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Interaction of *LEAFY*, *AGAMOUS* and *TERMINAL FLOWER1* in maintaining floral meristem identity in *Arabidopsis*

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SUMMARY

The *Arabidopsis* transcription factor LEAFY acts upstream of homeotic genes such as *AGAMOUS* to confer floral identity on meristems that arise after the transition to reproductive development. Compared to the genetic circuitry regulating the establishment of floral meristem identity, little is known about its maintenance. Previous experiments with *leafy* heterozygous plants and *agamous* mutants grown in conditions that reduce the floral inductive stimulus have shown that both genes are required to prevent reversion of floral to inflorescence meristems. Here, we present evidence that *LEAFY* maintains floral meristem identity independently of *AGAMOUS*, and that the primary role of LEAFY is either direct repression of shoot identity genes or repression of an intermediate factor that activates shoot identity genes. The latter conclusions were deduced from the phenotypes conferred by a gain-of-function transgene, *LEAFY:VP16*, that appears to act as a dominant negative, or antimorphic, allele during maintenance of floral meristem identity. These observations contrast with previous findings that LEAFY acts as a direct activator of floral homeotic genes, supporting the hypothesis that the transcriptional activity of LEAFY is dependent on specific co-regulators.

Key words: *Arabidopsis thaliana*, Flower development, Meristem identity, *LEAFY*, Floral reversion

INTRODUCTION

In flowering plants, almost the entire adult body derives from groups of undifferentiated cells called meristems, which include a small number of true stem cells. In the aerial portion of the plant, leaves and axillary shoots are formed during the initial, vegetative phase, and flowers after the plant has been induced to make the transition from vegetative to reproductive development. Several genes that are required to confer floral identity on newly arising meristems have been identified, including *LEAFY* (*LFY*) and *APETALA2* (*AP2*), and the three closely related *APETALA1* (*AP1*), *CAULIFLOWER* (*CAL*) and *FRUITFULL* (*FUL*) genes (Bowman et al., 1993; Ferrándiz et al., 2000; Huala and Sussex, 1992; Irish and Sussex, 1990; Schultz and Haughn, 1991; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993; Weigel et al., 1992). In the absence of these genes, all of which encode transcription factors (Gu et al., 1998; Kempin et al., 1995; Mandel et al., 1992; Okamuro et al., 1997; Parcy et al., 1998), floral meristems are partially or completely replaced by shoot meristems, while ectopic overexpression of several of them causes an opposite phenotype, replacement of shoots with flowers (Liljegren et al., 1999; Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995). The activity of the floral meristem-identity genes is counteracted by the shoot-identity gene *TERMINAL FLOWER1* (*TFL1*), which encodes a protein that is likely involved in signal transduction (Alvarez et al., 1992; Bradley et al., 1997; Liljegren et al., 1999; Ratcliffe et al., 1998; Ratcliffe et al., 1999; Shannon and Meeks-Wagner, 1991; Shannon and Meeks-Wagner, 1993).

In many species, including *Arabidopsis*, a floral inductive stimulus is required only transiently to cause a stable transition from vegetative to reproductive development. In contrast, a floral inductive stimulus is required continuously in several other species, and its absence can cause either inflorescence or floral reversion. In inflorescence reversion, a shoot ceases to produce flowers and reverts to the formation of leaves with axillary vegetative shoots. In floral reversion, individual flowers stop producing floral organs and initiate vegetative organs (Anthony et al., 1996; Battey and Lyndon, 1984; Battey and Lyndon, 1986; Battey and Lyndon, 1988; Battey and Lyndon, 1990; Pouteau et al., 1997; Pouteau et al., 1998). The latter observation indicates that floral meristem identity not only needs to be established, but also maintained.

Both inflorescence and floral reversion are rare in wild-type *Arabidopsis* (Bowman, 1994; Laibach, 1951), but floral reversion has been described in several mutant backgrounds (e.g., Bowman et al., 1993; Clark et al., 1993; Mizukami and

Ma, 1997; Okamuro et al., 1996). While these instances of floral reversion differ in details, they all have in common that the flowers do not revert to a leaf-producing vegetative shoot meristem, but to a flower-producing inflorescence meristem.

In contrast to the establishment of floral meristem identity, its maintenance has received relatively little attention. It is particularly intriguing that *LFY*, a cardinal factor in establishing floral meristem identity, is also required for its maintenance (Okamuro et al., 1996). It is unclear how direct this effect of *LFY* is, especially since *LFY* is a direct activator of the homeotic gene *AGAMOUS* (*AG*), which itself is required for stable floral meristem identity (Busch et al., 1999; Mizukami and Ma, 1997; Okamuro et al., 1996). *lfy* mutants are of limited use in studying this process, because, in these plants, floral meristem identity is not properly established in the first place. Here, we use a combination of mutants and transgenic plants to dissect the role of *LFY* in the maintenance of floral meristem identity. First, we show that *LFY* maintains floral meristem identity independently of homeotic gene activation. Second, we provide evidence that *LFY* is likely to perform this function by acting as a transcriptional repressor of shoot identity genes.

MATERIALS AND METHODS

Plant material

Plants were grown under a 3:1 mixture of Cool White and Gro-Lux (wide spectrum) fluorescent lights at 23°C. Unless otherwise noted, plants were grown in long days (16 hours light, 8 hours darkness). Short day conditions were 8 hours light, 16 hours darkness.

Wild type was either Landsberg *erecta* (L*er*) or Columbia (Col-0, Col-7). *ag-1* and *lfy-6* mutants in L*er* (Bowman et al., 1989; Weigel et al., 1992), and *tfl1-1* and *lfy-12* mutants in Col-0 have been described previously (Huala and Sussex, 1992; Shannon and Meeks-Wagner, 1991). *LFY:VP16* lines DW245.2.7 and DW245.2.25 (strong phenotype as homozygotes), and DW245.2.37 (weak phenotype) were in the Col-7 background (Parcy et al., 1998). Additional transgenic plants were generated by vacuum infiltration of Col-7, using vectors pDW245 (for LFY:VP16) and pFP17 (for LFY:mVP16) (Bechtold et al., 1993; Parcy et al., 1998). dCAPS genotyping of *ag-1* heterozygotes has been described elsewhere (Neff et al., 1998). *lfy-6* and *lfy-12* heterozygous and homozygous plants were identified by CAPS genotyping (Konieczny and Ausubel, 1993) as described previously (Blázquez et al., 1997) (http://www.weigelworld.org).

Plant analysis

Methods for in situ hybridization, scanning electron microscopy and light microscopy were according to Parcy and colleagues (Parcy et al., 1998). The *TFL1* probe was derived from p129D7 (Bradley et al., 1997).

Any flower that produced secondary flowers interior to the first whorl of organs was considered to be reverting. Partially reverted flowers continued to produce floral organs, while completely reverted flowers did not, and produced only secondary flowers.

