AP1* plants at all time points collected, appreciable levels of *TFL1* expression were not observed (Figure 4D; data not shown). Faint traces of *TFL1* expression associated with secondary shoot meristems were seen infrequently (Figure 4E). Therefore, constitutive *AP1* activity is able to largely suppress *TFL1* expression in primary and secondary shoot meristems of CL-grown plants. Traces of *TFL1* expression observed in a few secondary meristems may correlate with our observations of partial shoot-to-flower transformations at some basal leaf nodes of 35S::*AP1* plants (Figure 2C).

Mutations in *TFL1* Enhance the 35S::*AP1*–Conferred Phenotype

Because the phenotypes of 35S::*AP1* and *tfl1* plants mirror each other, and the above results indicate that constitutive *AP1* activity can largely suppress *TFL1* expression, we

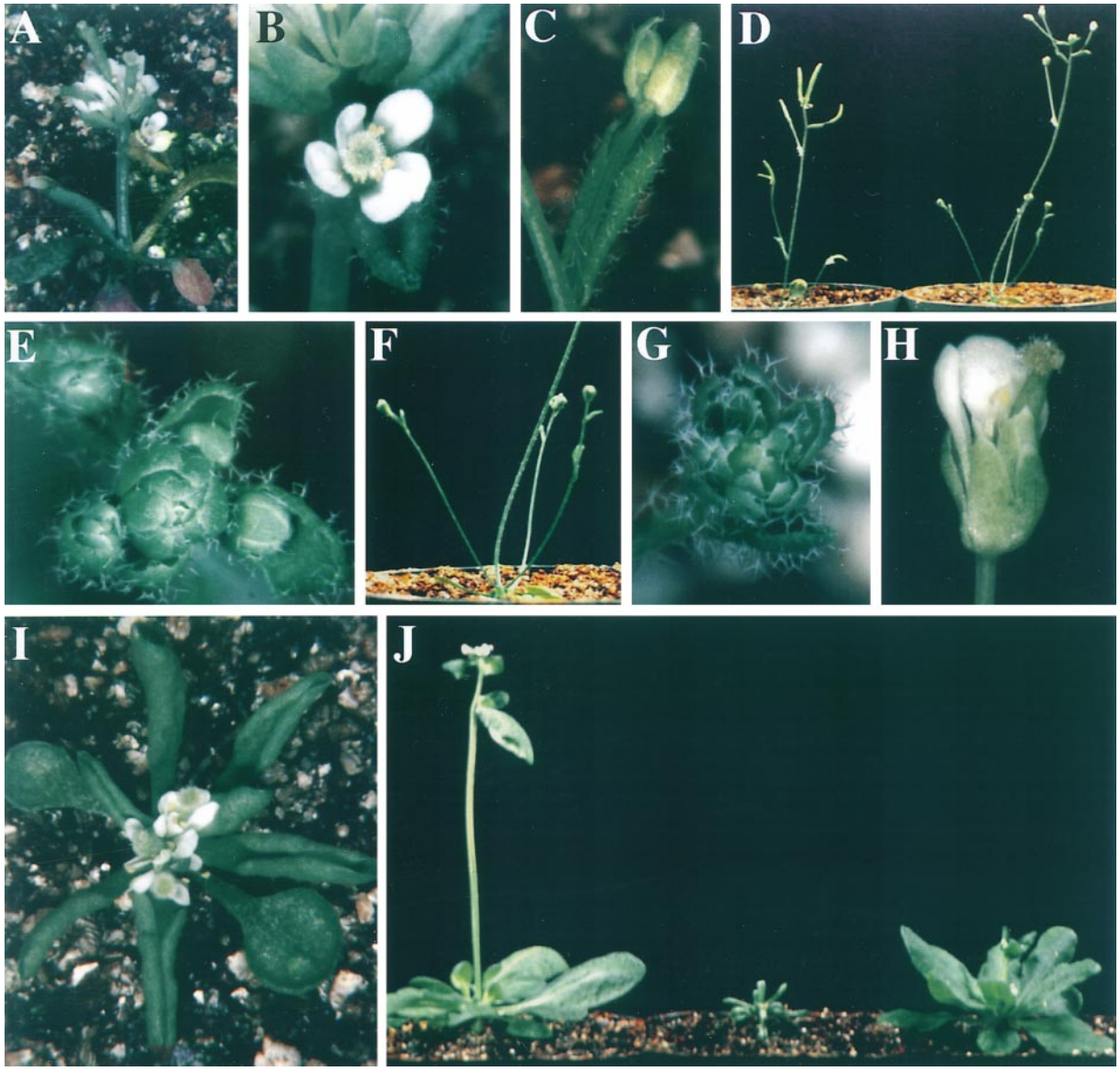

Figure 2. Phenotype of 35S::*AP1*, 35S::*AP1 lfy*, and 35S::*AP1* 35S::*LFY* Plants.

