Changing the spatial pattern of TFL1 expression reveals its key role in the shoot meristem in controlling Arabidopsis flowering architecture

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Abstract

Models for the control of above-ground plant architectures show how meristems can be programmed to be either shoots or flowers. Molecular, genetic, transgenic, and mathematical studies have greatly refined these models, suggesting that the phase of the shoot reflects different genes contributing to its repression of flowering, its vegetative-ness (‘veg’), before activators promote flower development. Key elements of how the repressor of flowering and shoot meristem gene TFL1 acts have now been tested, by changing its spatiotemporal pattern. It is shown that TFL1 can act outside of its normal expression domain in leaf primordia or floral meristems to repress flower identity. These data show how the timing and spatial pattern of TFL1 expression affect overall plant architecture. This reveals that the underlying pattern of TFL1 interactors is complex and that they may be spatially more widespread than TFL1 itself, which is confined to shoots. However, the data show that while TFL1 and floral genes can both act and compete in the same meristem, it appears that the main shoot meristem is more sensitive to TFL1 rather than floral genes. This spatial analysis therefore reveals how a difference in response helps maintain the ‘veg’ state of the shoot meristem.

Key words: Architecture, expression, flowering, identity, meristem, TFL1.

Introduction

The development of plants with different architectures reflects variation in underlying molecular patterns (Sussex and Kerk, 2001). These patterns are complex interactions of gene, protein, and metabolite systems (Kaufmann et al., 2010a; Sparks et al., 2013; Park et al., 2014). Analysis of these systems has identified many of the genes that control the formation of parts, their identity, position, and complexity (Benlloch et al., 2007; Studer et al., 2011; Alonso-Blanco and Mendez-Vigo, 2014). Such phenotypic traits have been selected during evolution and characterize different species, but, for any gene, what elements of its pattern are key in giving rise to a particular architecture?
Pattern elements include the level of gene expression, its timing, and in which cells it is expressed. For example, as plants pass through various developmental stages, different genes are expressed at appropriate levels and times, such as those maintaining the vegetative state of Arabidopsis (Poethig, 2010; Andres and Coupland, 2012). Other genes are expressed in specific domains to direct formation of organs in particular places, such as petals in flowers (O’Maoileidigh et al., 2014). The diversity of forms amongst species is the result of the evolution of these complex patterns. In addition to representing where, when, or how much of a gene is expressed, these patterns also determine potential new interacting genetic networks. Analysis of gene interactions, expression patterns, and loss- or gain-of-function phenotypes give us models for how these systems might operate. However, central to any models is the need to test how any pattern element contributes to generating a particular form. What is the effect of changing an element so a gene is expressed in novel domains or with different timing or levels? These questions have now been addressed for TFL1, a controller of plant architecture (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992; Schultz and Haughn, 1993; Ohshima et al., 1997).

TFL1 functions as a repressor of flowering and belongs to a small family of six genes in Arabidopsis (Kim et al., 2013). FT is a member of this family and is a key promoter of flowering, a florigen (Kardailsky et al., 1999; Kobayashi et al., 1999; Abe et al., 2005; Wigge et al., 2005; Shalit et al., 2009). The antagonism, different levels, and different expression patterns of these very similar proteins affect overall plant architecture (Hanzawa et al., 2005; Wigge et al., 2005; Shalit et al., 2009; Jaeger et al., 2013; Ho and Weigel, 2014). TFL1 can act through transcription to repress floral genes, and can modulate protein cellular trafficking patterns (Sohn et al., 2007; Hanano and Goto, 2011). Controlling the patterns and levels of TFL1 interactions in relation to floral meristem genes is predicted to be crucial (Prusinkiewicz et al., 2007; Koes, 2008; Jaeger et al., 2013; Park et al., 2014).

In wild-type (WT) Arabidopsis, TFL1 expression is limited to shoots (Simon et al., 1996; Bradley et al., 1997; Ratcliffe et al., 1999). During the vegetative phase, TFL1 is weakly expressed in the centre of the shoot meristem. This vegetative shoot meristem generates leaf primordia from its flanks to form a compact rosette. Upon integration of developmental and environmental signals, the shoot meristem makes cauline leaves (CLs; bearing shoot meristems in their axils) and the shoot elongates (bolts) (Poethig, 2010; Andres and Coupland, 2012; O’Maoileidigh et al., 2014). The level of TFL1 expression is up-regulated at this stage. TFL1 expression remains high in the shoot meristem as it generates floral meristems from its flanks, and TFL1 becomes strong in the stem.

