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Separation of shoot and floral identity in *Arabidopsis*

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SUMMARY

The overall morphology of an *Arabidopsis* plant depends on the behaviour of its meristems. Meristems derived from the shoot apex can develop into either shoots or flowers. The distinction between these alternative fates requires separation between the function of floral meristem identity genes and the function of an antagonistic group of genes, which includes *TERMINAL FLOWER 1*. We show that the activities of these genes are restricted to separate domains of the shoot apex by different mechanisms. Meristem identity genes, such as *LEAFY*, *APETALA 1* and *CAULIFLOWER*, prevent *TERMINAL FLOWER 1* transcription in floral meristems on the apex periphery. *TERMINAL FLOWER 1*, in turn, can inhibit the activity of meristem identity genes at the centre of the shoot apex in two ways; first by delaying their upregulation, and second, by preventing the meristem from responding to *LEAFY* or *APETALA 1*. We suggest that the wild-type pattern of *TERMINAL FLOWER 1* and floral meristem identity gene expression depends on the relative timing of their upregulation.

Key words: *Arabidopsis*, Phase change, *TERMINAL FLOWER 1* (*TFL1*), *APETALA 1* (*AP1*), LEAFY (*LFY*), *CAULIFLOWER* (*CAL*), Shoot meristem, Floral meristem, Flower development

INTRODUCTION

The aerial parts of *Arabidopsis* are ultimately derived from the primary shoot apical meristem which is established during embryogenesis (Sussex, 1989; Evans and Barton, 1997). Over the plant life cycle, a series of growth phases reflect the activity of this meristem (Poethig, 1990; Schultz and Haughn, 1993; Ratcliffe et al., 1998). Following germination, the shoot apical meristem generates leaf primordia, which bear axillary shoot meristems. The duration of this vegetative phase (V) depends on environmental conditions and is controlled via an extensive network of flowering-time genes (Koornneef et al., 1991; Martinez-Zapater et al., 1994; Simon et al., 1996; Ruiz-Garcia et al., 1997; Nilsson et al., 1998). These genes regulate when the shoot switches to reproductive development and becomes an inflorescence (I). During a first-inflorescence phase (I_1), 2-3 cauline leaf primordia are produced, before a second-inflorescence phase (I_2) in which floral meristems are generated. During I_2, it is necessary for the shoot meristem to retain a distinct identity from the floral meristems it produces. A key question concerns how this separation is achieved (Shannon and Meeks-Wagner, 1993; Okamuro et al., 1993).

The distinction between shoot and floral meristems is maintained by two complementary sets of genes. First, floral fate depends upon the action of meristem identity genes such as *LEAFY* (*LFY*), *APETALA 1* (*AP1*), and *CAULIFLOWER* (*CAL*) (Mandel et al., 1992; Weigel et al., 1992; Bowman et al., 1993; Gustafson-Brown et al., 1994; Kempin et al., 1995; Mandel and Yanofsky, 1995; Weigel and Nilsson; 1995). Secondly, a group of genes, including *TERMINAL FLOWER 1* (*TFL1*), prevent the shoot from becoming a flower by retarding progression through all growth phases (Ratcliffe et al., 1998).

The opposing functions of *TFL1* and floral meristem identity genes is reflected in their complementary expression patterns and phenotypic effects. In wild type, *TFL1* and the floral meristem identity genes are expressed in separate domains (Fig. 1). Both types of genes are most strongly expressed during inflorescence development, with *TFL1* in the centre of the apex and floral meristem identity genes on its periphery (Mandel et al., 1992; Weigel et al., 1992; Kempin et al., 1995; Bradley et al., 1997). A similar separation is also observed between low levels of *TFL1* and *LFY* expression during vegetative growth (Bradley et al., 1997; Blazquez et al., 1997, 1998; Hempel et al., 1997).

If the activity of floral meristem identity genes is reduced, flowers develop with various shoot-like characteristics. (Irish and Sussex 1990; Schultz and Haughn 1991, 1993; Mandel et al., 1992; Huala and Sussex, 1992; Weigel et al., 1992; Bowman et al., 1993; Shannon and Meeks-Wagner, 1993). In extreme cases, such as the *lfy*;*ap1*;*cal* triple mutant, inflorescence nodes comprise secondary shoots with subtending leaves, and flower-like structures are rarely made (Bowman et al., 1993). Compared to wild type, therefore, the *lfy*;*ap1*;*cal* shoot apex switches from the V to the I phase at about the normal time, but flowers of the I_2 phase are replaced by shoots. Conversely, when *LFY* or *AP1* are constitutively expressed from a 35S CaMV promoter, all growth phases are shortened and shoot meristems are converted into flowers (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995).

The phenotypes produced by constitutive expression of *LFY* and *AP1* are similar to the effects of a reduction in *TFL1* activity. In *tfl1* mutants, the V, I_1 and I_2 are dramatically shortened and the shoot enters a final floral (F) phase in which the apex itself becomes a floral meristem (Shannon and Meeks-Wagner 1991, 1993; Alvarez et al., 1992; Okamuro et al., 1993, Schultz and Haughn, 1991, 1993; Hicks et al., 1996; Bradley et al., 1997; Ohshima et al., 1997; Ratcliffe et al., 1998). Correlating with this, *LFY* and *AP1* become prematurely and ectopically expressed in the *tfl1* apex, indicating that *TFL1* normally inhibits their activity in that region (Weigel et al., 1992; Bowman et al., 1993; Gustafson-Brown et al., 1994; Bradley et al., 1997). By contrast, 35S*TFL1* produces an extension in all growth phases, resulting in more highly branched plants which form flowers much later than wild type. Upregulation of *LFY* and *AP1* is delayed in these plants, and an additional growth phase (I_1*) occurs in which shoots lacking subtending leaves are formed (Ratcliffe et al., 1998).

To determine how *TFL1* and floral meristem identity genes oppose each other, we have examined *TFL1* expression in plants constitutively expressing *LFY* or *AP1* and in mutants for *lfy*, *ap1*, or *ap1*;*cal*. We show that *LFY*, *AP1* and *CAL* can inhibit *TFL1* at a transcriptional level. By contrast, inhibition of floral meristem identity gene expression by *TFL1* occurs in two different ways. First, *TFL1* retards upregulation of these genes by delaying phase progression at the shoot apex. Secondly, we demonstrate that *TFL1* can prevent a response to *LFY* and *AP1* even when they are expressed at high levels. We suggest that the final pattern of *TFL1* and floral meristem identity gene expression in the wild-type shoot apex depends on the relative timing of their upregulation, and is maintained by distinctive mechanisms of mutual inhibition.

MATERIALS AND METHODS

Plant growth conditions and microscopy

Experiments were performed on *Arabidopsis* plants of the ecotype Columbia, except for the following: *lfy-7* (Wassilewskija), *cal1-1*;*ap1-1* (Wassilewskija; Landsberg *erecta*), and 35S*LFY* (Landsberg *erecta*). Seeds of mutant lines were ordered from the *Arabidopsis* Biological Resource Centre at Ohio State University. The 35S*LFY* and 35S*AP1* lines were kindly supplied by Detlef Weigel and Martin Yanofsky. For all phenotypic analyses, seeds were imbibed and stratified for 5 days at 4°C in the dark, then germinated and grown on soil (1.5 parts John Innes No. 1, 1 part vermiculite, 1 part grit) in a glasshouse at 20°C under standard long day conditions (16 hours light/8 hours dark). During the winter, lighting in the glasshouse was supplemented and extended by high pressure sodium lights. Material for in situ hybridisation time courses was harvested from plants grown in a cabinet at 20°C under standard long day conditions. Light in the growth cabinet was supplied by banks of fluorescent tubes at an intensity between 90-120 $\mu E/m^2$/second. Equivalent batches of plants from the growth cabinet and the glasshouse displayed no notable differences in growth.

The mean node number produced during different growth phases was determined for each batch of plants and a standard error was calculated with 95% confidence limits attached. Node numbers were scored for a minimum of 10 plants in each batch except in the cases of 35S*LFY*;35S*TFL1* and 35S*TFL1*;*lfy-7* where only 4 and 2 plants were scored respectively (see below). Scanning electron microscopy was performed as detailed by Green and Linstead (1990).

In situ hybridisation

RNA in situ hydridisation was performed on tissue sections of 7 μm thickness as detailed by Ratcliffe et al. (1998) and Coen et al. (1990). RNA signal was detected as a dark blue/black colour on a light blue background when viewed under the light microscope. Double labelling was based on the method of Fobert et al. (1994). The two riboprobes were visualised sequentially; tissue sections were photographed to record localisation of the first probe, heat treated, and the location of the second probe visualised. Expression of the second gene could then be compared to that of the first.

Construction of 35S*AP1*;35S*TFL1* plants

Pollen from a hemizygous 35S*TFL1* plant of line JI.At1 was crossed to homozygous 35S*AP1* plants of line 563.I1.11A (Ratcliffe et al., 1998; Mandel and Yanofsky, 1995). Both parent lines contained a single copy of their respective transgenes in a Columbia background. Out of 23 plants examined in the F_1 from this cross, 13 had a phenotype identical to that of the 35S*AP1* parent line and 10 displayed a consistent novel phenotype. These 10 plants were confirmed to be doubly transgenic by RNA in situ hybridisation. Node numbers produced by these plants during each growth phase were recorded. Doubly transgenic plants in the F_2 generation exhibited the same phenotypes as those observed during the F_1.