Plants were grown under CL (**[A]** to **[H]**) or SD (**[I]** and **[J]**) conditions.
(A) 35S::*AP1* plant (563.LI1.2) at day 18. After producing five leaves, this plant's primary shoot meristem has been converted into a compound terminal flower. Secondary shoot meristems present in the axils of cauline leaves also have been transformed into solitary flowers.
(B) Close-up of a secondary shoot-to-flower transformation shown in **(A)**. Converted flowers usually have an extra sepal and petal compared with wild-type flowers.
(C) Secondary shoot-to-flower conversion of a 35S::*AP1* plant (563.CI2.24) with characteristics of an abbreviated shoot. This structure most likely represents the partial conversion of a shoot meristem to a flower meristem.
(D) 35S::*AP1* (left) and 35S::*AP1 lfy* (right) plants at day 21. The shoot-to-flower conversions of 35S::*AP1* plants are largely unaffected by mutations in *LFY*, although some attenuation can be observed. 35S::*AP1 lfy* plants usually produce an extra cauline leaf before the primary shoot meristem is transformed into a terminal flower, and some of the converted floral meristems have shootlike traits.
(E) 35S::*AP1 lfy* terminal flower. Compared with the compound terminal flower formed in 35S::*AP1* plants (see **[A]** and **[D]**), the terminal flower formed by the primary shoot meristem of 35S::*AP1 lfy* plants retains some shootlike characteristics, because additional flower meristems arise in the axils of its outermost leaflike floral organs in a starburst pattern.
(F) 35S::*AP1 lfy* rosette flowers (close-up of 35S::*AP1 lfy* plant shown in **[D]**) showing internode elongation between outer organs. No further structures develop in the axils of these organs.

wondered whether mutations in *TFL1* could enhance further the abbreviated growth phases and shoot architecture of 35S::*AP1* plants. If constitutive *AP1* activity is already sufficient to downregulate *TFL1* expression, enhancement by mutations in *TFL1* should be minimal. We discovered that the degree of enhancement is affected by photoperiod. Compared with 35S::*AP1* and *tfl1* plants grown under CL, 35S::*AP1 tfl1* plants produce approximately two fewer leaves, and all secondary shoot-to-flower transformations are complete (Table 1 and Figure 3A). This relatively small enhancement reinforces our observations that *TFL1* expression is largely downregulated in CL-grown 35S::*AP1* plants but confirms that some *TFL1* activity remains. Under SD conditions, the enhancement is more striking, because 35S::*AP1 tfl1* plants flower with approximately nine fewer leaves than do 35S::*AP1* plants, and, whereas in both parents secondary shoot-to-flower transformations are notably attenuated, they are nearly complete in 35S::*AP1 tfl1* plants (Table 1 and Figure 3B). Furthermore, SD-grown 35S::*AP1 tfl1* plants produce significantly fewer floral nodes before the primary apex is converted into a terminal flower (Table 1).

DISCUSSION

LFY and *AP1* Act Redundantly and Positively to Regulate Each Other

Lateral meristems acquire a floral identity primarily through the activity of the meristem identity genes *LFY*, *AP1*, and *CAL*. Loss-of-function analyses indicate that these three genes act redundantly to affect the switch between production of leaf primordia with associated shoot meristems to the formation of flower meristems (Irish and Sussex, 1990; Schultz and Haughn, 1991, 1993; Huala and Sussex, 1992; Weigel et al., 1992; Bowman et al., 1993). We have discovered that the onset of *AP1* expression is delayed in *lfy* mutants, indicating that *LFY* is formally a positive regulator of *AP1*, which is consistent with molecular and genetic evidence provided by previous gain-of-function experiments. Shoot-to-flower transformations of plants constitutively expressing *LFY* are largely suppressed by mutations in *AP1*, indicating that *AP1* mediates many of the 35S::*LFY* effects (Weigel and Nilsson, 1995). Furthermore, ectopic expression of *AP1* has been observed in 35S::*LFY* plants, and increased levels of *AP1* were found in plants that express LFY::VP16, an activated form of LFY created by fusing LFY to the activation domain from the viral protein VP16 (Parcy et al., 1998). The close temporal sequence of *LFY* and *AP1* activation in wild-type plants implies that regulation of *AP1* by *LFY* could be direct (Gustafson-Brown, 1996; Simon et al., 1996; Hempel et al., 1997), and indeed, the LFY protein recently has been shown to bind to the *AP1* promoter (Parcy et al., 1998).

Besides directly regulating *AP1* expression, *LFY* also may indirectly promote *AP1* activity via negative regulation of *TFL1.* Transformations of *lfy* basal flowers into shoots previously have been attributed to inappropriate activation of *TFL1* in these lateral meristems because such transformations are partially reversed in *tfl1 lfy* mutants (Shannon and Meeks-Wagner, 1993). Indeed, *TFL1* is ectopically expressed in basal nodes of *lfy* inflorescences (Ratcliffe et al., 1999). Because nodes of *tfl1 lfy* mutants show more flowerlike characteristics than do corresponding nodes of *lfy* mutants, it seems likely that the onset of *AP1* expression may be partially restored in *tfl1 lfy* mutants. Thus, the delay in the onset of *AP1* expression in *lfy* mutants may be due to a combination of direct and indirect regulation of *AP1* by *LFY.*