RESULTS

AG and *LFY* have additive effects on floral reversion

Previous work has shown that both *LFY* and *AG* are required for the maintenance of floral meristem identity, as flowers of *lfy/LFY* heterozygous plants or *ag* homozygous mutants can revert to shoots under weak floral inductive conditions such as short days (Mizukami and Ma, 1997; Okamuro et al., 1996). To determine whether reduction of *LFY* activity and loss of *AG* function affect reversion independently, we compared *ag* mutants that were either wild-type for *LFY*, or heterozygous for a *lfy* null allele. Under our conditions, *LFY/LFY ag/ag* plants grown in short days reverted only rarely. In contrast, *lfy/LFY ag/ag* plants showed frequent floral reversion (Table 1, Fig. 1H), indicating that *LFY* prevents reversion even in the absence of *AG*.

Abnormal meristem development in *LFY:VP16* flowers

LFY is a direct activator of homeotic genes such as *AG* and *AP1* (Busch et al., 1999; Parcy et al., 1998; Wagner et al., 1999). The fact that LFY activates transcription in a heterologous system, yeast, only when fused to the VP16 transcriptional activation domain (Cousens et al., 1989; Triezenberg et al., 1988), or when co-expressed with another transcription factor, indicates that LFY has to interact with other proteins to regulate downstream targets (Busch et al.,

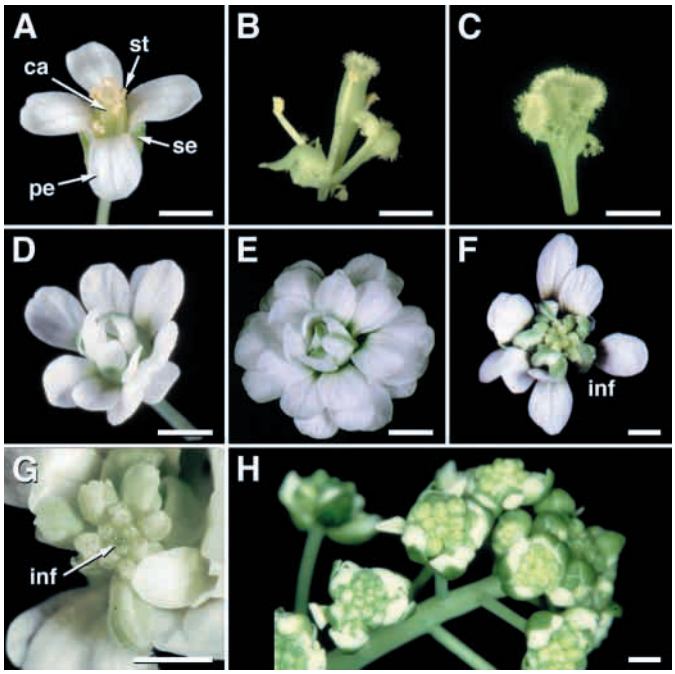

Fig. 1. Floral reversion in *ag-1* mutant backgrounds. (A) Wild-type flower with sepals (se), petals (pe), stamens (st) and carpels (ca) forming the central gynoecium. (B) *LFY:VP16*, intermediate phenotype. The outer whorl is occupied by carpelloid organs. Organs in whorl 2 are missing or replaced by stamens. (C) *LFY:VP16*, strong phenotype. The number of floral organs is reduced further. All of them are carpelloid, and no whorled structure is apparent. (D) *ag-1*. Stamens in whorl 3 are replaced by petals, and the central gynoecium by an internal flower that repeats the pattern of the primary flower. (E-G) *LFY:VP16 ag-1*, strong phenotype. Young flowers are similar to those of *ag* mutants and include petaloid sepals (E). In older flowers, a new inflorescence (inf) emerges from the center (F,G). (H) *lfy-6/+ ag-1* flower from a plant grown in short days. After a few whorls of floral organs had formed, the flower reverted to an inflorescence. All plants were grown in long days except (H). Scale bars 1 mm.

Table 1. Floral reversion in *ag-1* background

	lfy-6/LFY$^+$	*LFY*$^+$*/LFY*$^+$
Plants	19	13
Flowers examined per plant	20	20
Reverting flowers, range	0-20	0-2
Reverting flowers, average	10.5	0.5
Standard deviation	8.9	0.8

Plants were grown in short days. Reversion was counted on the first 20 flowers produced by the primary inflorescence of each plant. *lfy-6/LFY* and *LFY*$^+$*/LFY*$^+$ are significantly different from each other (Student's *t*-test, $P<0.00015$).

1999; Lohmann et al., 2001; Parcy et al., 1998). Fusion to the VP16 activation domain also allows LFY to activate *AG* independently of other factors in planta (Busch et al., 1999; Parcy et al., 1998). Since LFY is normally an activator of *AG*, the more potent LFY:VP16 can be interpreted as a hypermorphic form of LFY. However, if LFY transcriptional activity is context dependent, LFY:VP16 might have a dominant-negative effect by activating target genes that are directly repressed by unmodified LFY. There are several examples for conversion of a transcriptional repressor into an activator by fusion to the VP16 domain (e.g., Conlon et al., 1996; Estes et al., 2001; Ferreiro et al., 1998; Waltzer et al., 1995; Zuber et al., 1999). A process during which LFY might normally act as a repressor could be maintenance of floral meristem identity, during which LFY prevents floral reversion, presumably by repressing the transcription of shoot identity genes. If this is the case, *LFY:VP16* should have effects on floral reversion similar to those resulting from reduction of wild-type *LFY* activity.

The floral phenotype of *LFY:VP16* plants is mostly due to ectopic *AG* expression and consists of reduced floral organs and conversion of petals to stamens and sepals to carpels (Fig. 1A-C) (Parcy et al., 1998). Because there are no obvious signs of floral reversion in mature *LFY:VP16* flowers, we studied developing *LFY:VP16* flowers using the scanning electron microscope. Initiation of floral meristems in *LFY:VP16* plants was similar to wild type, but deviated from wild type subsequently. Instead of initiating four first-whorl organs in a cruciform manner, *LFY:VP16* produced an irregular number of first-whorl organs, often more than four (Fig. 2A,B,F). In wild type, the formation of the first whorl is followed by the initiation of petal primordia in the second whorl and stamen primordia in the third whorl (Smyth et al., 1990). Between the stamen primordia, floral meristem development is terminated by formation of the domed gynoecium primordium, which gives rise to the congenitally fused carpels (Fig. 2E,I). In *LFY:VP16*, there was no evidence of second- or third-whorl primordia demarcating the gynoecial dome (Fig. 2F,J). Instead, the meristem continued to grow apically, and new, fused primordia were initiated in a pattern that was at least partially spiral (Fig. 2J). These primordia eventually fused to produce an abnormal gynoecium that had an excess of style tissue at the expense of valve tissue (Fig. 2M,N). The abnormal