Construction of 35S*LFY*;35S*TFL1* plants

Pollen from a hemizygous 35S*TFL1* plant from line JI.At1 was crossed to a homozygous 35S*LFY* plant of line 151.2.5 (Ratcliffe et al., 1998, Weigel and Nilsson, 1995). Twelve F_1 plants were obtained, of which 8 exhibited the same phenotype as the 35S*LFY* parent. These individuals were confirmed as hemizygous for the 35S*LFY* transgene. Self-pollination of these plants yielded progeny that segregated 3:1 for kanamycin resistance and a 35S*LFY* versus wild-type phenotype. The 4 remaining F_1 plants were shown to be doubly transgenic by in situ hybridisation, and all exhibited a consistent phenotype. Node numbers produced by these plants in each growth phase were recorded.

The 35S*LFY* transgene was in the Landsberg *erecta* background, whereas 35S*TFL1* was in a Columbia background. To verify that the 35S*TFL1* phenotype was not significantly affected in F_1 hybrids derived from crosses between these different backgrounds, the 35S*TFL1* parent was also crossed to a wild-type segregant from the 35S*LFY* parent line. Plants containing the 35S*TFL1* transgene in the F_1 from this cross exhibited the same overall phenotype as the parental line, but made slightly fewer rosette and cauline leaves (approximately 20 rosette and 12 cauline leaves in the parental line compared to 17 rosette and 11 cauline in the F_1).

Construction of the 35S*TFL1*; *lfy-7* plants

Strong alleles for *lfy* prevent fertile flower formation, so a cross was made using a *lfy-7* heterozygote as the parent. The *lfy-7* allele was chosen since it has strong effects and contains a novel *Mse*I site, not found in the wild-type allele, which facilitates easy genotyping (Weigel et al., 1992). Primers, 5′-GTACGAGGATTAATGTCA-TGTAC-3′ and 5′-TTCGCCACGGTCTTTAGCAATTG-3′ were designed flanking the *Mse*I site which generated a 482 bp fragment during PCR. If the *lfy-7* allele was present, this fragment was cleaved into two smaller fragments on digestion with *Mse*I, whereas for wild type the fragment remained uncut.

Pollen from a *lfy-7*/+ plant was transferred to a hemizygous 35S*TFL1* plant of line JIAt.1, and F_1 seeds were germinated on kanamycin plates to eliminate those that lacked the transgene. After approximately 3 weeks, resistant F_1 plants were transferred to soil and grown to maturity. The F_1 plants were then genotyped to identify individuals carrying the *lfy-7* allele and seed derived from self-pollination was collected. The inflorescences of these plants were more highly branched than siblings which lacked the *lfy-7* allele. The F_2 population was grown on soil and examined after 5 weeks. Of 108

plants, 70 had no visible bolt, whereas the remaining 38 had a well developed inflorescence. Amongst the 38 plants, 14 had a *lfy* phenotype. From the 70 plants that had not bolted after 5 weeks, 20 were grown to maturity, of which 2 developed a distinct phenotype from the remaining 18. These 2 plants were genotyped and shown to be homozygous for the *lfy-7* allele.

RESULTS

Expression of *TFL1*, *LFY* and *AP1* in wild-type *Arabidopsis*

We compared the expression patterns of *TFL1*, *LFY* and *AP1* at the primary shoot apex during its three different growth phases (Fig. 1). Corresponding to these growth phases, three different patterns of gene expression were discerned by RNA in situ hybridisation. During the vegetative phase, up to about 7 days after sowing, *TFL1* was weakly expressed in the centre of the shoot meristem, and *LFY* was weakly expressed in young primordia at the periphery of the apex. No *AP1* RNA was detected at this stage. At about 8-9 days, correlating with the I_1 phase, *TFL1* was upregulated in the centre of the shoot meristem. However, *LFY* expression remained weak and *AP1* expression remained absent for about 2-3 days following *TFL1* upregulation. Following the onset of the I_2 phase at 10-12 days, high levels of *LFY* and *AP1* expression were observed in groups of cells at the periphery, destined to form flowers, and *TFL1* was still expressed at high levels in the centre of the apex. Expression of *AP1* closely followed *LFY* upregulation, but whereas *LFY* was expressed throughout young floral meristems (Stage 1, Smyth et al., 1990), *AP1* was first expressed only in an adaxial portion of these meristems at the junction with the shoot meristem. Slightly later, by stage 2-3, *AP1* was present throughout floral meristems.

From the beginning of the I_2 phase, the primary shoot was visibly elongated (bolting) when seen in longitudinal sections. At this point, secondary shoot meristems were seen in axils of the leaf primordia which had initiated during the V and I_1 phases. These leaf primordia did not contain high levels of *LFY* or *AP1*, but strong *TFL1* expression was observed in their young axillary shoot meristems. The axillary shoot meristems exhibited a similar expression pattern to the primary apex: high levels of *TFL1* were established early on, and followed later by strong expression of *LFY* and *AP1* in floral meristems at their periphery.

Over-expression of *LFY* inhibits *TFL1*

At all stages in the life cycle, *TFL1* and *LFY* were expressed in separate domains of the shoot apex, with *TFL1* in the central region and *LFY* in cells on the periphery. This raised the possibility that *LFY* might restrict *TFL1* expression. We tested this by examining *TFL1* expression in transgenic lines that constitutively express *LFY* from a CaMV 35S promoter (Weigel and Nilsson, 1995).

As described previously, 35S*LFY* plants produce flowers earlier than wild type (Weigel and Nilsson, 1995). The vegetative phase of the primary apex is 2-3 leaves shorter than in wild type, and many plants make a short bolt with an occasional cauline leaf, before terminating in a cluster of one or more flowers (Fig. 2). Other 35S*LFY* plants produce a terminal flower structure on a pedicel directly from the rosette,

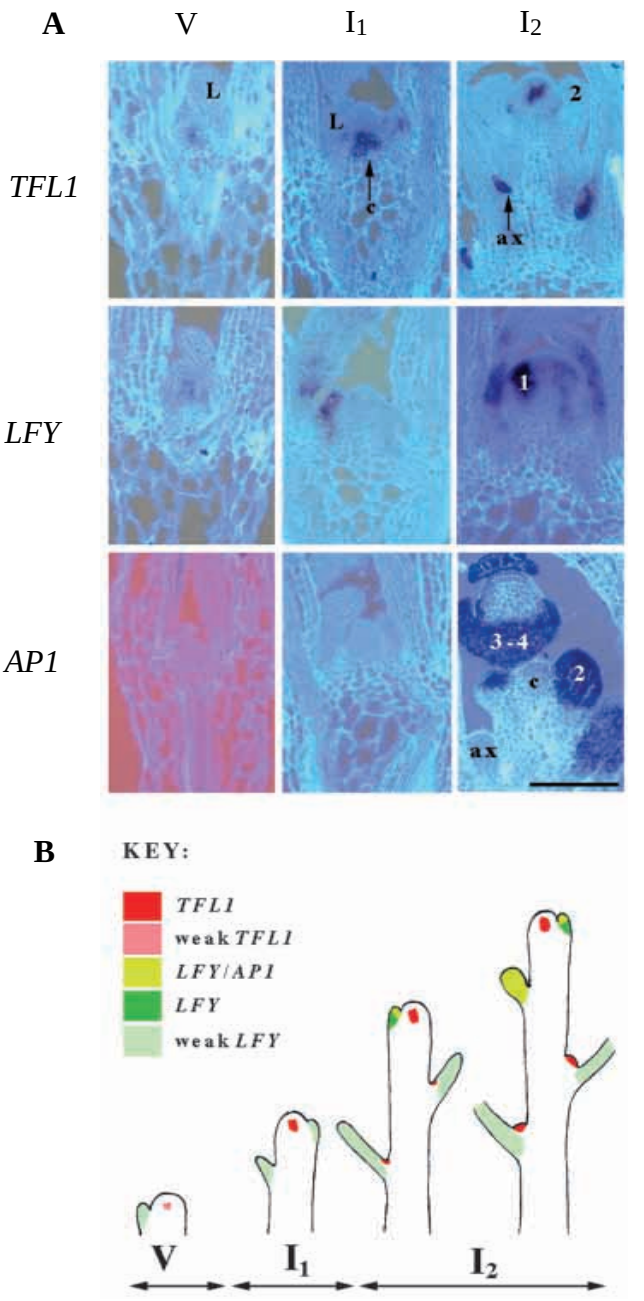

Fig. 1. Relative expression domains of *TFL1* and floral meristem identity genes during the *Arabidopsis* life cycle. (A) Wild-type RNA in situ hybridisation patterns of *TFL1*, *LFY* and *AP1* RNA in longitudinal sections through the *Arabidopsis* apex during the vegetative (V), first-inflorescence (I_1) and second-inflorescence (I_2) phases. Note that *TFL1* appears to be upregulated in the I_1 phase before *LFY* and *AP1* upregulation occur in the I_2. *TFL1* is expressed in the centre (c) of the shoot apex and axillary shoot meristems (ax) whereas *LFY* and *AP1* are strongly expressed on the periphery of the apex in young floral meristems (1, 2, staged according to Smyth et al., 1990). Low level *LFY* expression is also visible in leaf primordia (L). Note also, that in older shoots (not shown) *TFL1* RNA is often detected in the vascular tissue. In older developing flowers (stages 3-4) *AP1* RNA is restricted to the outer whorls. Scale bar, approximately 100 μm. (B) Sketch summarising the relative RNA expression patterns of *TFL1*, *LFY* and *AP1* in a growing *Arabidopsis* shoot apex at the V, I_1 and I_2 phases.