We have found that whereas *LFY* is a positive regulator of *AP1*, *AP1* is also a positive regulator of *LFY. LFY* is prematurely expressed in the converted flower meristems of 35S::*AP1* plants. Moreover, although genetic analyses of CL- and SD-grown 35S::*AP1 lfy* mutants demonstrate that *AP1* acts primarily downstream of *LFY*, a feedback loop of *AP1* activating *LFY* is also apparent and is especially visible under noninductive growth conditions. After an abbreviated vegetative phase, SD-grown plants constitutively expressing *AP1* produce several floral nodes without subtending bracts before the primary shoot meristem either senesces or forms a terminal flower. These floral nodes occur at positions occupied by leaves with associated shoots in wild-type plants, and such nodes are replaced by leaves with leafy shoots or are absent in 35S::*AP1 lfy* plants, indicating that *LFY* mediates the bract suppression and floral identity of these

Figure 2. (continued).

(G) Typical 35S::*AP1 lfy* flower. Most flowers produced by 35S::*AP1 lfy* plants consist of leaflike floral organs produced in a spiral arrangement with a few carpelloid organs in the center, as is characteristic of *lfy* flowers.

(H) Rare 35S::*AP1 lfy* flower, with concentrically arranged floral organs, petals, and a stamen.

(I) 35S::*AP1* 35S::*LFY* plant at day 29. All primary and secondary shoots are converted into flowers, and leaves are small and tightly curled.

(J) 35S::*AP1* (left), 35S::*AP1* 35S::*LFY* (center), and 35S::*LFY* (right) plants at day 40. 35S::*AP1* 35S::*LFY* plants show an enhancement of both parental phenotypes. Under SD conditions, the primary shoots of 35S::*AP1* and 35S::*LFY* plants produce an inflorescence of flowers before conversion to a terminal flower. The primary shoot meristems of 35S::*AP1* 35S::*LFY* plants produce significantly fewer leaves and flowers before forming a terminal flower. 35S::*AP1* 35S::*LFY* plants produced a total of 9.8 $\pm$ 1.5 leaves compared with 15.2 $\pm$ 2.2, 18.2 $\pm$ 2.7, and 44.3 $\pm$ 2.2 total leaves produced by 35S::*AP1*, 35S::*LFY*, and wild-type plants, respectively.

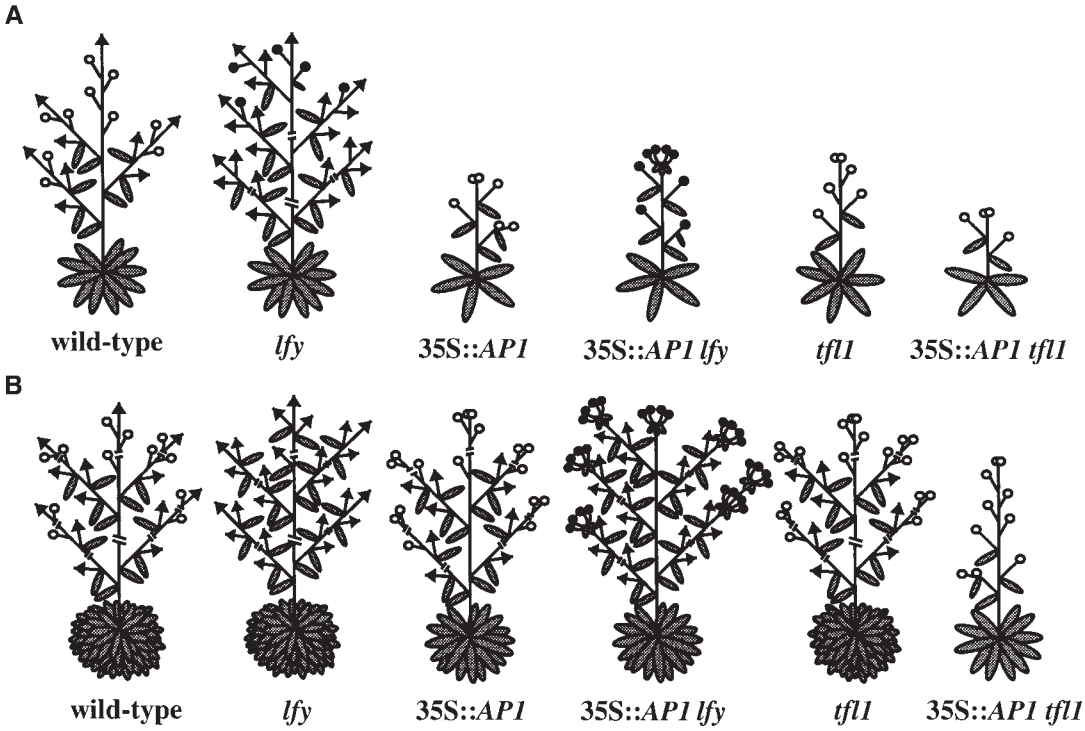

Figure 3. Shoot Architecture of Wild-Type, *lfy*, 35S::*AP1*, 35S::*AP1 lfy*, *tfl1*, and 35S::*AP1 tfl1* Plants.

