RESEARCH ARTICLE

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

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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 (V2), with production of rosette leaves being the first (V1). Secondary shoot meristems formed in the axils of cauline and rosette leaf primordia reiterate the V2 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 V2 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 V2 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 V2 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).
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 V₁ 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 (V₁) 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–confferred 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–confferred 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 Ify 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 Ify null (ify-12; Huala and Sussex, 1992) mutants. Compared with wild-type plants grown under continuous light (CL), Ify-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 Ify mutants, appreciable AP1 expression was not detected until approximately day 15. When AP1 RNA begins to accumulate in Ify 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 Ify 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 (ify-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
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 sepal) 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 tf1-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 Ify 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 tf1 Ify mutants (Shannon and Meeks-Wagner, 1993; Figures 2E and 3). Secondary shoot-to-flower conversions of 35S::AP1 Ify 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 Ify plants appear identical to flowers produced by Ify 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 Ify plants, such as those that develop in the axils of the terminal flower's outer whorl organs, can display characteristics more typical of weak Ify flowers. Approximately one flower per 35S::AP1 Ify plant clearly resembles flowers of intermediate or weak Ify 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 Ify 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 tf1 Ify 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 Ify mutants are further enhanced (Huala and Sussex, 1992; Weigel et al., 1992; Schultz and Haughn, 1993). Besides a dramatic increase in the number

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Table 1. Effect of Ify and tf1 on Leaf Number and Shoot Morphology of 35S::AP1 Plants in CL and SD conditions

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Rosette Leaves</th>
<th>Cauline Leaves</th>
<th>Total Leaves</th>
<th>Lateral Shoots</th>
<th>Floral Nodes</th>
<th>Total Nodes</th>
</tr>
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<tbody>
<tr>
<td>CL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>8.0 ± 0</td>
<td>2.5 ± 0.7</td>
<td>10.5 ± 0.7</td>
<td>2.5 ± 0.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ify</td>
<td>8.5 ± 0.8</td>
<td>7.0 ± 0.6</td>
<td>15.5 ± 0.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>35S::AP1</td>
<td>3.3 ± 0.7</td>
<td>2.1 ± 0.3</td>
<td>5.4 ± 0.7</td>
<td>0.2 ± 0.4</td>
<td>1.9 ± 0.6</td>
<td>5.4 ± 0.7</td>
</tr>
<tr>
<td>35S::AP1 Ify</td>
<td>3.3 ± 0.8</td>
<td>3.5 ± 0.5</td>
<td>6.8 ± 0.8</td>
<td>1.2 ± 1.0</td>
<td>2.3 ± 0.8</td>
<td>6.8 ± 0.8</td>
</tr>
<tr>
<td>Wild type</td>
<td>10.5 ± 0.6</td>
<td>2.9 ± 0.5</td>
<td>13.4 ± 0.8</td>
<td>2.9 ± 0.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>tf1</td>
<td>7.2 ± 0.7</td>
<td>1.5 ± 0.5</td>
<td>8.7 ± 0.7</td>
<td>0</td>
<td>3.0 ± 0.8</td>
<td>10.2 ± 1.2</td>
</tr>
<tr>
<td>35S::AP1</td>
<td>5.2 ± 0.6</td>
<td>2.7 ± 0.8</td>
<td>7.9 ± 1.3</td>
<td>1.0 ± 0.9</td>
<td>1.8 ± 1.2</td>
<td>8.0 ± 1.3</td>
</tr>
<tr>
<td>35S::AP1 tf1</td>
<td>3.6 ± 0.5</td>
<td>1.7 ± 0.4</td>
<td>5.3 ± 0.5</td>
<td>0</td>
<td>1.7 ± 0.4</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>59.8 ± 2.7</td>
<td>9.6 ± 0.9</td>
<td>69.4 ± 2.1</td>
<td>9.6 ± 0.9</td>
<td>28.2 ± 5.7</td>
<td>97.6 ± 7.5</td>
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<tr>
<td>Ify</td>
<td>60.8 ± 7.0</td>
<td>56.8 ± 1.9</td>
<td>117.6 ± 6.8</td>
<td>55.2 ± 3.5</td>
<td>1.6 ± 2.1</td>
<td>117.6 ± 6.8</td>
</tr>
<tr>
<td>35S::AP1</td>
<td>10.3 ± 1.4</td>
<td>2.7 ± 0.7</td>
<td>13.0 ± 1.8</td>
<td>2.7 ± 0.7</td>
<td>16.1 ± 10.5</td>
<td>29.1 ± 11.5</td>
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<tr>
<td>35S::AP1 Ify</td>
<td>11.2 ± 1.7</td>
<td>4.8 ± 0.8</td>
<td>16.0 ± 2.4</td>
<td>4.7 ± 0.8</td>
<td>0.2 ± 0.4</td>
<td>16.0 ± 2.4</td>
</tr>
<tr>
<td>Wild type</td>
<td>58.5 ± 4.3</td>
<td>11.6 ± 0.9</td>
<td>70.1 ± 4.1</td>
<td>11.6 ± 0.9</td>
<td>40.7 ± 3.1</td>
<td>110.7 ± 4.2</td>
</tr>
<tr>
<td>tf1</td>
<td>50.4 ± 3.2</td>
<td>8.4 ± 0.9</td>
<td>58.8 ± 4.1</td>
<td>7.7 ± 1.0</td>
<td>27.1 ± 2.6</td>
<td>85.3 ± 4.6</td>
</tr>
<tr>
<td>35S::AP1</td>
<td>18.5 ± 2.8</td>
<td>5.9 ± 1.5</td>
<td>24.4 ± 4.1</td>
<td>5.9 ± 1.5</td>
<td>31.2 ± 1.9</td>
<td>55.6 ± 4.9</td>
</tr>
<tr>
<td>35S::AP1 tf1</td>
<td>11.4 ± 1.6</td>
<td>3.9 ± 1.0</td>
<td>15.3 ± 2.5</td>
<td>0.9 ± 1.4</td>
<td>7.0 ± 1.9</td>
<td>19.3 ± 4.4</td>
</tr>
</tbody>
</table>