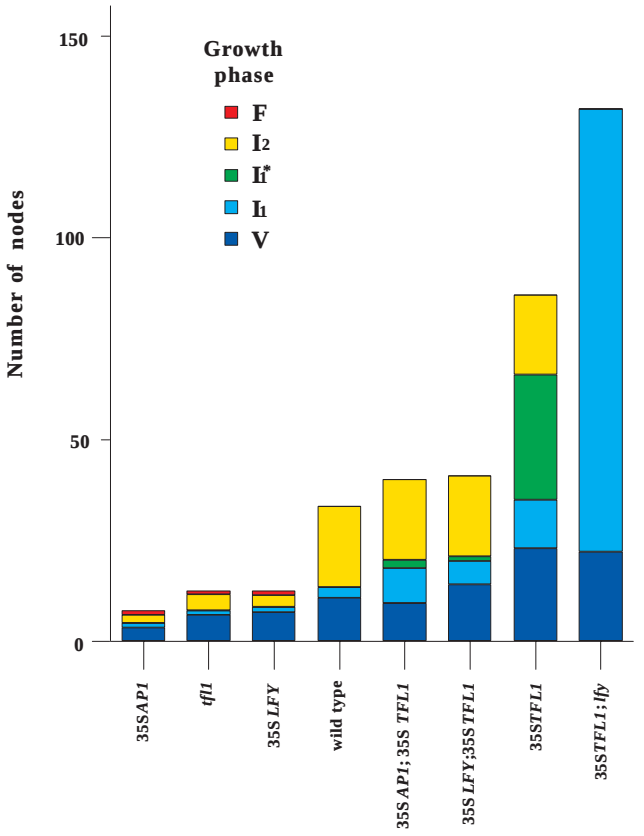

Fig. 2. Effects of *TFL1* and floral meristem identity gene activity on growth phase duration of the primary shoot apex. Coloured bars represent the number of nodes produced in each growth phase under long day conditions: V, vegetative phase during which rosette leaves produced; I_1, first inflorescence phase when cauline leaves are produced; I_1*, phase when shoots lacking a cauline leaf are made; I_2, second inflorescence phase when flowers are made; F, floral phase when apex itself becomes a flower. Details of node number were as follows: 35S*AP1* made: 3.3±0.5 V nodes; 1.3±0.3 I_1 nodes; 1-2 I_2 nodes; 1 F node. *tfl1* made: 6.5±0.5 V nodes; 1.1±0.4 I_1 nodes; 1-4 I_2 nodes; 1 F node. 35S*LFY* made: 7.1±0.4 V nodes; 2.4±0.3 I_1 nodes; 1-3 I_2 nodes; 1 F node. Wild type made: 10.7±0.3 V nodes; 2.7±0.1 I_1 nodes; >20 I_2 nodes. 35S*AP1*;35S*TFL1* made: 9.5±0.7 V nodes; 8.1±0.7 I_1 nodes; 4.2±0.9 I_1* nodes; >20 I_2 nodes. 35S*LFY*;35S*TFL1* made: 14.0±3.2 V nodes, 5.8±2.0 I_1 nodes; 1.3±2.4 I_1* nodes; >20 I_2 nodes. 35S*TFL1* made: 22.9±1.5 V nodes, 12.2±1.1 I_1 nodes; 30.8±2.2 I_1* nodes; >20 I_2 nodes. 35S*TFL1*;*lfy-7* made: approx. 20 V nodes and >110 I_1 nodes. Note that *lfy-7* plants (not shown on chart) made 8.7±0.7 V nodes and 10.3±1.1 I_1 nodes before carpelloid flowers.

without an intervening inflorescence. A further aspect of the 35S*LFY* phenotype is that each secondary meristem in the axil of a leaf develops into a flower rather than a shoot. Because the 35S*LFY* line gave poor seed set when the transgene was homozygous, it was difficult to obtain sufficient seed from homozygotes to follow the time course of *TFL1* expression. Instead, seed was collected from a 35S*LFY* hemizygote which yielded 35*LFY* and wild-type plants in a 3:1 ratio. Individuals containing the transgene were distinguished from wild-type segregants by probing separate sections from each apex with *LFY*; the transgenic plants exhibited constitutive *LFY* expression whereas the wild-type segregants did not (Fig. 3A,C).

Expression of *TFL1* was not detected at any time, over a full range of developmental stages in the 35*LFY* plants (Fig. 3B). More than 30 different 35S*LFY* plants were analysed, on alternate days between 2 and 16 days from sowing, and all lacked visible *TFL1* expression. This result was verified in three separate in situ experiments. Wild-type segregants from the population afforded a positive control and exhibited normal *TFL1* profiles (Fig. 3D). Thus, the lack of *TFL1* RNA in the 35S*LFY* lines confirmed that *LFY* might restrict *TFL1* expression.

Constitutive *TFL1* can overcome constitutive *LFY* activity

The above results showed that constitutive *LFY* activity could inhibit *TFL1* transcription. Nevertheless, it was unclear whether this revealed a functionally important aspect of *LFY* activity or whether the absence of *TFL1* was an indirect consequence of all 35S*LFY* meristems rapidly assuming a floral meristem identity. To distinguish between these possibilities, we constructed plants that were doubly transgenic for a 35S*LFY* and a 35S*TFL1* transgene. In these plants, *TFL1* would be constitutively expressed and uncoupled from transcriptional repression by *LFY*. If *LFY* activity was sufficient to confer floral meristem identity, then the double transgenics should be identical to the 35S*LFY* line. Alternatively, if the repression of *TFL1* by *LFY* was also important, then the double transgenics should show an altered phenotype.

The 35S*TFL1* line was crossed to 35S*LFY* and the phenotype examined in the F_1. The 35S*LFY*;35S*TFL1* double transgenics were clearly different to the 35S*LFY* parent line (Figs 2, 3E). They produced approximately twice as many rosette leaves in the V phase as the 35S*LFY* parent. The V phase was also longer than for wild type but was shorter than in the 35S*TFL1* parent (Fig. 2). Following the V phase, the 35S*LFY*;35S*TFL1* apex did not directly terminate in a solitary flower, but entered an I_1 phase during which about 5 cauline leaves with axillary shoots were initiated. This compared to about 0-2 cauline leaves in 35S*LFY* plants, 2-3 in wild type, and about 12 in 35S*TFL1*. Next, 1-2 I_1* nodes were made, before a large number of fertile flowers in the I_2 phase. Unlike the 35S*LFY* parent, the 35S*LFY*;35S*TFL1* primary apex remained indeterminate. However, secondary apices occasionally terminated in a club-like fasciated cluster of fused carpels after production of 20-30 flowers (Fig. 3F). Additionally, secondary inflorescences sometimes twisted back on themselves to form a fasciated loop (not shown) before recovering and continuing indeterminate growth. Double transgenic plants in the F_2 generation exhibited the same overall phenotype as the F_1, but displayed a greater degree of variation, assumed to be due to relative copy number of the two transgenes. Nevertheless, all F_2 double transgenics had an extensive primary inflorescence. In some cases, though, the I_1 nodes consisted of a cauline leaf subtending a shoot which terminated in a flower-like structure. In other instances, the I_1 nodes consisted of a cauline leaf and axillary shoot as seen in the F_1.

These data indicated that constitutive *TFL1* activity could prevent *LFY* from conferring a floral identity on young meristems, even though *LFY* was expressed in comparable quantities to that in the 35S*LFY* parent (Fig. 3A,G,H). As the

plant aged, however, *TFL1* became less able to prevent a response to *LFY* and normal flowers formed. This might account for the 35S*LFY*;35S*TFL1* plants having a less severe phenotype than the 35S*TFL1* parent, and for the occasional termination of secondary shoots in carpels.

LFY is involved in inhibition of *TFL1* in floral meristems

The phenotype of the 35S*LFY*;35S*TFL1* plants demonstrated that *TFL1* activity could interfere with the response of meristems to *LFY*. During the I_2 phase of wild type, therefore, it is important that *TFL1* expression is prevented in cells at the flanks of the apex which express *LFY*. The absence of *TFL1* RNA from the 35S*LFY* plants suggested that *LFY* itself might have such an inhibitory function. To assess this possibility, we analysed *TFL1* expression in a strong *lfy* mutant. In a wild-type inflorescence, *LFY* is expressed in very young floral meristems (from stages 0-1). If *LFY* acted to inhibit *TFL1*, we predicted that ectopic *TFL1* expression might be observed in equivalent groups of cells at the periphery of the *lfy* mutant apex.

A prominent feature of strong *lfy* mutants is an expanded I_1 phase in which an increased number of cauline leaves subtending secondary shoots are produced. Eventually, infertile carpelloid flower-like structures form rather than shoots, but normal fertile flowers never develop (Schultz and Haughn, 1991, 1993; Weigel et al., 1992; Blazquez et al., 1997). Since *lfy* homozygotes do not set seed, expression studies were performed on a segregating population of plants derived from a self-fertilised *lfy-7* heterozygote.