(A) Plants grown under CL conditions. Plants constitutively expressing *AP1* flower significantly earlier than do wild-type plants and show an abbreviation of all growth phases, mirroring the phenotype of plants with loss-of-function mutations in *TFL1.* Mutations in *LFY* generally do not affect the early-flowering and shoot-to-flower transformations of CL-grown 35S::*AP1* plants; at best, 35S::*AP1 lfy*–converted flowers display additional shootlike traits.

(B) Plants grown under SD conditions. Early-flowering and shoot-to-flower transformations of 35S::*AP1* plants and *tfl1* mutants are notably attenuated by short photoperiods, although all primary and secondary shoots are abbreviated compared with wild-type shoots. 35S::*AP1 tfl1* plants grown under SD conditions exhibit a dramatic enhancement of both parental phenotypes because they flower earlier and display many shoot-to-flower transformations, implying that a significant level of *TFL1* activity is present in SD-grown 35S::*AP1* plants. Under short photoperiods, a feedback loop of *AP1* activating *LFY* is visible by comparing the shoot architecture of 35S::*AP1 lfy*, 35S::*AP1*, and wild-type plants. The primary shoot meristems of 35S::*AP1 lfy* plants produce approximately two more cauline leaves with associated shoots than do 35S::*AP1* plants (Table 1) before terminating in a leafy starburst, as seen in CL-grown 35S::*AP1 lfy* plants (Figure 2E). As additional shoots subtended by bracts in 35S::*AP1 lfy* plants occur at positions in 35S::*AP1* plants occupied by floral nodes, which in wild-type plants are occupied by leaves with associated shoot meristems, *LFY* activation via *AP1* appears responsible for the identity of these 35S::*AP1* floral nodes.

35S::*AP1*–conferred floral nodes. Our *AP1* gain-of-function results are also consistent with the previous observation that the initial expression of *LFY* is significantly reduced in *ap1 cal* double mutants, indicating that *AP1* and *CAL* are redundant for the upregulation of *LFY* (Bowman et al., 1993).

Although the activation of *AP1* by *LFY* may be direct, determining whether the upregulation of *LFY* by *AP1* is direct or indirect awaits further characterization of *LFY* regulatory regions. Moreover, whereas *AP1* is sufficient to activate *LFY* selectively within flower meristems, *LFY* expression is not upregulated throughout 35S::*AP1* plants. This suggests that *AP1* normally interacts with another flower-specific factor to upregulate *LFY* or, alternatively, that *AP1* negatively regulates a repressor of *LFY*, such as *TFL1*, and this in turn leads to the upregulation of *LFY.*

AP1 Is Regulated by a *LFY*-Independent Pathway

Because the onset of *AP1* expression is delayed in *lfy* mutants but is not absent, one or more factors act redundantly with *LFY* to activate *AP1* expression in lateral meristems. Good candidates for such factors include the flowering-time genes. It has been proposed that two genes in one of the in-

ductive flower-promoting pathways, *FT* and *FWA*, regulate *AP1* transcription independently of *LFY* because *lfy ft* and *lfy fwa* double mutants completely lack flowerlike structures and *AP1* expression has not been observed in either double mutant (Ruiz-García et al., 1997). These results indicate that *FT* and *FWA* may be primarily responsible for the redundant activation of *AP1* in *lfy* mutants (Ruiz-García et al., 1997). However, recent experiments have revealed that the *FWA* locus is hypomethylated in two ethyl methanesulfonate–induced *fwa* alleles (Koornneef et al., 1998), and it has been suggested that such mutants may represent gain-of-function alleles (Levy and Dean, 1998). Our observation that mutations in *FWA* completely suppress the early-flowering and shoot-to-flower transformations of plants constitutively expressing *AP1* (A. Pinyopich, S.J. Liljegren, and M.F. Yanofsky, unpublished results) supports the proposal that the wild-type *FWA* gene product may normally act as a floral repressor (Levy and Dean, 1998) rather than as an *AP1* activator.

Negative Regulation of *TFL1* by *AP1*

In wild-type plants, *TFL1* and *AP1* are expressed in nonoverlapping domains, where they act to promote inflorescence and flower meristem identity, respectively. We have shown that when *AP1* is constitutively expressed, it can negatively regulate *TFL1*, because *TFL1* RNA is largely absent in CL-grown 35S::*AP1* plants. However, we also have found that mutations in *TFL1* enhance the phenotypes of CL- and SD-grown 35S::*AP1* plants, confirming that some *TFL1* activity remains, despite constitutive *AP1* expression. Results consistent with these also have been described for 35S::*AP1* plants grown in long days (LD; 16 hr of light and 8