This pattern of TFL1 expression appears to reflect its function. In tfl1 mutants, the shoot meristem makes fewer rosette leaves (RLs; see Table 1 for abbreviations) and plants bolt early compared with the WT (Shannon and Meeks-Wagner, 1991; Schultz and Haughn, 1993). Also, tfl1 mutants make fewer CLs and only a few flowers (Fs) before the shoot meristem converts to a floral meristem to give a terminal flower (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992; Schultz and Haughn, 1993). Thus TFL1 is needed to maintain and regulate shoot identity throughout the different phases of the plant life cycle to generate a particular architecture.

Models suggest how the TFL1 expression pattern controls Arabidopsis architecture (Ratcliffe et al., 1999; Liljegren et al., 1999; Ferrandiz et al., 2000; Prusinkiewicz et al., 2007). TFL1 delays the action of floral signals at the shoot meristem that promotes bolting, and TFL1 prevents their activity in the shoot meristem so that floral genes are not expressed in the shoot but only in lateral meristems. This maintains shoot identity and prevents the shoot meristem converting to a flower. An integrated model summarizes these interactions as acting upon the vegetative character (‘veg’) of the shoot apex (Prusinkiewicz et al., 2007). TFL1 contributes to ‘veg’ to delay flowering. Models also show how genes affecting floral meristem development, such as LFY, AP1, CAL, or FUL, prevent TFL1 expression in floral meristems. For example, both LFY and AP1 repress TFL1 by direct binding to its promoter (Kaufmann et al., 2010b; Winter et al., 2011). Also, a series of MADS box transcription factors promoting floral meristem identity suppress TFL1 in emerging floral meristems in Arabidopsis, and similarly in other species (Liu et al., 2013). This mutual inhibition results in clear domains of expression and activity, and a shoot architecture of leaves and branches at the base and an elongated stem with flowers on its sides. These models are consistent with mutant phenotypes and the expression patterns of these genes in various backgrounds (Blazquez et al., 2006). However, how do these myriad of interactions tie in with the spatial network of TFL1 action?

These models are supported by the phenotypes of plants ectopically expressing floral genes or TFL1 (Weigel and Nilsson, 1995; Mandel and Yanofsky, 1995; Ratcliffe et al., 1998, 1999; Liljegren et al., 1999; Parcy et al., 2002). Most of these ectopic studies have used the p35S promoter which is expressed constitutively, in most tissues, though patterns can vary (van Leeuwen et al., 2001). In p35S::LFY or p35S::AP1 plants, all phases are shorter (like tfl1 mutants), with plants

| Table 1. Abbreviations used for growth phases and plant organs scored |
|-------------|-----------------|------------------------------------------|---|
| Phase | Phase abbreviation | Lateral organs made by shoot meristem | Organ abbreviation |
| Vegetative rosette | V | Leaves | L |
| Inflorescence bolting and bearing cauline leaves | I1 | Cauline leaves | CL |
| Inflorescence/ap1-like structures without subtending leaves | I1* | Shoots or ap1-like flowers without subtending cauline leaves | I1* shoots, ap1-like F |
| Inflorescence with flowers | I2 | Flowers | F |
bolting early and shoot meristems converting to flowers (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995). In p35S::TFL1 plants, all phases are longer, with more RLs and CLs. These plants also make a novel II* phase of shoots without subtending CLs and ap1-like structures (Ratcliffe et al., 1998). In double transgenics such as p35S::pAP1;35S::TFL1, intermediate phenotypes occur, again suggesting that TFL1 and floral genes are antagonistic in their effects on meristem identity (Liljegren et al., 1999; Ratcliffe et al., 1999). However, the p35S promoter does not tell us where these genes act; is it in the same or different tissues? Neither does p35S tell us how genes may have quantitative effects or whether their expression at different times has different effects on architecture.