At early time points, *lfy* mutants were morphologically indistinguishable from wild type and *TFL1* was upregulated in a normal pattern in all plants examined. Later, at 14-16 days, longitudinal sections revealed that the primary apices were bolting (Fig. 4A-F). At this time, when wild-type segregants were initiating floral meristems of the I_2 phase, *lfy* mutants could be recognised through their altered inflorescence morphology and increased numbers of cauline leaf primordia (Fig. 4E,F). *TFL1* RNA was present in the primary shoot apex of the *lfy* mutant, in the same position as in the wild-type apex (Fig. 4A,C). Nevertheless, despite the absence of functional *LFY*, *TFL1* was not ectopically expressed in the youngest groups of cells on the periphery of the apex which were equivalent to stage 0-1 wild-type floral meristems (arrows, Fig. 4A,C). A double-labelling experiment using a *TFL1* probe followed by *AP1*, verified that these individuals were *lfy* mutants; *AP1* RNA was either absent or very much reduced (arrow, Fig. 4B). By contrast, double-labelling on plants with a wild-type morphology from the same time points, showed strong *AP1* expression in young floral meristems (arrow, Fig. 4D). Although *TFL1* was not expressed in the youngest nodes on the periphery of the *lfy* apex, it was expressed in the axillary meristems of older nodes by the time the cauline leaf

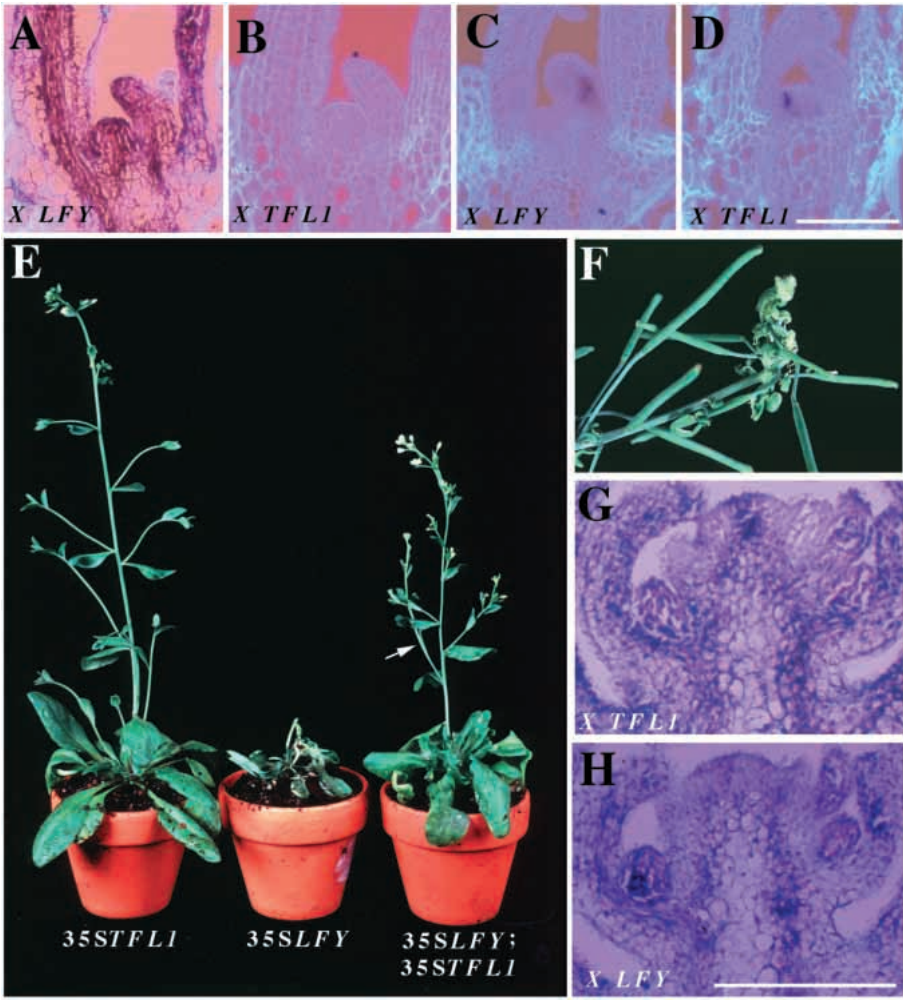

Fig. 3. Effects of constitutive *LFY* activity in a wild-type versus a 35S*TFL1* background. (A-D) *TFL1* and *LFY* expression in 35S*LFY* plants (A,B) compared to wild-type segregants (C, D). Longitudinal sections taken for RNA in situ hybridisation are shown at a representative time point of 6 days. (A) *LFY* expression in 35S*LFY*. (B) Section from same 35S*LFY* apex shown in A probed with *TFL1*. (C) weak *LFY* expression in leaf primordia of wild-type control. (D) *TFL1* expressed in apex of wild-type control. (E) 35S*TFL1*, 35S*LFY* and 35S*LFY*;35S*TFL1* plants at approximately 40 days after sowing. Individuals shown were taken from an F_2 population. Note that the 35S*LFY* plant is senescing and siliques are amongst the rosette leaves, whereas the 35S*LFY*;35S*TFL1* plant is still growing and producing shoots (arrow) rather than flowers in the axils of cauline leaves. (F) Carpelloid structure which occasionally terminated secondary shoots of 35S*LFY*;35S*TFL1* plants. (G,H) Longitudinal sections from a 35S*LFY*;35S*TFL1* shoot probed with *TFL1* or *LFY*. Similar levels of expression are observed from both transgenes, although expression from the 35S promoter is not completely homogeneous. Note that crosses were also made to produce 35S*TFL1*;*tfl1* plants (data not shown); these displayed no obvious phenotypic differences to the 35S*TFL1* plants. It is likely, therefore, that the endogenous *TFL1* gene made only a minimal contribution to the 35S*LFY*;35S*TFL1* phenotype compared to the effect of the 35S*TFL1* transgene. Scale bars, approximately 100 μm.

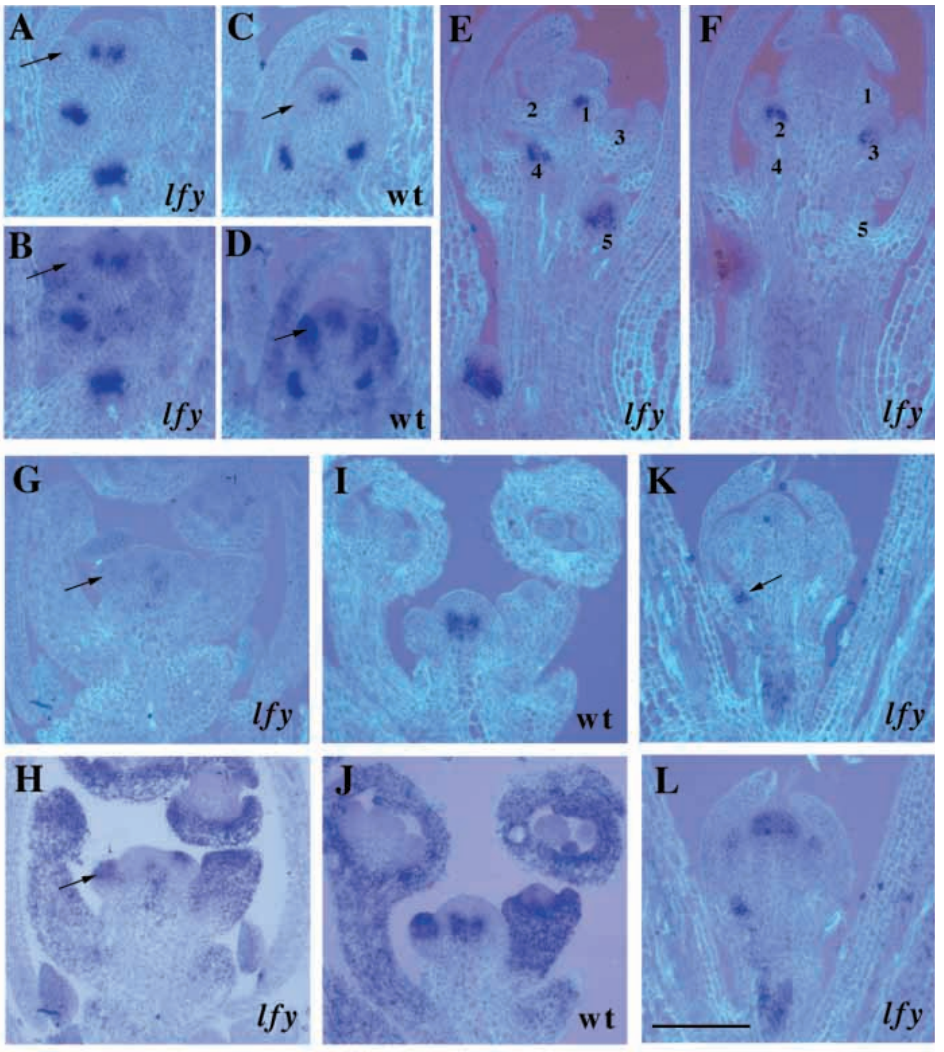

Fig. 4. Expression of *TFL1* and *AP1* in *lfy-7* mutant inflorescences. (A-D) Expression patterns in a *lfy-7* inflorescence compared to wild type at approximately 14 days (early I_2 phase). (A) *TFL1* expression in *lfy-7* mutant. Note that *TFL1* is expressed in axillary shoot meristems but not in youngest nodes (arrow) on the periphery of the shoot apex. (B) Section shown in A after double-labelling with *AP1*. Note that although some background expression is seen, *AP1* is not strongly expressed. (C) Expression of *TFL1* in wild-type control at 14 days. (D) Section shown in C after double labelling with *AP1*. In contrast to the *lfy-7* mutant, *AP1* is strongly expressed in youngest nodes (arrow) of wild-type apex at this stage. (E,F) *TFL1* expression in representative sections, approximately 20 μm apart, cut from a *lfy-7* apex at 16 days. Note that in this pair of sections, *TFL1* expression is visible in at least 5 axillary meristems (numbered) subtended by leaf primordia (I_1 nodes), compared to wild type (not shown) where only 2-3 of these nodes are present. (G-J) Expression patterns in older *lfy-7* apex compared to wild type at approximately 21 days. (G) *TFL1* expression in *lfy-7* mutant. Note that *TFL1* is weak in the primary apex, and absent in the youngest nodes on the periphery of the apex (arrow). (H) Section from G double labelled with *AP1*; expression can be seen in the youngest nodes (arrow). (I) Wild-type control at 21 days probed with *TFL1*. (J) Section from I double labelled with *AP1*. (K,L) Expression patterns in a secondary shoot from a mature *lfy-7* inflorescence at approximately 35 days. (K) *TFL1* expression is visible in a tertiary shoot meristem (arrow) and is also present in the vascular tissue. Distinct *TFL1* expression is not present in the apex. (L) Double labelling of section in K with *AP1*. Note that *AP1* is expressed in the apex, correlating with the formation of terminal carpelloid structures. Scale bar, approximately 100 μm.