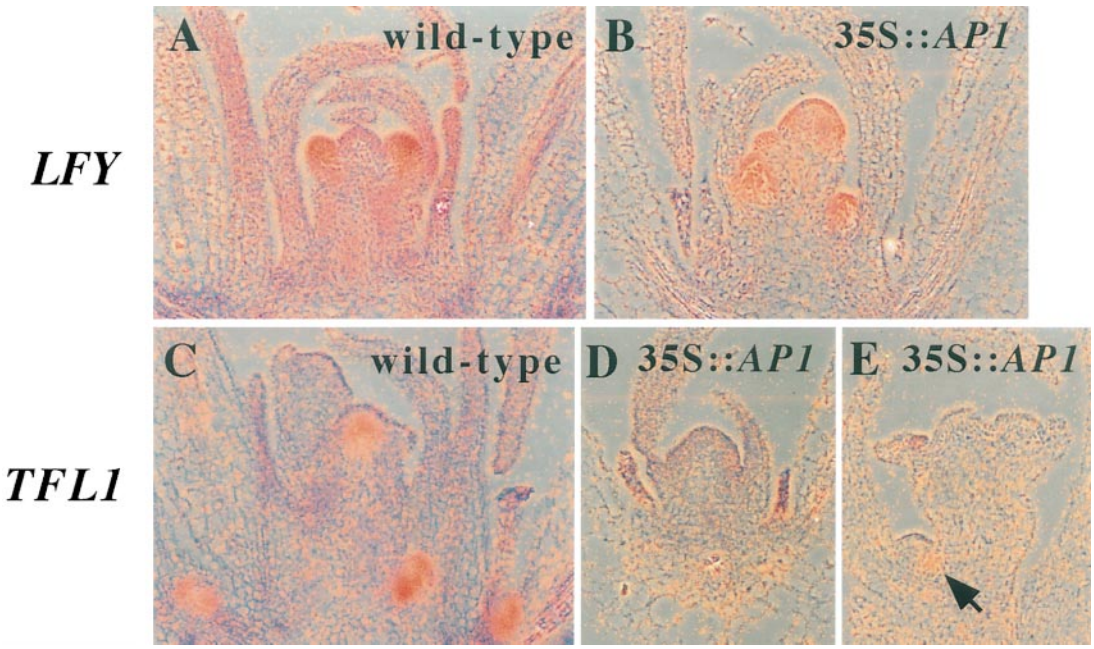

Figure 4. Expression of *LFY* and *TFL1* in Wild-Type and 35S::*AP1* Plants.

Sections of wild-type primary apices at day 12 (**[A]** and **[C]**) and 35S::*AP1* plants at days 6 **(D)** and 8 (**[B]** and **[E]**) probed with *LFY* (**[A]** and **[B]**) or *TFL1* (**[C]** to **[E]**) antisense RNA are shown.

(A) *LFY* is expressed in flower meristems arising on the flanks of the wild-type primary shoot apex.

(B) In 35S::*AP1* plants, *LFY* expression can be observed in converted floral meristems and at the primary apex at time points before it is first seen in flower meristems of wild-type plants.

(C) *TFL1* is expressed below the primary apex and in regions corresponding to secondary shoot meristems in wild-type plants, as described previously by Bradley et al. (1997).

(D) In 35S::*AP1* plants, *TFL1* expression was not observed in primary shoot apices and, in addition, was usually not seen in secondary meristems.

(E) Occasionally, faint traces of *TFL1* expression could be detected in 35S::*AP1* secondary meristems (see arrow), which may correlate with partial shoot-to-flower transformations that can occur at basal leaf nodes.

hr of dark): *TFL1* expression is briefly observed at the shoot apex and then is absent at subsequent time points (Ratcliffe et al., 1999). *AP1* and *CAL* may normally have overlapping roles in barring *TFL1* expression from floral meristems, because 35S::*CAL* plants also flower early and show shoot-to-flower conversions (Savidge, 1996). Moreover, the conversion of flower meristems into inflorescence meristems that occurs in *ap1 cal* mutants is reversed by mutations in *TFL1*, suggesting that ectopic activity of *TFL1* contributes to the inflorescence meristem proliferations that occur in this double mutant (Bowman et al., 1993). Recent experiments have indeed shown that whereas *ap1* mutants maintain a wild-type pattern of *TFL1* expression, *TFL1* is ectopically expressed in the lateral meristems and inflorescence meristem proliferations of *ap1 cal* double mutants, demonstrating the redundancy of *AP1*/*CAL* regulation of *TFL1* (Ratcliffe et al., 1999).

LFY also plays a role in negative regulation of *TFL1* because *TFL1* expression has not been observed in LD-grown 35S::*LFY* plants, whereas *TFL1* RNA is present in the basal nodes of *lfy* mutants (Ratcliffe et al., 1999). Because we have demonstrated that the onset of *AP1* expression is delayed in *lfy* mutants, the presence of *TFL1* transcripts in *lfy* basal nodes may be due to the simultaneous loss of both *LFY* and *AP1* activity at these positions rather than to the loss of *LFY* alone. Furthermore, the notable suppression of 35S::*LFY* shoot-to-flower transformations by mutations in *AP1* suggests that the absence of *TFL1* RNA in 35S::*LFY* plants is also due to concerted *AP1* and *LFY* activity.