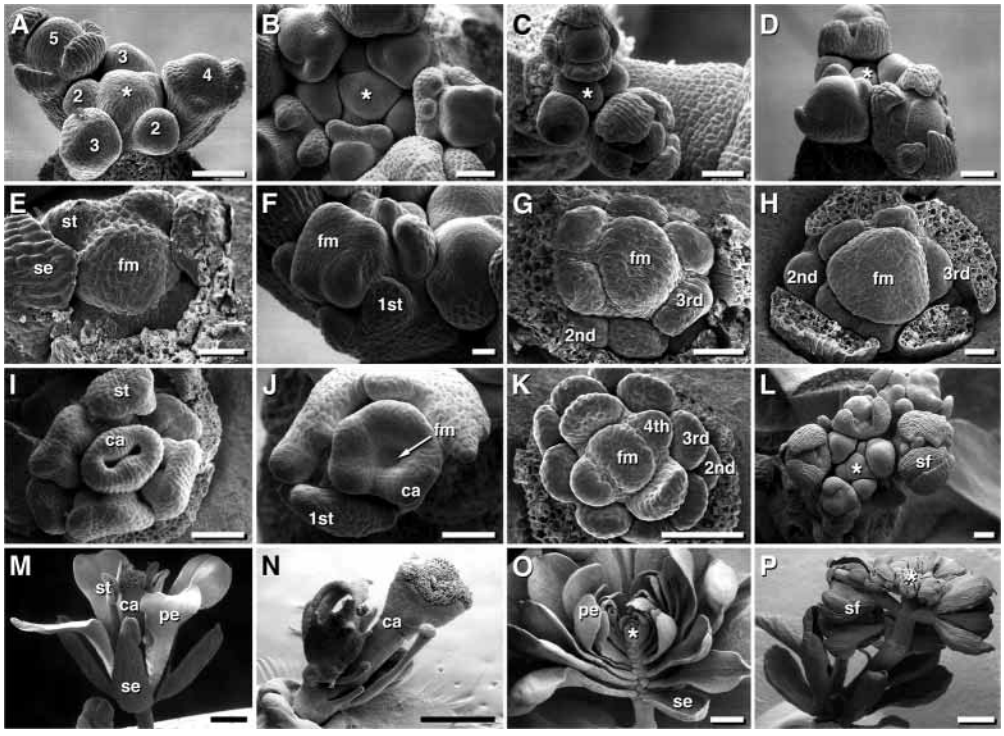

Fig. 2. Scanning electron micrographs of developing mutant and transgenic flowers. The top row shows inflorescence shoot apices (meristem indicated with an asterisk) surrounded by developing flowers. Numbers indicate floral stages (Smyth et al., 1990). (A,E,I,M) Wild type. (E) Stage 5 flower, with two sepals (se) dissected away to reveal the developing stamens (st) and the floral meristem (fm), which has begun to form the central gynoecium consisting of congenitally fused carpels. (I) Stage 7 flower, in which the floral meristem has terminated with the formation of carpels (ca). (M) Mature flower with four whorls or organs, including sepals, petals (pe), stamens and carpels. (B,F,J,N) Strong *LFY:VP16* line. Note supernumerary organs in the first whorl (1st) of developing flowers (F). The floral meristem is enlarged compared to that of the wild type and is beginning to produce another set of four organ primordia. (J) Three partially fused carpels are found in the center, which appear to have almost spiral phyllotaxy. A floral meristem is still visible in the center. (N) A mature flower that consists of several carpels and carpelloid organs that lack a clear whorled arrangement. (C,G,K,O) *ag-1*. The floral meristem persists and produces many whorls of organs that develop into sepals and petals. The floral meristem is indicated by an asterisk in (O). (D,H,L,P) *LFY:VP16 ag-1*. After the meristem has produced several whorls of organs, it reverts to an inflorescence meristem (indicated by an asterisk) that produces secondary flowers (sf). Note the enlarged floral meristem in H. Scale bars 50 μm (A-D, I-L), 20 μm (E-H), and 500 μm (M-P).

early development of *LFY:VP16* flowers, with continued proliferation of the floral meristem and partially spiral phyllotaxis, suggests a meristem defect in these flowers. Specifically, the floral meristem may have partial shoot identity.

AG masks floral reversion induced by *LFY:VP16*

One possibility for why *LFY:VP16* flowers have initially abnormal floral meristem development, but ultimately do not revert into shoots is that the effects of *LFY:VP16* on reversion are counterbalanced by the effects of *AG*, the expression of which is dramatically increased in *LFY:VP16*. Not only is *AG* required for maintenance of floral meristem identity, but overexpression of *AG* can also be sufficient to convert shoots into flowers (Mizukami and Ma, 1997; Okamuro et al., 1996). To determine whether increased *AG* expression masks an effect of *LFY:VP16* on floral reversion, we examined *LFY:VP16 ag* plants. As described previously, *ag* completely suppresses the homeotic conversions seen in *LFY:VP16* (Fig. 1D,E) (Parcy et al., 1998). However, after a few whorls of floral organs were produced, *LFY:VP16 ag-1* flowers became proliferous and reverted either partially or completely to shoots (Fig. 1F,G). Partially reverted flowers continued to produce both floral organs and secondary flowers, while completely reverted flowers produced only secondary flowers in a spiral phyllotaxy. In contrast to *ag* or *lfy/LFY* flowers that revert only in short days, *LFY:VP16 ag* flowers reverted both in long and short days.

Scanning electron microscopy of developing *LFY:VP16 ag* flowers revealed that the number of first-whorl organs was irregular, as with *LFY:VP16 AG*$^+$ flowers, and often greater than four (Fig. 2C,D). In contrast to *LFY:VP16 AG*$^+$ or non-transgenic *ag* flowers, only a few whorls of floral organs were produced, before the floral meristem reverted to a shoot meristem and produced new floral primordia on its flanks (Fig. 2G,H,K,L). Second-order flowers repeated the pattern of the primary flowers, with a few whorls of floral organs followed by the production of higher-order floral primordia (Fig. 2L,P).