primordium and axillary shoot meristem were distinct (Fig. 4E,F). In wild type, only 2 to 3 such I_1 nodes were made before single flowers, but in the *lfy* mutant, more than 10 of these nodes were made before carpelloid flowers. Therefore, by this criterion, *TFL1* was ectopically expressed in *lfy* mutants, being present in axillary shoot meristems at the positions where floral meristems developed without *TFL1* expression in wild type.

At later time points, from about 20 days, the *lfy* inflorescence apex produced nodes which formed carpelloid flowers. Expression of *TFL1* was not observed in the meristems that formed these structures, but *AP1* expression was now detected (Fig. 4G,H), confirming the delay in *AP1* upregulation in *lfy* mutants (Mandel and Yanofsky, 1995). A reduction in *TFL1* expression was noted in the inflorescence meristem at these later time points (Fig. 4G). Wild-type segregants also often showed reduced levels of *TFL1* in the main apex, but this was usually less marked than in the *lfy* mutants (Fig. 4I,J). This suggested that *TFL1* levels might decline more rapidly in the *lfy* mutant, correlating with the eventual formation of carpelloid terminal structures in the inflorescence (Weigel et al., 1992). Secondary shoots harvested from mature *lfy* inflorescences at 30-40 days from sowing did not show marked *TFL1* expression in their apices, although expression was still detected in tertiary shoot meristems (Fig. 4K). By contrast, *AP1* RNA was expressed throughout the tip of such secondary shoots, in accordance with them terminating in carpelloid flowers (Fig. 4L).

Thus, at lower nodes of the *lfy* inflorescence, *TFL1* was ectopically expressed by the time shoot meristems were distinct from their subtending cauline leaf primordia. However, at the upper nodes of the *lfy* inflorescence, *TFL1* was not found in the meristems that expressed *AP1* and formed carpelloid flowers. This indicated that *AP1* could also be involved in the inhibition of *TFL1* expression in floral meristems.

Inhibition of *TFL1* in young floral meristems requires *AP1*/*CAL*

To check whether *AP1* was required to inhibit *TFL1* expression, we analysed a strong *ap1* mutant. In *ap1* mutants, the vegetative phase is slightly reduced, the I_1 phase is of

similar length to wild type, and during the I_2, flowers develop with shoot-like characteristics (Irish and Sussex, 1990; Bowman et al., 1993; Schultz and Haughn, 1993). At about 14 days after sowing, *LFY* was strongly expressed in meristems at the periphery of the I_2 shoot apex, as has been previously described (Weigel et al., 1992). However, *TFL1* was not ectopically expressed in these regions, even though *AP1* activity was compromised (Fig. 5A-C). Furthermore, *TFL1* expression was not detected in shoots developing within flowers of the *ap1* mutant (Fig. 5B). Strong *TFL1* expression was observed only in wild-type positions: in the centre of the primary apex and in secondary shoot meristems developing in the axils of leaf primordia formed during the V and I_1 phases.

These observations show that *AP1* activity is not needed to prevent *TFL1* expression in floral meristems. However, the *AP1* gene is known to show functional redundancy with a highly homologous gene, *CAL* (Bowman, 1992; Bowman et al., 1993; Kempin et al., 1995). This raised the possibility that *CAL* could also contribute to the inhibition of *TFL1*. We therefore investigated *TFL1* expression in *cal*;*ap1* double mutants.

In *ap1;cal* plants the V and I_1 phases are similar to wild type, but the meristems initiated during the I_2 phase do not behave as determinate floral meristems; instead they remain indeterminate and initiate further meristems. Depending upon the environmental conditions, the whole apex rapidly proliferates and assumes the appearance of a cauliflower. Eventually, however, flowers are produced and viable seed is set (Bowman, 1992; Bowman et al., 1993).

At day 8, no difference was detected in the early expression of *TFL1* and *LFY* between *cal*;*ap1* mutants and wild type. By about 12 days, *cal*;*ap1* mutant apices had entered the I_2 phase and sections showed that the primary shoot was elongating. As with the *ap1* mutant, high levels of *LFY* expression were detected throughout meristems produced by the apex during the I_2 (Fig. 5F), confirming the observations of Bowman et al. (1993). However, the pattern of *TFL1* expression was markedly different from that in the *ap1* mutant: *TFL1* was ectopically expressed in young meristems that were equivalent to floral meristems of about stage 1 (Fig. 5E). At later time points, *cal*;*ap1* apices were so extensively proliferated that it was very difficult to distinguish the primary shoot meristem. At these stages, *TFL1* expression was visible as a pattern of dots, at the centres of the supernumerary meristems (Fig. 5G,I). Scrutiny of serial sections (not shown) cut through entire *cal;ap1* apices revealed that *TFL1* and *LFY* were both present at high levels in all these meristems (individual sections contained *TFL1* RNA in only a limited number of meristems because the expression domain of *TFL1* usually only spans 2-3 sections). The results showed that the action of *AP1*/*CAL* prevents *TFL1* expression in meristems generated during the I_2 phase.

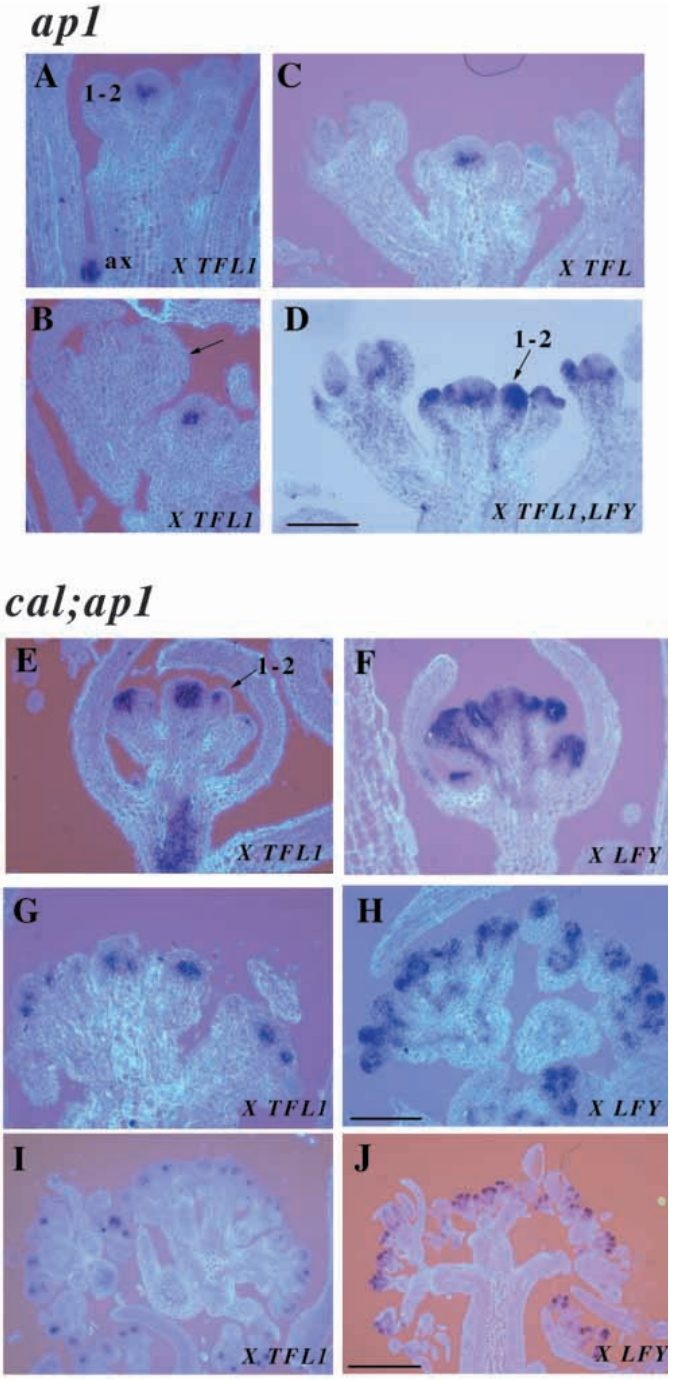

Fig. 5. Expression patterns of *TFL1* and *LFY* in *ap1* and *cal*;*ap1* mutants. (A-D) Expression in the *ap1-7* mutant. (A) Expression of *TFL1* in the *ap1* mutant apex at 14 days (early I_2). Expression is present in the primary apex and axillary shoot meristems (ax) but is absent from the youngest nodes equivalent to young stage 1-2 floral meristems. (B) *TFL1* expression in *ap1* apex at 16 days. Note that *TFL1* is not expressed in the shoot-like flowers (arrow) produced by *ap1*. (C,D) Double labelling to compare *TFL1* and *LFY* expression. Apex shown at 20 days but similar patterns were observed from start of I_2. (C)*TFL1* expression. (D) Section from C after double labelling with *LFY*. Note strong expression of *LFY* in youngest nodes equivalent to stage 1-2 floral meristems. (E-J) Expression in the *cal*;*ap1* mutant. Expression of *TFL1* (E) and *LFY* (F) in young inflorescence at 13 days. Note that *TFL1* and *LFY* are now expressed in young meristems (1-2; equivalent to stage 1-2 floral meristems). (G,H) Expression of *TFL1* and *LFY* at 16 days. (I,J) Expression of *TFL1* and *LFY* at 21 days. Both genes are expressed as a pattern of dots through the cauliflower-like proliferation. Scale bars, approximately 100 μm in A-H and approximately 500 μm in (I,J).