Specification of Flower Meristems

Assignment of floral fate to lateral meristems of Arabidopsis plants involves two distinct actions: suppression of leaf primordia and production of flower rather than shoot meristems. Loss-of-function studies demonstrate that *LFY* is primarily responsible for suppression of bract development, presumably by acting in a small subset of cells at the base of the lateral primordium (Schultz and Haughn, 1991; Weigel et al., 1992). In wild-type plants, *TFL1* is expressed in lateral shoot meristems once they become distinct from subtending leaf primordia (Bradley et al., 1997). Besides programming a floral fate, cooperative activity of the flower meristem identity genes *LFY*, *AP1*, and *CAL* bars *TFL1* expression from lateral meristems, preventing establishment of a shoot program. Within developing flower meristems, *LFY* may directly activate *AP1*, whereas *AP1*, *CAL*, and *LFY* may indirectly regulate each other in part through negative regulation of *TFL1.* Cooperative interactions between *LFY* and *AP1* are clearly illustrated by the dramatic phenotype of 35S::*AP1* 35S::*LFY* plants under noninductive growth conditions. Under SD conditions, constitutive expression of both *AP1* and *LFY* is much more effective at overriding the lowered reproductive competence of shoot meristems to produce flower meristems than is either activity alone.

METHODS

Growth Conditions

For all phenotypic analyses and in situ hybridization experiments, *Arabidopsis thaliana* seeds were vernalized for 3 to 5 days at 4°C after sowing. Plants were grown at 22 to 24°C under either continuous light (CL) or short-day (SD) conditions.

Transgenic Lines and Mutant Alleles

Agrobacterium tumefaciens–mediated plant transformation with pAM563 (Mandel and Yanofsky, 1995) by the vacuum infiltration method (Bechtold et al., 1993) was used to generate 19 new 35S::*APETALA1* (*AP1*) lines in the Landsberg *erecta* ecotype. One of the strongest lines (563.LI1.2) was chosen for further study. Of the 35S::*AP1* lines previously generated in the Columbia ecotype (Mandel and Yanofsky, 1995), 47 were rescreened under CL and SD conditions, and three of the strongest lines (563.CI1.5, 563.CI1.19, and 563.CI2.1) were chosen for follow-up studies. All 35S::*AP1* lines examined exhibit a semidominant phenotype, basically as described by Mandel and Yanofsky (1995). Plants hemizygous for the *AP1* transgene generally exhibit no differences in flowering time from homozygous 35S::*AP1* plants, as measured by total leaves produced, but shoot-to-flower transformations are more partial (data not shown).

The 35S::*LEAFY* (*LFY*) line (DW151.2.5L; Weigel and Nilsson, 1995) used is in the Landsberg *erecta* ecotype. The *lfy-12* (Huala and Sussex, 1992) and *terminal flower tfl1-1* (Shannon and Meeks-Wagner, 1991) alleles are in the Columbia ecotype. Previous analysis of the 35S::*AP1 lfy*–conferred phenotype was with the *lfy-26* allele in the Landsberg *erecta* ecotype (Mandel and Yanofsky, 1995; Lee et al., 1997).

In Situ Hybridization Experiments

For analysis of *AP1* expression, *lfy-12* and wild-type (Columbia) plants were grown under CL conditions and harvested at 11 to 14 days after pots were moved to the growth room. Further time points for *lfy-12* plants were collected at days 15 to 19. Before approximately day 16, *lfy* mutants were visibly indistinguishable from wild-type plants; thus, plants homozygous for the *lfy-12* allele were identified by cleaved amplified polymorphic sequence marker genotyping (Konieczny and Ausubel, 1993), as described by Blázquez et al. (1997). Sections from two plants were analyzed for each time point. A similar study showed a delay in the onset of *AP1* expression in plants carrying a weak allele of *LFY*, *lfy-5* (data not shown; Gustafson-Brown, 1996). For analyses of *LFY* and *TFL1* expression, 35S::*AP1* plants (563.CI1.5) and wild-type (Columbia) plants were grown under CL conditions and harvested at 4, 6, 8, 10, 12, and 14 days after pots were moved to the growth room. Sections from two or three plants were analyzed for each time point with each probe.

Longitudinal sections of plant tissue were probed with ^{35}S-labeled *TFL1*, *AP1*, or *LFY* antisense RNA. *AP1* and *LFY* probes were synthesized as described previously (Gustafson-Brown et al., 1994; Blázquez et al., 1997). The *TFL1* probe was synthesized with T7 RNA polymerase from a SacI-digested pSL66 template to generate a transcript containing 392 nucleotides of the 3′ end of the *TFL1* cDNA. pSL66 was created by ligating a full-length *TFL1* cDNA fragment into pGEM-Teasy (Promega) after polymerase chain reaction amplification of the cDNA from a *TFL1* expressed sequence tag (T44654). Fix-

ation of tissue, preparation of 8-μm sections, hybridization, and washes were performed as described previously (Drews et al., 1991), with minor modifications. Slides were exposed for 10 days (*AP1*), 14 days (*LFY*), or 21 days (*TFL1*).