In this study, tests were carried out to determine how different elements of the TFL1 expression pattern contribute to plant architecture. Three different promoters were used to change the regulation of TFL1 expression, and when and where it can act. Further, by using floral promoters to express TFL1, it was directly tested how floral genes and TFL1 compete in the same tissues, and at the same time. By using tfll and WT backgrounds, the action of TFL1 in the shoot meristem could also be directly compared with its effects in lateral meristems, using the same constructs. It is shown that TFL1 can act outside of the shoot meristem, affecting the fate of lateral primordia. Therefore, TFL1 interactors and signalling components to affect ‘veg’ are not restricted to the shoot meristem. TFL1 prevented leaves from becoming flowers and delayed floral gene action. However, floral genes eventually overcame TFL1 action in lateral meristems. Despite ectopic expression, plants can tolerate quite different TFL1 expression patterns and yet still generate a raceme. The use of different spatial promoters has allowed the suggestion that TFL1 is expressed in its specific pattern to engineer sharp transitions from shoots to flowers, with the main and lateral meristems having different responses at the flowering transition.

### Materials and methods

#### Plant materials and analyses

Arabidopsis ecotype Col (WT) and tfll-1 (Shannon and Meeks-Wagner, 1991) were used as controls and hosts for transformation of promoter::TFL1 constructs. Lines were compared with p35S::TFL1 and grown in the greenhouse under controlled temperatures of 20–28 °C and long-day (LD) photoperiods supplemented with light as necessary (400 W Philips HDK/400 HPI (R) (N) or cool light from fluorescent tubes at an intensity of 90–120 mmol m⁻² s⁻¹) to give 16h light/8h dark as described (Ratcliffe et al., 1998).

The WT and tfll mutants were transformed with pLFY::TFL1 or pAP1::TFL1, and 14–33 transformants were obtained. These transformants were analysed and 7–12 single insertion locus lines were identified for each construct. For pANT::TFL1, only WT plants were directly transformed. Subsequently, lines were used to introduce pANT::TFL1 into tfll mutants by crossing. Five to seven lines for all constructs were preliminarily analysed to show consistent results (Supplementary Fig. S1 available at JXB online). Data from two representative strong lines for each construct were collected when all lines were grown at the same time and under the same LD greenhouse conditions, allowing a direct, quantitative comparison of phenotypes. Lines were sown and analysed in 3–5 independent experiments, and the results obtained showed that the phenotypes of all lines were highly consistent relative to controls. Abbreviations used for phenotypes of growth phases and plant organs scored are given in Table 1.

#### Promoter::TFL1 constructs

The ANT promoter was a 4.2 kb 5’ region (pYM-94-1) kindly provided by Yukiko Mizukami (Grandjean et al., 2004). The LFY promoter was a 2.3 kb 5’ region (pDW132) kindly provided by Detlef Weigel (Blazquez et al., 1997). The API promoter was a 1.7 kb 5’ region (pKY72) kindly provided by Marty Yanofsky (Hempen et al., 1997). The TFL1 cDNA (Hanzawa et al., 2005) was amplified with primers Y34 (AGTGGATCCATGGAGAATATGGGAACT) and Y37 (ATGGAATTCCTAGCGTTTGCGTGCAG) to add XhoI and BglII sites 5’ of ATG and 3’ of the stop codon, respectively, and cloned into pGEM-T (Stratagene). This XhoI–BglII fragment was cloned into a vector with a multiple cloning site and the p35S terminator to give pK6. The different promoter fragments were cloned into this vector as XhoI–BglII (pANT), SalI–BamHI (pLFY), or HindIII–BamHI (pAPI), respectively. The full promoter::TFL1 fragments were then transferred as XhoI–BamHI (pANT::TFL1), XhoI–BamHI (pLFY::TFL1), or HindIII–BamHI (pAPI::TFL1) to the binary vector pGreen0229 (Basta resistant; Hellens et al., 2000). This gave pK31 (pANT::TFL1), pK30 (pLFY::TFL1), and pK26 (pAPI::TFL1), which were transformed into Agrobacterium GV3101 with pSOUP and used to transform Arabidopsis plants by dipping as described (Clough and Bent, 1998).