TFL1 is transiently upregulated in the *tfl1* mutant

We have shown that floral meristem identity genes prevent expression of *TFL1* in meristematic cells produced on the periphery of the shoot apex during I_2. In a complementary manner, *TFL1* acts within the shoot meristem to retard transition through growth phases and hence delays the expression of floral meristem identity genes on the periphery of the shoot apex (Ratcliffe et al., 1998). Whether a meristem develops as a flower or a shoot could therefore depend on whether *TFL1* or floral meristem identity gene expression is established first. In the *tfl1* mutant, *LFY* and *AP1* are expressed throughout the main apex, which eventually develops into a flower (Mandel et al., 1992; Weigel et al., 1992; Bradley et al., 1997). To investigate how the timing of *TFL1* compared to *LFY* and *AP1* expression, we performed in situ hybridisations on the *tfl1* mutant.

The *tfl1-1* allele contains a point mutation which disrupts *TFL1* function but which might not prevent transcription (Shannon and Meeks-Wagner, 1991; Bradley et al., 1997). *TFL1* expression was not detected in *tfl1-1* mutant apices during the vegetative phase of development. However, by day 6, *TFL1* was strongly upregulated in the *tfl1-1* shoot apex, about 2 days earlier than in wild type (Fig. 6A). By day 8-9, *TFL1* was no longer visible, and *LFY* was seen throughout the main apex, correlating with its floral fate (data not shown; Bradley et al., 1997). High levels of *LFY* expression were not observed before upregulation of *TFL1*.

The transient early expression of *TFL1* in the *tfl1* mutant can be explained in the following way. The reduction in *TFL1* activity instigates a premature exit from vegetative growth, therefore the *TFL1* gene responds by being upregulated earlier than in wild type. However, in the absence of TFL1 activity, floral meristem identity genes also become rapidly upregulated in the shoot apex and inhibit *TFL1* transcription. Therefore, if *TFL1* activity was not established in a shoot apex before floral meristem identity genes are upregulated, those genes could inhibit its expression.

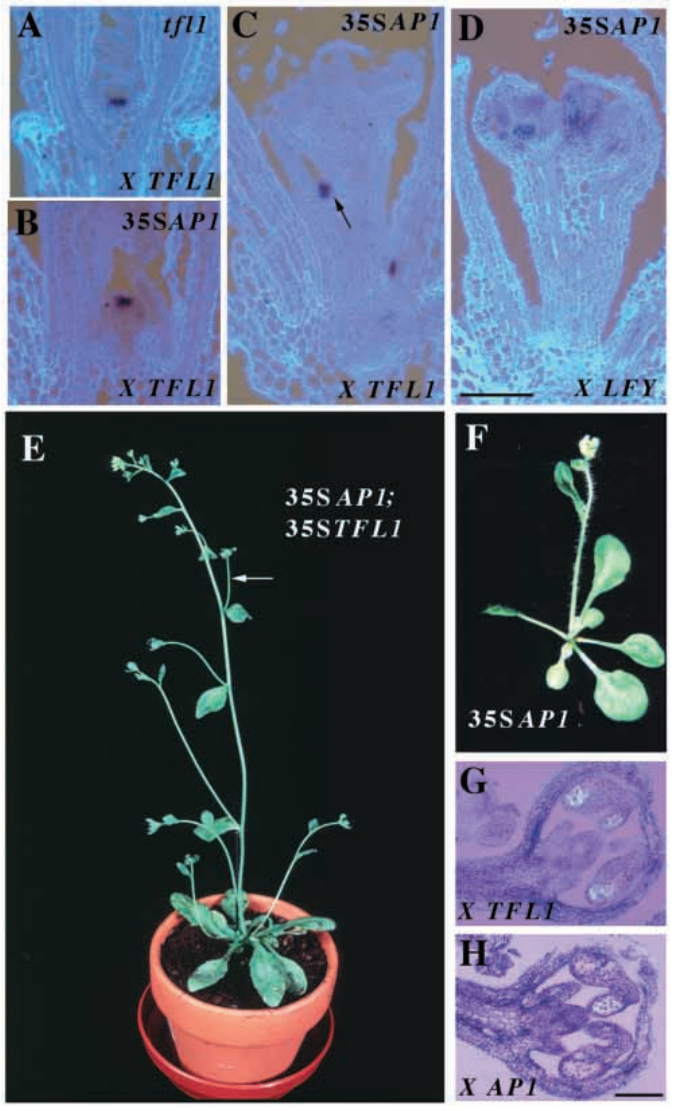

Fig. 6. Effects of constitutive *AP1* activity in a wild-type versus a 35S*TFL1* background. (A,B) Expression pattern of *TFL1* at 6 days in the *tfl1* mutant (A) and in 35S*AP1* (B). (C) *TFL1* expression in 35S*AP1* at 8-10 days. Main apex is now floral, but *TFL1* is strongly expressed in axillary meristems (arrow). (D) *LFY* expression in the primary apex of 35S*AP1* at 10 days. (E) 35S*AP1*;35S*TFL1* plant at approximately 40 days. Note that axillary meristems develop as shoots (arrow) instead of single flowers. (F) 35S*AP1* plant at approximately 15 days; the primary apex has terminated in a flower. (G,H) *TFL1* and *AP1* expression in tissue from a 35S*AP1*;35S*TFL1* plant. Both transgenes are expressed at similar levels. Scale bars, 100 μm.

TFL1 is transiently upregulated in 35S*AP1* plants

As described previously (Mandel and Yanofsky, 1995), plants with constitutive *AP1* expression resemble *tfl1* mutants: they develop very rapidly under long day conditions, usually only making 3-4 rosette leaves, and then a single cauline leaf before terminating in a cluster of 3-4 flowers (Figs 2, 6F). If the phenotype exhibited by 35S*AP1* plants was due to inhibition of *TFL1* transcription, we anticipated that 35S*AP1* plants would lack high levels of *TFL1* RNA.

TFL1 RNA was not detected in very young 35S*AP1* seedlings at 2-4 days from sowing (not shown). By day 6, however, *TFL1* was strongly expressed in the 35S*AP1* shoot apex, as seen in the *tfl1* mutant (Fig. 6B). This strong expression persisted for a narrow time interval and had disappeared from the main apex by days 8-10 (Fig. 6C). At this time, longitudinal sections clearly showed that the 35S*AP1* plants were bolting and that *LFY* was expressed in the main shoot apex in accordance with its floral fate (Fig. 6D). Strong *TFL1* expression was seen in secondary meristems in axils of leaf primordia at days 8-10 (Fig. 6C). However, by day 12, *TFL1* expression was not discerned anywhere in the plant (not shown).

The window of high *TFL1* levels in the 35S*AP1* plants implied that *AP1* alone was unable to prevent upregulation of *TFL1* in a shoot meristem at early stages of development. It was likely therefore, that the shortened growth phases observed in the 35S*AP1* plants were not entirely due to repression of *TFL1* transcription and were at least partly caused by the effects of ectopic *AP1* on other factors which determine flowering time or interfere with *TFL1* action.

Constitutive *TFL1* can overcome constitutive *AP1* activity

To determine the extent to which the 35S*AP1* phenotype was due to repression of *TFL1*, we constructed plants containing

both 35S*TFL1* and 35S*AP1*. If the factors responsible for the accelerated flowering of 35S*AP1* acted purely by interfering with the action of TFL1 (i.e. events downstream of *TFL1* transcription), we anticipated that the double transgenics would show the same phenotype as the 35S*AP1* line.

The phenotype of the 35S*AP1*;35S*TFL1* double transgenic was markedly different from that of the 35S*AP1* parent line in terms of vegetative phase duration and inflorescence morphology (Figs 2, 6E). First, the V phase of 35S*AP1*;35S*TFL1* plants was 2-3 times longer than in the 35S*AP1* parent line, being about the same duration as in wild-type controls. Next, the 35S*AP1*;35S*TFL1* apex underwent an I_1 phase, producing several cauline leaves which subtended meristems that developed as shoots rather than flowers. This I_1 phase was much longer than in the 35S*AP1* parent and was 0.75 times the length of the 35S*TFL1* I_1 phase (Fig. 2). Subsequently, the 35S*AP1*;35S*TFL1* apex entered a brief I_1* phase (where 1-2 flower-like shoots lacking cauline leaves were initiated) prior to an I_2 in which a large number of flowers were made. All shoots on the 35S*AP1*;35S*TFL1* plants remained indeterminate.