Phenotypic and Genetic Analyses

The 35S::*AP1* line 563.CI2.1 was used in crosses with both *lfy-12* and *tfl1-1* mutants. For each phenotypic analysis presented, five to 15 plants homozygous for the *AP1* transgene and the mutant allele were observed, along with similar numbers of control plants. For 35S::*AP1 lfy* phenotypic analyses, progeny of plants homozygous for the *AP1* transgene and heterozygous for the *lfy* allele also were observed, and the absence of the *lfy* allele in 35S::*AP1* control plants was verified by genotyping (see above). For 35S::*AP1 tfl1* phenotypic analyses, progeny of plants homozygous for the *AP1* transgene and the *tfl1* allele were observed. 35S::*AP1 tfl1* plants were initially identified by phenotype, and the genotype was confirmed by *tfl1-1*–derived cleaved amplified polymorphic sequence marker genotyping, as described by Neff et al. (1998). The molecular lesion associated with the *tfl1-1* allele is described by Bradley et al. (1997) and Ohshima et al. (1997).

Homozygous 35S::*AP1* plants (563.LI1.2) were pollinated by 35S::*LFY* plants to generate F_1 plants hemizygous for both *LFY* and *AP1* transgenes. For phenotypic analysis, 10 35S::*AP1* 35S::*LFY* plants were observed compared with plants hemizygous or homozygous for either the *AP1* or *LFY* transgene. Because significant differences in total leaves produced were not observed between plants hemizygous and homozygous for either respective transgene, only results for hemizygous 35S::*AP1* and 35S::*LFY* plants are presented.

ACKNOWLEDGMENTS

We thank Detlef Weigel, François Parcy, and Ove Nilsson for comments on the manuscript, for helpful discussions, and, along with Max Busch, Desmond Bradley, Oliver Ratcliffe, and Enrico Coen, for sharing results before publication. Transgenic lines and mutant alleles were supplied by Ove Nilsson, Detlef Weigel, and the Arabidopsis Biological Resource Center (Ohio State University, Columbus). We thank Detlef Weigel for providing the plasmid pDW124, Igor Kardailsky for providing *tfl1-1* genotyping primers, and Alejandra Mandel for assistance in the early stages of this research. Laura Block, Sonia Guimil, and Tina Kuhlmann provided excellent technical assistance. This work was supported by a Lucille P. Markey fellowship to S.J.L., an Ananda Mahidol Foundation fellowship to A.P., and grants from the National Science Foundation (No. IBN9728402), the National Institutes of Health (No. GM55328), and the U.S. Department of Agriculture (No. 96-35304-3701) to M.F.Y.

Received December 1, 1998; accepted March 18, 1999.

REFERENCES

Alvarez, J., Guli, C.L., Yu, X.-H., and Smyth, D.R. (1992). *terminal flower*: A gene affecting inflorescence development in *Arabidopsis thaliana.* Plant J. **2,** 103–116.

Bechtold, N., Ellis, J., and Pelletier, G. (1993). *In planta Agrobacterium*-mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. C. R. Acad. Sci. III **316,** 1194–1199.

Bernier, G. (1988). The control of floral evocation and morphogenesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. **39,** 175–219.

Blázquez, M.A., Soowal, L.N., Lee, I., and Weigel, D. (1997). *LEAFY* expression and flower initiation in *Arabidopsis.* Development **124,** 3835–3844.

Bowman, J.L., Alvarez, J., Weigel, D., Meyerowitz, E.M., and Smyth, D.R. (1993). Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. Development **119,** 721–743.

Bradley, D., Ratcliffe, O., Vincent, C., Carpenter, R., and Coen, E. (1997). Inflorescence commitment and architecture in *Arabidopsis.* Science **275,** 80–83.

Drews, G.N., Bowman, J.L., and Meyerowitz, E.M. (1991). Negative regulation of the Arabidopsis homeotic gene *AGAMOUS* by the *APETALA2* product. Cell **65,** 991–1002.

Grbic, V., and Bleecker, A.B. (1996). An altered body plan is conferred on *Arabidopsis* plants carrying dominant alleles of two genes. Development **122,** 2395–2403.

Gustafson-Brown, C. (1996). Characterization of the *Arabidopsis* Floral Homeotic Gene *APETALA1.* PhD Dissertation (La Jolla, CA: University of California at San Diego).

Gustafson-Brown, C., Savidge, B., and Yanofsky, M.F. (1994). Regulation of the Arabidopsis floral homeotic gene *APETALA1.* Cell **76,** 131–143.

Hempel, F.D., and Feldman, L.J. (1994). Bi-directional inflorescence development in *Arabidopsis thaliana*: Acropetal initiation of flowers and basipetal initiation of paraclades. Planta **192,** 276–286.

Hempel, F.D., Weigel, D., Mandel, M.A., Ditta, G., Zambryski, P.C., Feldman, L.J., and Yanofsky, M.F. (1997). Floral determination and expression of floral regulatory genes in *Arabidopsis.* Development **124,** 3845–3853.