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

Sections of wild-type primary apices at days 11 to 14 appear at top; sections of Ify 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 Ify 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 Ify 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 Ify 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 Ify 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 Ify 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 Ify 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 axis 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 axis 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 whereas 35S::AP1 plants 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 plants, indicating that LFY mediates the bract suppression and floral identity of these press 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 leaf 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 mutants (Shannon and Meeks-Wagner, 1993). Indeed, TFL1 is ectopically expressed in basal nodes of leaf inflorescences (Ratcliffe et al., 1999). Because nodes of tfl1 mutants show more flowerlike characteristics than do corresponding nodes of mutant plants, it seems likely that the onset of AP1 expression may be partially restored in tfl1 mutants. Thus, the delay in the onset of AP1 expression in leaf 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 plants 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 plants, indicating that LFY mediates the bract suppression and floral identity of these

Figure 2. (continued).

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

(H) Rare 35S::AP1 leaf 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 ± 1.5 leaves compared with 15.2 ± 2.2, 18.2 ± 2.7, and 44.3 ± 2.2 total leaves produced by 35S::AP1, 35S::LFY, and wild-type plants, respectively.
1014 The Plant Cell

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-

Figure 3. Shoot Architecture of Wild-Type, lfy, 35S::AP1, 35S::AP1 lfy, tf1l, and 35S::AP1 tf1l 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 tf1l mutants are notably attenuated by short photoperiods, although all primary and secondary shoots are abbreviated compared with wild-type shoots. 35S::AP1 tf1l 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.
Interactions among Meristem Identity Genes

Inductive 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 non-overlapping 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 hr of darkness).

![Figure 4. Expression of LFY and TFL1 in Wild-Type and 35S::AP1 Plants.](image)

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 Ify mutants (Ratcliffe et al., 1999). Because we have demonstrated that the onset of AP1 expression is delayed in Ify mutants, the presence of TFL1 transcripts in Ify 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.

**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.C11.5, 563.C11.19, and 563.C12.1) 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 Ify-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 Ify-conferred phenotype was with the Ify-26 allele in the Landsberg erecta ecotype (Mandel and Yanofsky, 1995; Lee et al., 1997).

**In Situ Hybridization Experiments**

For analysis of AP1 expression, Ify-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 Ify-12 plants were collected at days 15 to 19. Before approximately day 16, Ify mutants were visibly indistinguishable from wild-type plants; thus, plants homozygous for the Ify-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, Ify-5 (data not shown; Gustafson-Brown, 1996). For analyses of LFY and TFL1 expression, 35S::AP1 plants (563.C11.5) 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 35S-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-
tion 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 Ify-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 Ify phenotypic analyses, progeny of plants homozygous for the AP1 transgene and heterozygous for the Ify allele also were observed, and the absence of the Ify 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 F1 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.

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