The 35S*TFL1* transgene offset the early flowering of 35S*AP1* such that bolting in the 35S*AP1*;35S*TFL1* plants occurred at the same time as in wild type (Fig. 2). Therefore, the factors causing early flowering of 35S*AP1* plants could not all act downstream of *TFL1*. However, the observation that the 35S*AP1*;35S*TFL1* phenotype was less severe than that of the 35S*TFL1* parent confirmed that 35S*AP1* did influence other components in addition to *TFL1* transcription. This was also consistent with the observation that the 35S*AP1* line had an even shorter vegetative phase than the *tfl1* mutant (Fig. 2). As with 35S*LFY*;35S*TFL1*, the absence of terminal flowers in the 35S*AP1*;35S*TFL1* plants showed that once a shoot meristem had switched to reproductive growth, *AP1* expression was not sufficient to confer floral identity if that group of cells also expressed *TFL1*.

Constitutive *TFL1* prevents *AP1* activation in the *lfy* mutant

In a wild-type plant, upregulation of *LFY* is closely followed by *AP1* expression on the periphery of the shoot apex. In the *lfy* mutant, however, there is a delay between initiation of inflorescence development and the onset of *AP1* expression. This shows that *LFY* has a role in *AP1* activation but that *AP1* can eventually be upregulated even if *LFY* is absent (Weigel et al., 1992; Mandel et al., 1992; Gustafson-Brown et al., 1994; Simon et al., 1996). In 35S*TFL1* plants there is a delay in the onset of both *LFY* and *AP1* expression (Ratcliffe et al., 1998). To determine whether the delay in *AP1* expression was solely a consequence of the delay in *LFY* expression we introduced the 35S*TFL1* transgene into a strong *lfy* mutant background. If the effects on *AP1* were mediated via *LFY* alone, it was anticipated that 35S*TFL1*;*lfy* plants would eventually form carpelloid flowers that expressed *AP1*, at a similar position to the flowers in the 35S*TFL1* parent.

Plants containing the 35S*TFL1* transgene in a *lfy*-7 mutant background had a vegetative phase of approximately the same length as that in 35S*TFL1* (Fig. 2). However, 35S*TFL1*; *lfy* plants never formed the infertile carpelloid flowers seen on the *lfy* mutant. Instead, the 35S*TFL1*; *lfy* apex continued indefinitely to produce I_1 nodes comprising a cauline leaf subtending a shoot (Fig. 7A,B). More than 110 I_1 nodes were produced by the primary apex of 35S*TFL1*; *lfy* plants and they survived for about 5 months, senescing 1-2 months later than sibling 35S*TFL1* plants which had *LFY* activity (Fig. 2). To check whether the secondary shoots produced by the 35S*TFL1*; *lfy* plants at an equivalent position to flowers on the 35S*TFL1*

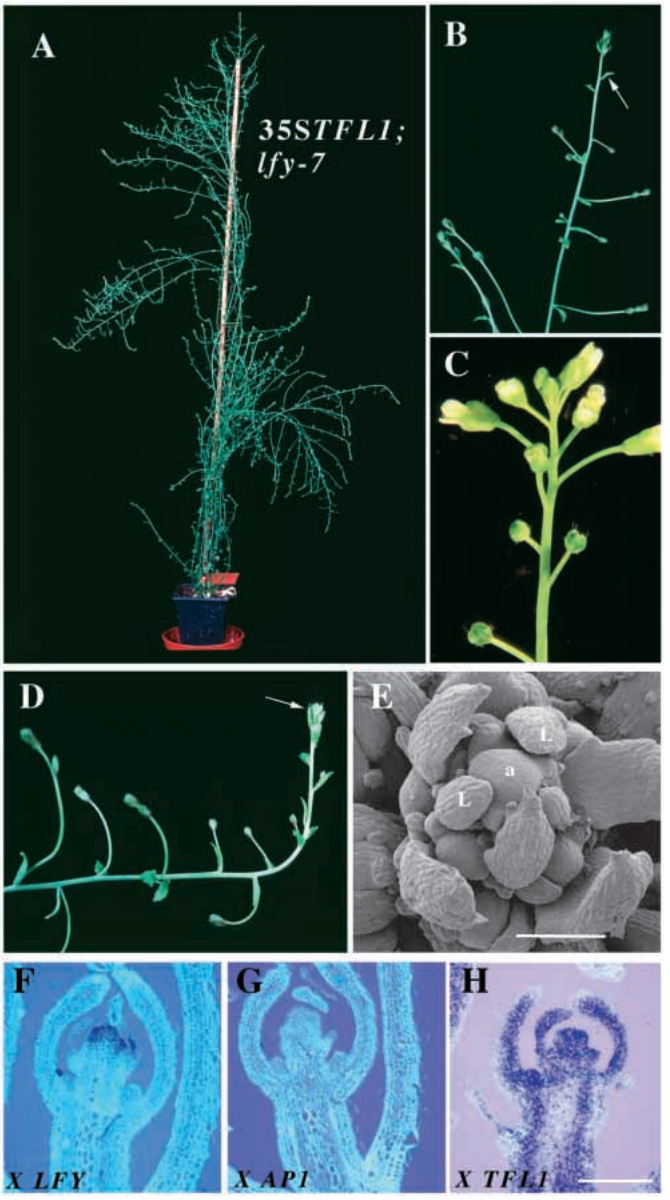

Fig. 7. Phenotype and expression patterns of 35S*TFL1*;*lfy-7* plants. (A) 35S*TFL1*;*lfy-7* at about 77 days. Eighty to ninety cauline leaves were visible by eye at this stage. (B) Close-up of 35S*TFL1*;*lfy-7* primary apex at 77 days. Note that uppermost nodes comprise shoots subtended by a cauline leaf (arrow) rather than flowers. (C) Close-up of 35S*TFL1* apex at 77 days. At this time the 35S*TFL1* plants had produced more than 80 visible nodes and the uppermost 10 of these comprised normal flowers. (D) Structure of secondary shoots from approximately node 90 on primary apex of 35S*TFL1*;*lfy-7* at about 100 days. Arrow indicates tip of structure taken for SEM and in situ analysis. (E) SEM of secondary shoot tip from approximately node 90 at 100 days. Note that no floral structures are visible and that leaf primordia (L) are being produced by the apex (a). (F-H) Expression patterns of *LFY*, *AP1* and *TFL1* in secondary shoot apex of 35S*TFL1*;*lfy-7* at node 90. Note that *AP1* expression is not detectable. Scale bars, approximately 100 μm.

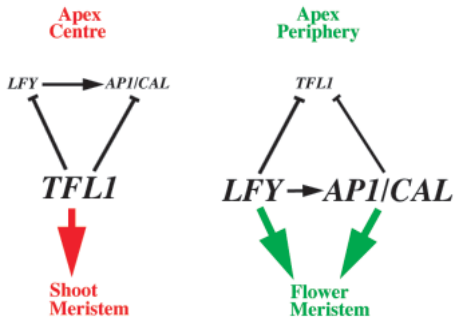

Fig. 8. Separation of flower and shoot meristem development at the shoot apex. Arrows represent positive interactions, blunt ended lines indicate inhibition. In the centre of the shoot apex, *TFL1* is upregulated prior to *LFY* and *AP1*/*CAL*. *TFL1* maintains development of the shoot by delaying the onset of growth phases where floral meristem identity genes are active. On the periphery of the shoot, meristem identity genes promote development of cells into floral meristems. Transcription of *TFL1* in these structures is prevented through the action of *AP1*/*CAL* and *LFY*.

(Fig. 7C), had any floral characteristics, we examined such secondary shoots by SEM (Fig. 7D,E). Secondary shoots, harvested (after 100 days) from the 35S*TFL1*; *lfy* primary apex at about node 90, showed no floral characteristics and were re-iterating the growth pattern of the primary apex by initiating leaf primordia at their periphery.

It was shown previously that 35S*TFL1* plants express high levels of *LFY* and *AP1* by the time they develop normal flowers (Ratcliffe et al., 1998). To examine if carpelloid flowers failed to form in 35S*TFL1*; *lfy* because *TFL1* prevented the expression of *AP1*, we examined secondary shoots by in situ hybridisation at the same position as the SEM analysis. These secondary shoots displayed constitutive *TFL1* expression and contained high levels of *LFY* RNA (comparable to levels observed in the *lfy-7* inflorescence; not shown) but *AP1* was not expressed (Fig. 7F-H). By contrast, *AP1* was expressed in sections taken from a comparable position on a mature *lfy-7* mutant inflorescence (Fig. 4L). This result implied that in the absence of *LFY* function, constitutive *TFL1* could inhibit the expression of *AP1*.

The lack of *AP1* expression in 35S*TFL1*;*lfy* plants reveals that *TFL1* can delay *AP1* activation by pathways other than through *LFY* (Fig. 8). Such pathways could involve activation of *AP1* by flowering-time genes such as *FT* and *FWA* (Ruiz-Garcia et al., 1997; Nilsson et al., 1998). When mutations in these genes are combined with *lfy* mutations, very similar characteristics are generated to those of the 35S*TFL1*;*lfy-7* plants (Ruiz-Garcia et al., 1997). The repression of *AP1* activation by *TFL1* might therefore involve inhibition of *FT* and *FWA*.