Huala, E., and Sussex, I.M. (1992). *LEAFY* interacts with floral homeotic genes to regulate Arabidopsis floral development. Plant Cell **4,** 901–913.

Irish, V.F., and Sussex, I.M. (1990). Function of the *apetala-1* gene during *Arabidopsis* floral development. Plant Cell **2,** 741–753.

Kempin, S.A., Savidge, B., and Yanofsky, M.F. (1995). Molecular basis of the *cauliflower* phenotype in *Arabidopsis.* Science **267,** 522–525.

Konieczny, A., and Ausubel, F.M. (1993). A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J. **4,** 403–410.

Koornneef, M., Blankestijn-de Vries, H., Hanhart, C.J., Soppe, W., and Peeters, A.J.M. (1994). The phenotype of some late-flowering mutants is enhanced by a locus on chromosome 5 that is not effective in the Landsberg *erecta* wild-type. Plant J. **6,** 911–919.

Koornneef, M., Alonso-Blanco, C., Peeters, A.J.M., and Soppe, W. (1998). Genetic control of flowering time in *Arabidopsis.* Annu. Rev. Plant Physiol. Plant Mol. Biol. **49,** 345–370.

Lee, I., Wolfe, D.S., Nilsson, O., and Weigel, D. (1997). A *LEAFY* co-regulator encoded by *UNUSUAL FLORAL ORGANS.* Curr. Biol. **7,** 95–104.

Levy, Y.Y., and Dean, C. (1998). The transition to flowering. Plant Cell **10,** 1973–1989.

Mandel, M.A., and Yanofsky, M.F. (1995). A gene triggering flower formation in *Arabidopsis.* Nature **377,** 522–524.

Mandel, M.A., Gustafson-Brown, C., Savidge, B., and Yanofsky, M.F. (1992). Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1.* Nature **360,** 273–277.

Martínez-Zapater, J.M., Coupland, G., Dean, C., and Koornneef, M. (1994). The transition to flowering in *Arabidopsis.* In *Arabidopsis*, E.M. Meyerowitz and C.R. Somerville, eds (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 403–433.

Neff, M.M., Neff, J.D., Chory, J., and Pepper, A.E. (1998). dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: Experimental applications in *Arabidopsis thaliana* genetics. Plant J. **14,** 387–392.

Ohshima, S., Murata, M., Sakamoto, W., Ogura, Y., and Motoyoshi, F. (1997). Cloning and molecular analysis of the *Arabidopsis* gene *Terminal Flower 1.* Mol. Gen. Genet. **254,** 186–194.

Parcy, F., Nilsson, O., Busch, M.A., Lee, I., and Weigel, D. (1998). A genetic framework for floral patterning. Nature **395,** 561–566.

Poethig, R.S. (1990). Phase change and the regulation of shoot morphogenesis in plants. Science **250,** 923–930.

Ratcliffe, O.J., Amaya, I., Vincent, C.A., Rothstein, S., Carpenter, R., Coen, E.S., and Bradley, D.J. (1998). A common mechanism controls the life cycle and architecture of plants. Development **125,** 1609–1615.

Ratcliffe, O.J., Bradley, D.J., and Coen, E.S. (1999). Separation of shoot and floral identity in Arabidopsis. Development **126,** 1109–1120.

Ruiz-García, L., Madueño, F., Wilkinson, M., Haughn, G., Salinas, J., and Martínez-Zapater, J.M. (1997). Different roles of flowering-time genes in the activation of floral initiation genes in Arabidopsis. Plant Cell **9,** 1921–1934.

Savidge, B. (1996). Floral Meristem Specification and Floral Organ Development in Arabidopsis. PhD Dissertation (La Jolla, CA: University of California at San Diego).

Schultz, E.A., and Haughn, G.W. (1991). *LEAFY*, a homeotic gene that regulates inflorescence development in Arabidopsis. Plant Cell **3,** 771–781.

Schultz, E.A., and Haughn, G.W. (1993). Genetic analysis of the floral initiation process (FLIP) in *Arabidopsis.* Development **119,** 745–765.

Shannon, S., and Meeks-Wagner, D.R. (1991). A mutation in the Arabidopsis *TFL1* gene affects inflorescence meristem development. Plant Cell **3,** 877–892.

Shannon, S., and Meeks-Wagner, D.R. (1993). Genetic interactions that regulate inflorescence development in Arabidopsis. Plant Cell **5,** 639–655.

Simon, R., Igeño, M.I., and Coupland, G. (1996). Activation of floral meristem identity genes in *Arabidopsis.* Nature **384,** 59–62.

Weigel, D., and Meyerowitz, E.M. (1993). Activation of floral homeotic genes in *Arabidopsis.* Science **261,** 1723–1726.

Weigel, D., and Nilsson, O. (1995). A developmental switch sufficient for flower initiation in diverse plants. Nature **377,** 495–500.

Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F., and Meyerowitz, E.M. (1992). *LEAFY* controls floral meristem identity in Arabidopsis. Cell **69,** 843–859.