LFY:VP16 is not a neomorphic allele

A concern with any gain-of-function allele is that it has activity unrelated to the normal function of the gene, or neomorphic activity (Muller, 1932). This would be the case, for example, if *LFY:VP16* interacted with promoters or proteins that are not targets of unmodified *LFY*. A hallmark of neomorphic mutations is that they are not affected by the dosage of the wild-type gene. We have previously shown that the homeotic organ conversions in *LFY:VP16* flowers are strongly dependent on the copy number of endogenous wild-type *LFY*, indicating that this phenotype is not due to neomorphic activity of *LFY:VP16* (Parcy et al., 1998). To confirm that *LFY:VP16* similarly does not act as a neomorph with respect to floral reversion, but rather as an antimorph, we studied a weak *LFY:VP16* line, which does not show any homeotic organ conversions in a wild-type background, but produces an intermediate phenotype when in a *lfy* heterozygous background, and a strong phenotype when in a *lfy* homozygous mutant background (Fig. 3B) (Parcy et al., 1998). When introduced into an *ag* mutant background, this *LFY:VP16* line showed no evidence of reversion. However, when we reduced the copy number of wild-type *LFY* from two to one in the *ag* line carrying the weak *LFY:VP16* insertion, frequent reversion

Fig. 3. Phenotypic effects of *LFY:VP16* are dependent on endogenous *LFY*. Structures shown are 'single flowers' from a weak *LFY:VP16* line. (A) In an otherwise wild-type background, the flowers of this line are similar to those of wild type. (B) In *LFY:VP16 lfy-12*, each flower consists only of congenitally fused carpels. (C,D) In *LFY:VP16 lfy-12 /+ ag-1*, single flowers are replaced by structures consisting of flower buds interspersed with floral organs. The first-whorl organs of the flower shown in C have fallen off; only secondary flowers remain. (E,F) In *LFY:VP16 lfy-12 ag-1*, only a small number of floral organs were produced before the floral meristem reverted to produce only floral buds. Scale bars 1 mm (A-C,F), and 3 mm (D,E).

was observed (Fig. 3C,D). An even more dramatic reversion was seen in a *lfy* homozygous mutant background; the floral meristem stopped producing floral organs and gave rise only to new floral buds (Fig. 3E,F). This observation demonstrates that *LFY:VP16* and endogenous *LFY* compete for the same targets in the reversion process, as they do in specifying floral organ identity. We also note that, in a *lfy* mutant background, *LFY:VP16* produces a phenotype that is very different from weak loss-of-function alleles, for which an extensive allelic series has been described (Huala and Sussex, 1992; Levin and Meyerowitz, 1995; Schultz and Haughn, 1991; Schultz and Haughn, 1993; Weigel et al., 1992).

The *LFY:VP16* phenotype in an *AG*$^+$ background is strongly transgene dosage-dependent, such that homozygous transgenic plants always show a considerably stronger phenotype than hemizygous plants (Parcy et al., 1998). A similar effect as on homeotic organ conversions was seen with floral reversion, when we examined three *LFY:VP16 ag* families that segregated for transgene insertions with intermediate phenotypes outside the *ag* background. Only a minority of plants in the three families showed reversion, consistent with only homozygous transgenic plants reverting. In contrast, in two *LFY:VP16 ag* families segregating for insertions with strong phenotypes outside the *ag* background, all plants showed reversion.

As a further test to determine whether the *LFY:VP16* effect was indeed due to the altered transcriptional activation potential conferred by the VP16 fusion, we transformed *ag* heterozygotes with the *LFY:mVP16* construct, which carries a truncated and inactive variant of the VP16 activation domain (Parcy et al., 1998). None of 12 *LFY:mVP16 ag* primary transformants showed any sign of reversion. In contrast, 3 out of 4 *LFY:VP16 ag* primary transformants showed complete or partial reversion. Fisher's exact test shows the two genotypes

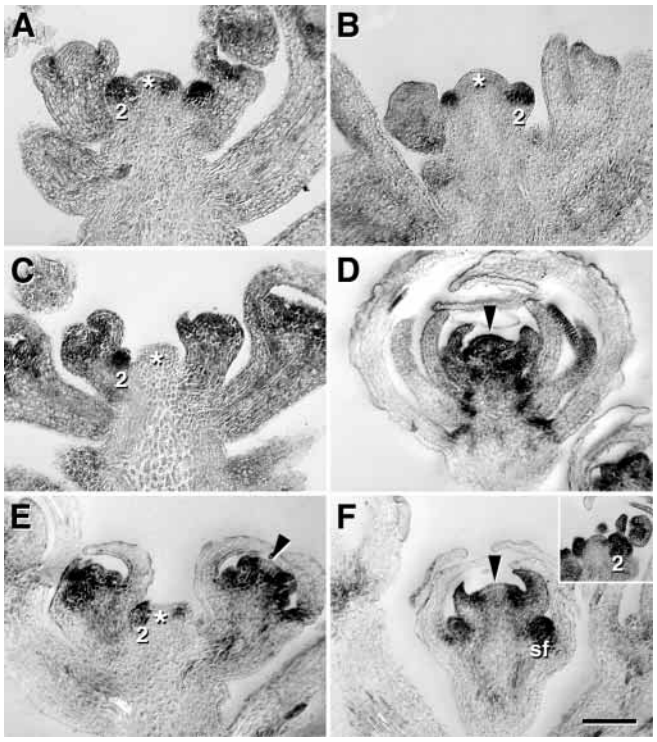

Fig. 4. Expression of *AP1* RNA in sections of inflorescence apices of wild-type (A), strong *LFY:VP16* (B), *ag-1* (C), and strong *LFY:VP16 ag-1* (E) plants (meristems indicated by asterisks). Numbers indicate floral stages (Smyth et al., 1990). (D,F) Sections of individual *ag-1* (D) and *LFY:VP16 ag-1* (F) flowers. In wild type *AP1* is activated as soon as flowers arise and expressed throughout floral primordia until stage 2. In *ag* mutants, *AP1* expression persists in the center of floral meristems (arrowhead in D). (E,F) In *LFY:VP16 ag* plants, *AP1* RNA is eliminated from the center of floral meristem after only a few whorls of organs have formed (arrows). Note secondary flowers (sf) in F. The inset in F shows the central meristem in a fully reverted flower, with a pattern of *AP1* expression similar to that of a wild-type inflorescence apex. Scale bar in F represents 50 μm for all panels, except for the inset in F (24 μm).