DISCUSSION

We have shown that the activities of *TFL1* and floral meristem identity genes are maintained in separate regions of the shoot apex by distinct mechanisms of mutual inhibition. Expression of *TFL1* is prevented at the periphery of the apex through the action of *LFY* or *AP1* and *CAL*. Conversely, *TFL1* acts to inhibit activity of floral meristem identity genes at the centre of the shoot apex (Fig. 8).

Restriction of *TFL1* activity

Inhibition of *TFL1* occurs at the level of the RNA transcript; either transcription of the *TFL1* gene or the accumulation of its message is prevented. This conclusion is supported by complementary results: *TFL1* expression becomes ectopic in mutants for *LFY* or *AP1/CAL*, and is inhibited when these floral meristem identity genes are constitutively expressed.

In *lfy* mutants, *TFL1* is ectopically expressed at lower nodes of the inflorescence, which form secondary shoots rather than flowers. When these nodes are initiated, instead of forming a floral meristem, cells at the periphery of the apex become partitioned into a leaf primordium and axillary meristem (Schultz and Haughn, 1991; Weigel et al., 1992; Coen and Carpenter; 1993). *TFL1* is not expressed at the earliest stages of these nodes, but is observed in the axillary meristems by the time they are distinct from their subtending leaf primordia. This delay could be rationalised if some transcription factor, required for *TFL1* expression, is active only in meristems and not leaf primordia. At upper nodes of the *lfy* inflorescence, carpelloid flowers form instead of shoots, but *TFL1* expression is not observed in the meristems from which such floral structures develop. By these later nodes, however, *AP1* and *CAL* have been activated, suggesting that they can prevent *TFL1* expression even in the absence of *LFY* activity.

Ectopic *TFL1* expression is observed in the *cal*;*ap1* infloresence, showing that *AP1* and *CAL* are indeed needed to inhibit *TFL1*. In the *cal*;*ap1* mutant, cells on the periphery of the inflorescence become meristems and do not form subtending leaf primordia. *TFL1* is established at very early stages in the development of these meristems. Because *LFY* is upregulated as normal, these structures might be considered equivalent to the floral meristems which are initiated in the wild-type I_2 phase. Therefore, the co-expression of *LFY* and *TFL1* demonstrates that *LFY* alone is not sufficient to prevent *TFL1* transcription in young meristems. At later stages, though, flowers are formed by the *cal*;*ap1* inflorescence, showing that *TFL1* activity is eventually inhibited.

TFL1 transcription is not detected in 35S*LFY* and is only briefly observed in 35S*AP1* plants, strengthening our conclusion that *TFL1* is inhibited by floral meristem identity genes (Fig. 8). The lack of *TFL1* expression during the vegetative phase of 35S*LFY* and 35S*AP1* suggests that high levels of floral meristem identity genes can prevent low levels of *TFL1* transcription. Alternatively, vegetative *TFL1* transcription might be present in 35S*AP1* and 35S*LFY* but below the threshold of detection. The period of strong *TFL1* expression in 35S*AP1* plants, corresponding to the I_1 phase, differentiates them from 35S*LFY* plants in which no *TFL1* expression is detected. If *AP1* and *LFY* can both repress *TFL1* transcription, why is *TFL1* upregulation observed in 35S*AP1* but not 35S*LFY* plants? One possible explanation is that other factors are required with *AP1* to act as co-repressors of *TFL1* transcription. Such factors could be absent during the I_1 phase, and only appear during later phases. Constitutive *LFY* activity, on the other hand, might directly induce such factors and possibly *AP1* and *CAL* as well, allowing inhibition of *TFL1* transcription at all developmental stages. Supporting this possibility, it has recently been demonstrated that *LFY* can transcriptionally activate *AP1* (Parcy et al., 1998).

Because LFY and AP1/CAL are thought to behave as transcription factors they could potentially restrict *TFL1* expression by binding to its promoter (Weigel et al., 1992;

Mandel et al., 1992; Kempin et al., 1995; Parcy et al., 1998). Alternatively, the inhibition of *TFL1* might be less direct and mediated via other factors which are transcriptionally regulated by the LFY and AP1/CAL proteins. Future characterisation of the *TFL1* promoter should distinguish between these possibilities and reveal whether or not it contains binding sites for LFY, AP1 or CAL.

Restriction of floral meristem identity gene activity

TFL1 can inhibit the floral meristem identity genes at two levels. First, *TFL1* can retard the upregulation of these genes. Previous studies on 35S*TFL1* and *tfl1* plants suggest that *TFL1* acts in the shoot meristem to retard phase changes over the entire life cycle (Schultz and Haughn, 1993; Bowman et al., 1993; Ratcliffe et al., 1998). As such, it delays the onset of phases where *LFY* and *AP1* are expressed on the periphery of the apex, and ultimately, their expression in the shoot meristem itself. Thus, when high levels of *TFL1* are present, as in 35S*TFL1* plants, *LFY* and *AP1* are upregulated much later than in wild-type. Conversely, when *TFL1* function is reduced, as in the *tfl1* mutant, *LFY* and *AP1* are upregulated prematurely and ectopically in the shoot apex.

Secondly, we demonstrate here that *TFL1* can prevent a response to floral meristem identity genes. This is revealed by the phenotype of plants constitutively expressing both *TFL1* and floral meristem identity genes. In these plants, *TFL1* and *LFY* or *AP1* are free from normal transcriptional regulation. Immediately following the vegetative phase, the main apex and axillary meristems do not develop into flowers, as occurs in the 35S*LFY* and 35S*AP1* parent lines. Instead the primary and axillary meristems develop as shoots; *TFL1* therefore prevents a response to *LFY* and *AP1* even though they are present at high levels. Although *TFL1* and floral meristem identity genes are not normally co-expressed, this interference by *TFL1* might be significant in dampening any mis-expression of *LFY*/*AP1* in the apex, ensuring that these genes remain functional in distinct apical and peripheral domains.

Normal flowers are produced at later inflorescence nodes of plants constitutively expressing both *TFL1* and floral meristem identity genes. By this time, therefore, the ability of *TFL1* to prevent a response has diminished. Furthermore, flowers are formed earlier than in the 35S*TFL1* parent line, indicating that constitutive expression of *LFY* or *AP1* does, to some extent, attenuate the extension of growth phases caused by *TFL1*. This attenuation could be due to the effects of constitutive *LFY* and *AP1* on other components which influence growth phase duration independently or downstream of *TFL1*.

Taken together, the above results show that *TFL1* can prevent a meristem from assuming floral identity both by retarding the upregulation of *LFY* and *AP1*/*CAL* and by preventing a response to these genes. It is unlikely, however, that TFL1 behaves as a direct repressor of transcription. More probably, TFL1 associates with membrane protein complexes, since it has similarity to animal proteins with such properties (Bradley et al., 1996, 1997; Ohshima et al., 1997; Pnueli et al., 1998). In this way, the TFL1 protein could act non-autonomously as part of a system transmitting signals from the centre to the periphery of the apex. Such a mechanism could indirectly influence whether floral meristem identity genes are upregulated or functional in peripheral cells. This might be clarified by studying the cellular localisation of the TFL1 protein, and analysis of its binding affinity for putative target molecules.

Regulation of meristem fate

The separation of flower and shoot meristem identities requires the floral meristem identity genes and *TFL1* to be expressed in distinct domains (Shannon and Meeks-Wagner, 1993; Okamuro et al., 1993). Mutual inhibition between these genes raises the question of how their expression domains are established. One possibility is that the final pattern of expression observed at the shoot apex reflects the relative timing of *TFL1* and floral meristem identity gene upregulation: whichever gene activity is established first in a region would prevent upregulation of the other (Fig. 8).

It appears that *TFL1* is upregulated in the centre of the wild-type shoot apex during the I_1 phase before *LFY* and *AP1/CAL* are strongly expressed on the periphery. By the I_2 phase, when *AP1* and *LFY* are upregulated, the prior establishment of *TFL1* could prevent their expression within the shoot meristem. Therefore, *LFY* and *AP1/CAL* upregulation would be restricted to cells on the periphery of the shoot apex which had not established *TFL1* activity. Under the influence of LFY, these peripheral cells would form floral meristems rather than the leaf primordia of earlier phases. Activity of CAL/AP1 along with LFY would then prevent *TFL1* transcription in these floral meristems, allowing them to develop into flowers. In addition to the primary apex, meristems formed in the axils of leaves before the I_2 phase, would also establish *TFL1* expression prior to *LFY* and *AP1*/*CAL* upregulation, accounting for their development as secondary shoots.

The relative timing of *TFL1* and floral meristem identity gene expression could also account for the transient high levels of *TFL1* transcription observed in the *tfl1* mutant. In the mutant, the V phase is shortened and *TFL1* is upregulated earlier than in a wild-type apex. Therefore, in addition to maintaining the duration of V, *TFL1* is itself upregulated in response to the switch to I_1. However, once *TFL1* has been upregulated, the mutant TFL1 protein cannot then prevent expression of *LFY* and *AP1* within the shoot meristem. The activity of these genes therefore switches *TFL1* off. A period of strong *TFL1* expression is observed in the mutant apex because there is a time-lag before *LFY* and *CAL*/*AP1* become upregulated and inhibit *TFL1* transcription. It is possible that similar mechanisms of mutual inhibition between *TFL1* homologues and floral meristem identity genes ensure separation of shoot and flower identities in other species. Thus, the relative timing of upregulation between these genes could account for some of the variation in inflorescence architecture observed amongst different angiosperm plants (Coen and Nugent, 1994).

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