#### RNA in situ hybridization

RNA in situ hybridization experiments with TFL1, LFY, AP1, and ANT antisense and sense probes were carried out as described (Ferrandiz et al., 2000). Note that quantification of in situ signals is not possible. Signal from probes cannot be compared to say if one is at different levels of expression, even though all probes are made at the same time, in the same way, as their base sequences. Also, the same probe on two different plants is still difficult to compare as tissue fixation and tissues vary.

### Results

Three promoters, pANT, pLFY, and pAP1, were used to express TFL1 in novel patterns during Arabidopsis development. These allowed TFL1, normally expressed only in the centre of shoot meristems, to be expressed ectopically in leaf primordia and floral meristems. In the WT, pANT is expressed in leaf primordia on the flanks of the shoot during all phases of growth (Elliott et al., 1996; Klucher et al., 1996; Long and Barton, 2000; Mizukami and Fischer, 2000; Grandjean et al., 2004). pLFY is weakly expressed in leaf primordia, but strongly in young floral meristems (Blazquez et al., 1997; Hempen et al., 1997). pAPI is only expressed in floral meristems, from stage 1 (Mandel et al., 1992; Hempen et al., 1997). The promoter fragments used have been shown largely to direct these expression patterns (Hempen et al., 1997; Krizek 1999; Benlloch et al., 2011). Further, the data below also showed that these promoters could drive expression of TFL1 ectopically in such patterns. In the tfll mutant background, both pLFY and pAPI become ectopically active in the shoot meristem (Weigel et al., 1992; Bowman et al., 1993; Gustafson-Brown et al., 1994; Bradley et al., 1997; Liljegren et al., 1999). The 35S promoter (p35S) was also used as a control to drive general, constitutive TFL1 expression in the WT during all phases and in most tissues as described (Ratcliffe et al., 1998).
et al., 1988). This promoter is considered strong and general, but can be variable, especially due to position effects (van Leeuwen et al., 2001).

Abbreviations were used in scoring phenotypes in terms of growth phases and types of organs generated from lateral primordia and meristems (Table 1).

Different promoters complement the TFL1 vegetative phase defect

The effects of the promoter: TFL1 constructs on vegetative development were investigated in terms of whether they extend the vegetative phase so that more RLs were generated.

WT plants made ~11 RLs in long days (Fig. 1). No significant changes in leaf number were observed in any lines carrying any of the three constructs (Fig. 1). The range of leaf numbers was greater in plants carrying pANT::TFL1 (9–16 leaves compared with 10–13 for the WT), but the averages were not statistically significant. This variability for pANT::TFL1 was seen in different experiments. In contrast, plants carrying p35S::TFL1 (which is strongly expressed in all tissues, including primordia and the shoot meristem) had an extension of the vegetative rosette (V) phase to 19 RLs, as previously shown (Fig. 1; Ratcliffe et al., 1998).

TFL1 extended the V phase in the WT, but only when expressed via p35S. There are a number of differences between p35S and the other promoters, including the timing and expression domain. To determine which aspect of p35S was important, either other promoters that had each of these features could be sought or these same promoters could be used but the genetic background could be altered to change their pattern. It was possible to change the pattern of these tested promoters by putting them into the tfl1 mutant background. In tfl1, pLFY and pAPI express TFL1 in primordia and throughout the shoot meristem (a change in domain), and earlier than in the WT (change in timing).

Analysis of pANT::TFL1 or pLFY::TFL1 in the tfl1 mutant showed that the V phase was extended compared with tfl1 (Fig. 1). The tfl1 mutant had a shorter V phase compared with transformants (Fig. 2E, F). After ~16–20 d, the tfl1 mutant had already flowered and made seed pods (silique). At this time point, tfl1 lines carrying pLFY::TFL1 or pAPI1::TFL1 had just started to bolt and were making flowers that had not yet matured. The common effect in all lines was to restore the V phase to WT (Fig. 1). Interestingly, pAPI1::TFL1 could also restore the V phase to WT in the strongest examples, and weaker lines always made significantly more RLs than tfl1. Unlike p35S, all other promoter lines made WT numbers of RLs, not more.