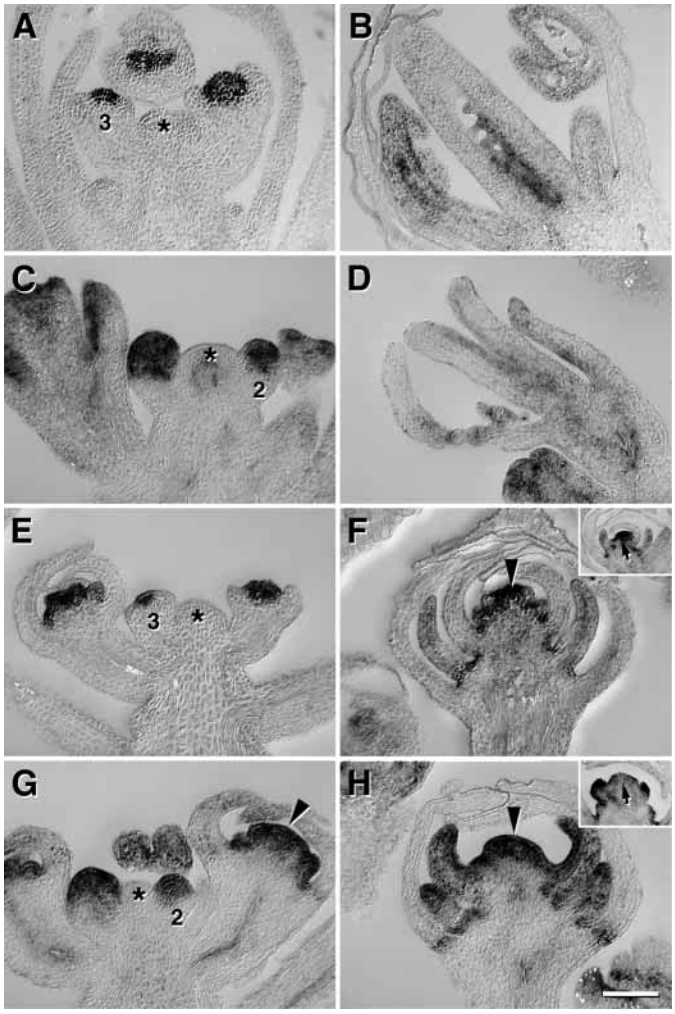

Fig. 5. Expression of *AG* RNA in wild type (A,B), strong *LFY:VP16* (C,D), *ag-1* (E,F) and strong *LFY:VP16 ag-1* (G,H). Left column shows sections of shoot apices with inflorescence meristems (indicated by asterisks) surrounded by young flowers. Right column shows sections of individual flowers. Numbers indicate floral stages (Smyth et al., 1990). *AG* is activated precociously and ectopically in *LFY:VP16*. (F,G) Mutant *AG* RNA persists in the center of *ag-1* and *LFY:VP16 ag-1* flowers (arrowheads). (H) Mutant *AG* RNA disappears from the center of *LFY:VP16 ag-1* flowers only at a late stage, after the reverted meristem has begun to produce secondary flowers (inset in H, compare with inset in F, arrows). Scale bar in H represents 50 μm for all panels, except for insets in F and H (24 μm).

to be significantly different regarding reversion with P=0.007, from which we conclude that the VP16 transcriptional activation potential is necessary to induce floral reversion. Taken together with the observation that reversion in an *ag* background is observed both in plants that are compromised in wild-type *LFY* function (in *lfy* heterozygotes) and in *LFY:VP16* plants, we conclude that *LFY:VP16* is a dominant-negative allele of *LFY* with respect to floral reversion.

Expression of floral markers in *LFY:VP16 ag*

We extended our morphological analysis of reverting flowers in *LFY:VP16 ag* by analyzing the expression of two floral marker genes, *AP1* and *AG*. *AP1* is initially activated throughout floral meristems, but becomes confined to the two outer whorls of organs, sepals and petals, from stage 3 on (Fig. 4A) (Mandel et al., 1992). Repression of *AP1* at this time is due to activation of *AG* in the center of the flowers (Fig. 5A) (Drews et al., 1991; Gustafson-Brown et al., 1994).

Because *AG* activity is absent in *ag* mutants, *AP1* continues to be expressed in the meristem of *ag* flowers (Fig. 4C,D) (Gustafson-Brown et al., 1994). In contrast to the persisting *AP1* expression in the center of *ag* flowers, we found that *AP1* RNA disappeared from the center of *LFY:VP16 ag* interior flowers even before the meristem had completely reverted to an inflorescence meristem (Fig. 4E). *AP1* RNA was already repressed in the center of *LFY:VP16 ag* flowers while these still produced floral organs (Fig. 4F). At later stages, the pattern of *AP1* expression in fully reverted *LFY:VP16* flowers resembled the *AP1* pattern in wild-type inflorescence apices, with *AP1* being restricted to new floral primordia forming on the flanks of the reverted floral meristem (Fig. 4F, inset).

AG, which is expressed in the center of wild-type flowers, is activated precociously and throughout young flowers in *LFY:VP16* (Fig. 5C) (Parcy et al., 1998). In the proliferating

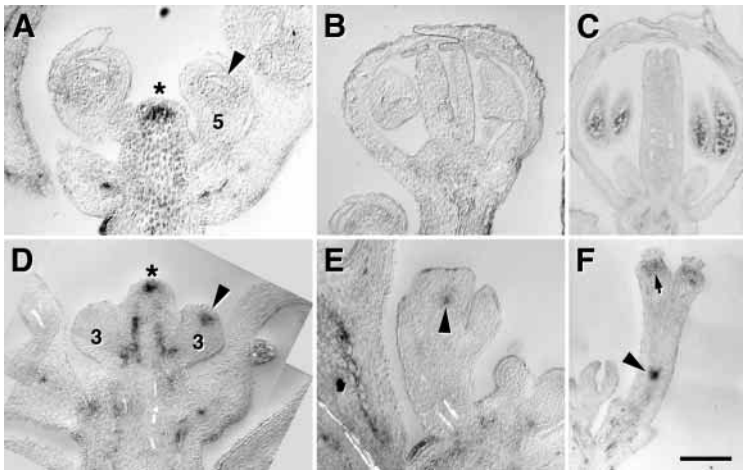

Fig. 6. Expression of *TFL1* RNA in wild type (A-C) and strong *LFY:VP16* (D-F) plants. Left column shows sections of shoot apices with inflorescence meristems (indicated by asterisks). Middle and right columns show sections of individual flowers. Numbers indicate floral stages (Smyth et al., 1990). In wild type, *TFL1* RNA is restricted to a group of subapical cells in the inflorescence meristem, and absent from flowers (arrowhead in A). In *LFY:VP16* flowers, there is ectopic *TFL1* expression, initially in a pattern similar to that in the shoot apical meristem (arrowheads in D,E). In the more advanced flower in (F), there is weak *TFL1* expression at the tip of the gynoecium (arrow). Occasionally, as in this flower, there is also a small group of *TFL1*-expressing cells at the base of the central gynoecium (arrowhead), possibly indicating a group of persisting meristematic cells. Scale bar in F represents 50 μm (A,C,D,F), and 24 μm (B,E).

meristems of *ag* flowers, mutant *AG* RNA continues to be expressed (Fig. 5E,F) (Gustafson-Brown et al., 1994). Initially, a similar pattern was seen in *LFY:VP16 ag* flowers, but in older flowers, mutant *AG* RNA disappeared from the central meristem (Fig. 5H inset), indicating that it had lost floral identity. The disappearance of *AG* RNA from the center of flowers, however, appeared to be delayed relatively to that of *AP1*.

Role of *TFL1* in *LFY:VP16*-induced floral reversion

One of the few cloned genes known to promote shoot meristem identity (or repress floral meristem identity) is *TFL1* (Bradley et al., 1997; Ratcliffe et al., 1998; Ratcliffe et al., 1999). Having shown that *LFY:VP16* has a dominant-negative effect on the maintenance of floral meristem identity, we wondered whether *TFL1* might mediate this effect. In wild type, *TFL1* is expressed in inflorescence meristems, but not developing flowers (Fig. 6A-C) (Bradley et al., 1997). Surprisingly, we found that *TFL1* was activated in *LFY:VP16* floral primordia, even though there was little morphological evidence for reversion in these flowers (Fig. 6D,E). In older flowers, weak ectopic *TFL1* expression was observed near the tip of the gynoecium, and, less often, in a group of cells at the base of the abnormal gynoecium (Fig. 6F).

To examine whether ectopic activation of *TFL1* is responsible for *LFY:VP16*-induced floral reversion, we generated *LFY:VP16 ag tfl1* plants. In contrast to *LFY:VP16 ag*, the flowers produced by *LFY:VP16 ag tfl1* plants never showed complete reversion to inflorescence shoots (Fig. 7C-G). Although the flowers on the main shoot produced a large number of secondary flowers, they did not stop producing floral organs, and secondary flowers remained interspersed with floral organs (Fig. 7F,G). That reversion was reduced, but not abolished, in *LFY:VP16 ag tfl1* indicates that ectopic activation of *TFL1* is partially responsible for floral reversion of *LFY:VP16* flowers. However, *TFL1* does not appear to be sufficient to trigger overt reversion, since *LFY:VP16 AG*$^{+}$ flowers do not revert, despite ectopic *TFL1* activation. Similarly, expressing *TFL1* under the control of *AG* regulatory sequences in the center of *ag* mutant flowers is not sufficient to cause reversion (R. Hong, M. Busch and D. W., unpublished results).

DISCUSSION

One of the main differences between shoot and floral meristems is that the central stem cell population in shoot meristems persists, whereas it is only transiently maintained in flowers. However, persisting stem cell proliferation in, for

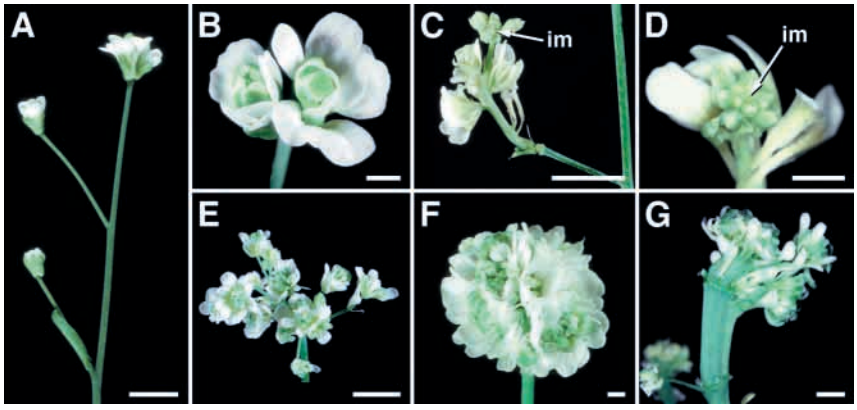

Fig. 7. A mutation in *TFL1* attenuates floral reversion in *LFY:VP16 ag-1* plants. (A) *tfl1-1 ag-1* inflorescence. The inflorescence has terminated with a single flower. Note that the most basal flower has formed in the axil of a leaf. (B) The floral phenotype of *tfl1-1 ag-1*, including that of this terminal flower, is the same as in *ag-1* flowers. (C,D) Flowers from a strong *LFY:VP16 ag-1* plant (either *tfl1-1/TFL1* or *TFL1/TFL1*; not genotyped). After the flower has produced a variable number of whorls of organs, the floral meristem reverts to an inflorescence meristem (im), which produces only floral buds. (E-G) Modified flowers from a strong *LFY:VP16 ag-1 tfl1-1* plant. Reversion to a true inflorescence that produces only secondary flowers is never observed, although the floral meristem produces many flowers interspersed with floral organs. In old flowers, fasciation is sometimes observed (G). Scale bars 1 mm (B,G,F), and 5 mm (A,C-E).

example, *clv1* or *ag* mutants, does not normally lead to floral reversion, but rather to the continued production of more floral organs (Bowman et al., 1989; Bowman et al., 1991; Clark et al., 1993; Mizukami and Ma, 1995; Sieburth et al., 1995). That floral reversion can be induced in some of these mutants, either by combining them with other mutants or by reducing floral inductive cues, suggests that the central stem cell population does not become irreversibly specified as floral, but rather that maintenance of floral meristem identity requires the continued activity of genes such as *LFY*. The results presented in this study support this hypothesis.

Floral reversion in *Arabidopsis*

Two types of situations in which floral meristems behave as inflorescence meristems have been described in *Arabidopsis*. One example is provided by *ap1 cal* double mutants, or even more strikingly, *ap1 cal ful* triple mutants, in which the inflorescence shoot meristem produces primordia that often behave very similarly to secondary inflorescence meristems (Bowman et al., 1993; Ferrándiz et al., 2000). This phenotype is reminiscent of floral reversion in many other species, typically induced by the removal of floral inductive cues (Battey and Lyndon, 1990). The *ap1 cal* and *ap1 cal ful* phenotypes are characterized by a failure to establish robust *LFY* expression, with concomitant ectopic *TFL1* expression in lateral meristems. That changes in *LFY* and *TFL1* expression are responsible for the *ap1 cal* and *ap1 cal ful* defects has been confirmed by demonstrating that overexpression of *LFY* or inactivation of *TFL1* strongly suppresses meristem proliferation in *ap1 cal* and *ap1 cal ful* mutants (Bowman et al., 1993; Ferrándiz et al., 2000). On the other hand, overexpression of *AG*, which acts downstream of *LFY*, is insufficient to suppress any aspect of the *ap1 cal ful* phenotype (Ferrándiz et al., 2000).

In contrast to *ap1 cal* or *ap1 cal ful* mutants, other mutants produce floral primordia that revert to an inflorescence shoot meristem only after several whorls of floral organs have been produced. This group of mutants includes *ag* mutants and *lfy* heterozygotes grown under short days, and *ap1 clv1* mutants (Clark et al., 1993; Okamuro et al., 1996). Similarly, *LFY:VP16 ag* floral meristems produce at least two whorls of organs before reverting to an inflorescence meristem. Also in contrast to *ap1 cal* or *ap1 cal ful*, inactivation of *TFL1* has only modest effects on floral reversion of *LFY:VP16*. Together, these observations indicate that the *LFY:VP16* allele uncouples the role of *LFY* in establishing and maintaining floral meristem identity.

Role of *LFY* in maintaining floral meristem identity

How does LFY contribute to the maintenance of floral meristem identity? LFY is a DNA-binding protein that directly regulates transcription of downstream genes (Busch et al., 1999; Parcy et al., 1998; Wagner et al., 1999). One of these targets is *AG*, which represses floral reversion (Mizukami and Ma, 1997; Okamuro et al., 1996). The additive effects of reducing *LFY* copy number and inactivating *AG* on floral reversion indicate that *LFY* does not maintain floral meristem identity solely by ensuring a sufficient level of *AG* expression. This result is consistent with the finding that *AG* RNA expression is not obviously altered in young flowers of *lfy/LFY* plants grown in short days (Okamuro et al., 1996).

There are two alternative explanations for the increased floral reversion in *ag/ag lfy/LFY* or *ag/ag LFY:VP16* plants. One possibility is that *LFY* and *AG* act entirely independently on floral reversion. Another possibility is that *LFY* acts as an *AG* substitute to maintain floral meristem identity in *ag* mutants. Indeed, *LFY* continues to be expressed in the center of (long-day grown) *ag* floral meristems (D. W., unpublished results). If maintenance of high levels of *LFY* expression in these meristems is compromised in short days, this could cause floral reversion in *ag* mutants, further exacerbated when *LFY* copy number is reduced.

LFY:VP16 is an activated version of LFY that can activate targets such as *AG* and *AP1* more strongly than wild-type LFY and that can therefore be classified as a hypermorphic allele with respect to activation of these targets (Parcy et al., 1998). In contrast, *LFY:VP16* appears to be an antimorphic allele with respect to maintenance of floral meristem identity, because it acts in a manner opposite to that of wild-type *LFY*.

Considering the evidence for LFY:VP16 being a transcriptional activator and the fact that at least one shoot identity gene, *TFL1*, is derepressed in *LFY:VP16* floral meristems, we postulate that transcriptional repression by LFY is involved in preventing reversion of a floral to an inflorescence meristem. If we assume further that floral reversion involves the activation of a hypothetical set of shoot identity genes, we can envision two scenarios through which LFY affects these genes (Fig. 8). In the first scenario, LFY negatively regulates shoot identity genes indirectly by activating a transcriptional repressor that downregulates or represses shoot identity genes. In the second scenario, LFY's primary effect is transcriptional repression, either through

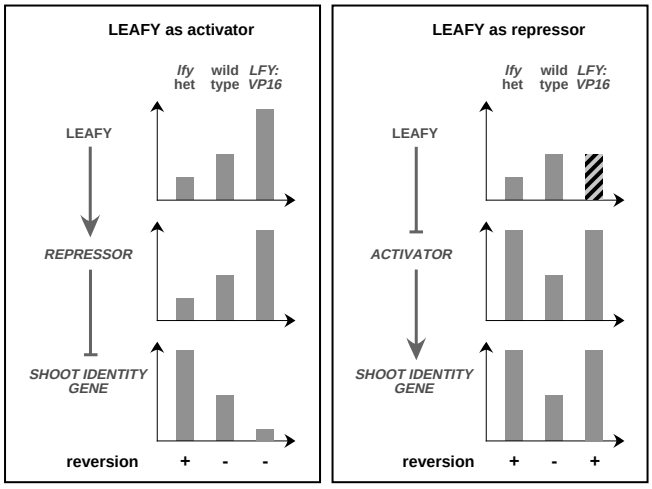

Fig. 8. Two scenarios for LFY action during maintenance of floral meristem identity. Diagrams show levels of LFY activity and expression levels of shoot identity genes along with levels of an intermediate regulator. If LFY's primary activity in this process is as a transcriptional activator, LFY:VP16 should be more potent than wild-type LFY, and reversion should not occur, because levels of shoot identity genes remain low. If LFY's primary activity is as a transcriptional repressor, LFY:VP16 should have the opposite effect of wild-type LFY, and cause elevated shoot identity gene expression, similar to a reduction in LFY activity in *lfy* heterozygotes. In the latter case, a scenario without an intermediate activator is formally equivalent.

direct repression of shoot identity genes, or though repression of a positive regulator of shoot identity genes. In both cases, reducing *LFY* activity in *lfy* heterozygotes would lead to floral reversion because of increased activity of shoot identity genes. However, if LFY's primary effect was transcriptional activation of a hypothetical reversion repressor, LFY:VP16 should be even more effective than wild-type LFY in activating this repressor, and we would not expect reversion. In contrast, if LFY's primary effect were repression of a hypothetical reversion activator, LFY:VP16 would have an opposite effect from wild-type LFY. Because LFY:VP16 acts in a dominant-negative fashion – floral reversion is observed both when wild-type LFY is reduced (in *lfy* heterozygous plants) and in *LFY:VP16* plants – we believe it most plausible that LFY's primary effect in maintaining floral meristem identity is transcriptional repression.

It is unknown whether LFY has some intrinsic repression potential. We have shown previously that LFY is able to bind to *AG* and *AP1* regulatory sequences, but LFY on its own is not sufficient for transcriptional activation in a heterologous system, yeast (Busch et al., 1999; Lohmann et al., 2001; Parcy et al., 1998). We therefore proposed that LFY interacts with other factors to differentially affect the expression of downstream targets, a hypothesis that we recently confirmed by demonstrating that LFY directly interacts in activation of *AG* with another transcription factor, the homeo domain protein WUSCHEL (Lohmann et al., 2001). By analogy, we hypothesize that LFY interacts with an unknown co-repressor in preventing floral meristem reversion. Little is known about transcriptional repression in plants, but the *Arabidopsis* genome encodes putative co-repressors such as LEUNIG (Conner and Liu, 2000).

Which are the genes repressed by LFY to prevent floral reversion? *TFL1* is derepressed both in *lfy* mutants (Ratcliffe et al., 1999) and in *LFY:VP16* plants (this work). However, although the *TFL1* promoter contains putative LFY binding sites, these are not bound by LFY in vitro (M. A. Busch and D. W., unpublished data), suggesting either that *TFL1* is not a direct target of LFY, or that other proteins are required for interaction of LFY with *TFL1* regulatory sequences. To further understand the interaction of *LFY* and *TFL1*, it will be necessary to define the regulatory sequences sufficient for normal *TFL1* expression.

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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.

Anthony, R. G., James, P. E. and Jordan, B. R. (1996). Cauliflower (*Brassica oleracea* var. *botrytis* L.) curd development: the expression of meristem identity genes. *J. Exp. Bot.* **47**, 181-188.

Battey, N. H. and Lyndon, R. F. (1984). Changes in apical growth and phyllotaxis on flowering and reversion in *Impatiens balsamina* L. *Ann. Bot.* **54**, 553-567.

Battey, N. H. and Lyndon, R. F. (1986). Apical growth and modification of the development of primordia during re-flowering of reverted plants of *Impatiens balsamina* L. *Ann. Bot.* **58**, 333-341.

Battey, N. H. and Lyndon, R. F. (1988). Determination and differentiation of leaf and petal primordia in *Impatiens balsamina*. *Ann. Bot.* **61**, 9-16.

Battey, N. H. and Lyndon, R. F. (1990). Reversion of flowering. *Bot. Rev.* **56**, 163-189.

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.* **316**, 1194-1199.

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

Bowman, J. L. (1994). *Arabidopsis*: *An Atlas of Morphology and Development*. New York: Springer.

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.

Bowman, J. L., Smyth, D. R. and Meyerowitz, E. M. (1989). Genes directing flower development in *Arabidopsis*. *Plant Cell* **1**, 37-52.

Bowman, J. L., Smyth, D. R. and Meyerowitz, E. M. (1991). Genetic interactions among floral homeotic genes of *Arabidopsis*. *Development* **112**, 1-20.

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

Busch, M. A., Bomblies, K. and Weigel, D. (1999). Activation of a floral homeotic gene in *Arabidopsis*. *Science* **285**, 585-587.

Clark, S. E., Running, M. P. and Meyerowitz, E. M. (1993). *CLAVATA1*, a regulator of meristem and flower development in *Arabidopsis*. *Development* **119**, 397-418.

Conlon, F. L., Sedgwick, S. G., Weston, K. M. and Smith, J. C. (1996). Inhibition of Xbra transcription activation causes defects in mesodermal patterning and reveals autoregulation of Xbra in dorsal mesoderm. *Development* **122**, 2427-2435.

Conner, J. and Liu, Z. (2000). LEUNIG, a putative transcriptional corepressor that regulates *AGAMOUS* expression during flower development. *Proc. Natl. Acad. Sci. USA* **97**, 12902-12907.

Cousens, D. J., Greaves, R., Goding, C. R. and O'Hare, P. (1989). The C-terminal 79 amino acids of the herpes simplex virus regulatory protein, Vmw65, efficiently activate transcription in yeast and mammalian cells in chimeric DNA-binding proteins. *EMBO J.* **8**, 2337-2342.

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.

Estes, P., Mosher, J. and Crews, S. T. (2001). *Drosophila single-minded* represses gene transcription by activating the expression of repressive factors. *Dev. Biol.* **232**, 157-175.

Ferrándiz, C., Gu, Q., Martienssen, R. and Yanofsky, M. F. (2000). Redundant regulation of meristem identity and plant architecture by *FRUITFULL*, *APETALA1* and *CAULIFLOWER*. *Development* **127**, 725-734.

Ferreiro, B., Artinger, M., Cho, K. and Niehrs, C. (1998). Antimorphic *goosecoids*. *Development* **125**, 1347-1359.

Gu, Q., Ferrándiz, C., Yanofsky, M. F. and Martienssen, R. (1998). The *FRUITFULL* MADS-box gene mediates cell differentiation during *Arabidopsis* fruit development. *Development* **125**, 1509-1517.

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

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**, 741-751.

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.

Laibach, F. (1951). Über sommer- und winterannuelle Rassen von *Arabidopsis thaliana* (L.) Heynh. Ein Beitrag zur Ätiologie der Blütenbildung. *Beitr. Biol. Pflanzen* **28**, 173-210.

Levin, J. Z. and Meyerowitz, E. M. (1995). *UFO*: an Arabidopsis gene involved in both floral meristem and floral organ development. *Plant Cell* **7**, 529-548.

Liljegren, S. J., Gustafson-Brown, C., Pinyopich, A., Ditta, G. S. and Yanofsky, M. F. (1999). Interactions among *APETALA1*, *LEAFY*, and *TERMINAL FLOWER1* specify meristem fate. *Plant Cell* **11**, 1007-1018.

Lohmann, J. U., Hong, R., Hobe, M., Busch, M. A., Parcy, F., Simon, R. and Weigel, D. (2001). A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. *Cell* **105**, 793-803.

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.

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

Mizukami, Y. and Ma, H. (1995). Separation of *AG* function in floral meristem determinacy from that in reproductive organ identity by expressing antisense *AG* RNA. *Plant Mol. Biol.* **28**, 767-784.

Mizukami, Y. and Ma, H. (1997). Determination of Arabidopsis floral meristem identity by *AGAMOUS*. *Plant Cell* **9**, 393-408.

Muller, H. J. (1932). Further studies on the nature and causes of gene mutations. *Proc. Int. Congr. Genet.* **6**, 213-255.

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.

Okamuro, J. K., Caster, B., Villarroel, R., Van Montagu, M. and Jofuku, K. D. (1997). The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **94**, 7076-7081.

Okamuro, J. K., den Boer, B. G. W., Lotys-Prass, C., Szeto, W. and Jofuku, K. D. (1996). Flowers into shoots: photo and hormonal control of a meristem identity switch in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **93**, 13831-13836.

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

Pouteau, S., Nicholls, D., Tooke, F., Coen, E. and Battey, N. (1997). The induction and maintenance of flowering in *Impatiens*. *Development* **124**, 3343-3351.

Pouteau, S., Tooke, F. and Battey, N. (1998). Quantitative control of inflorescence formation in *Impatiens balsamina*. *Plant Physiol.* **118**, 1191-1201.

Ratcliffe, O., Amaya, I., Vincent, C., Rothstein, S., Carpenter, R., Coen, E. and Bradley, D. (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.

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.

Sieburth, L. E., Running, M. P. and Meyerowitz, E. M. (1995). Genetic separation of third and fourth whorl functions of *AGAMOUS*. *Plant Cell* **7**, 1249-1258.

Smyth, D. R., Bowman, J. L. and Meyerowitz, E. M. (1990). Early flower development in *Arabidopsis*. *Plant Cell* **2**, 755-767.

Triezenberg, S. J., Kingsbury, R. C. and McKnight, S. L. (1988). Functional dissection of VP16, the *trans*-activator of herpes simplex virus immediate early gene expression. *Genes Dev.* **2**, 718-729.

Wagner, D., Sablowski, R. W. M. and Meyerowitz, E. M. (1999). Transcriptional activation of APETALA1 by LEAFY. *Science* **285**, 582-584.

Waltzer, L., Bourillot, P. Y., Sergeant, A. and Manet, E. (1995). RBP-J kappa repression activity is mediated by a co-repressor and antagonized by the Epstein-Barr virus transcription factor EBNA2. *Nucleic Acids Res.* **23**, 4939-4945.

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.

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

Zuber, M. E., Perron, M., Philpott, A., Bang, A. and Harris, W. A. (1999). Giant eyes in *Xenopus laevis* by overexpression of *XOptx2*. *Cell* **98**, 341-352.