The expression patterns of TFL1 were analysed in the different lines by RNA in situ hybridization to see how they related to the plant phenotypes. In the vegetative phase of all WT lines, no early endogenous or transgenic TFL1 expression was clearly seen, except for general, constitutive p35S::TFL1 (Fig. 3A–E). Thus the lack of any TFL1 effect on the vegetative phase was most probably simply a lack of detectable expression. This may reflect the use of promoter fragments, sensitivity of detection, or even mRNA stability at early stages.

In the tfl1 mutant background, no endogenous mutant tfl1-1 mRNA was seen at the early phase (Fig. 4A). Similarly, no transgenic TFL1 or tfl1-1 signal (together referred to as TFL1/tfl1-1) was seen at early time points, suggesting that it was below the detection limit (Fig. 4A–D). Thus, despite undetectable expression, all promoter::TFL1 lines in tfl1 had V phases restored to WT. Therefore, tfl1 may be easily complemented by different promoters, but the length of the V phase may be generally robust due to many flowering pathways controlling the ‘veg’ character of this growth phase (Prusinkiewicz et al., 2007).

Increased cauline leaf numbers by ectopic TFL1

After the V phase, the Arabidopsis shoot enters the first inflorescence (I1) phase, making CLs that have secondary shoots in their axils, on an elongated stem (bolt). WT plants made about three CLs on the main stem (Figs 1, 2). In the WT background, ectopic expression of TFL1 during this phase increased the number of CLs (Fig. 1). The strongest lines carrying pANT::TFL1 and pLFY::TFL1 showed an increase of 1–2 CLs compared with the WT. The range of CL numbers was only 2–4 for the WT, but 2–7 for pANT::TFL1. Therefore, these transformed plants displayed a more branching architecture compared with the WT (Fig. 2A–C). In contrast, WT lines containing pAPI1::TFL1 had no change in their numbers of CLs and appeared as WT in I1 (Figs 1, 2D). Plants carrying p35S::TFL1 had a dramatic increase in the length of this phase (Fig. 1).

The tfl1 mutants made only one CL compared with three in the WT (Figs 1, 2). Also, in tfl1 mutants, all CLs had single flowers in their axils, while WT plants had shoots (Fig. 2A, E, G). In the tfl1 background, pANT::TFL1 and most pLFY::TFL1 lines had CL numbers similar to the WT (Fig. 1). Also, these lines had shoots in their CL axils (Fig. 2H). In tfl1, pAPI1::TFL1 did not restore CL numbers to WT, but their numbers were significantly increased in the strongest lines compared with tfl1 (Fig. 1). Also, for the stronger lines, CLs usually had shoots in their axils (Fig. 2I). There was only one exception (of a flower) in 146 individuals scored. For the weaker lines, CLs often had axillary shoots, but flowers or API1-like flowers (see below) were found at frequencies of 25–45% (Fig. 2I insert).

For plants in the I1 phase, endogenous WT TFL1 expression was seen on leaves in all the shoot meristems (Fig. 3F–I). In the WT, TFL1 mRNA was observed in the main shoot meristem and stem tissues, and in the axillary shoot meristems of CLs (Fig. 3F) and in the other tissues of the CLs and flower meristems (Fig. 3G). A similar pattern was seen for the different lines, but each line had a different pattern of ectopic TFL1 expression superimposed on the endogenous TFL1 mRNA pattern. For pANT::TFL1, ectopic TFL1 expression was seen in CLs (Fig. 3G). In pLFY::TFL1 there was also TFL1 expression in CLs, while pAPI1::TFL1 lines had no TFL1 mRNA in leaves, only hints of ectopic expression in the first floral meristems as plants entered I2 (Fig. 3H, I). The p35S::TFL1 lines showed expression throughout most tissues (Fig. 3I). Therefore, these expression patterns appeared to correlate with small effects on the I1 phase for pANT and
Regulating TFL1 expression to affect Arabidopsis architecture

Fig. 1. Ectopic TFL1 affects plant organ numbers. The number of rosette leaves (RLs), cauline leaves (CLs), I1* structures (shoots without subtending CLs or ap1-like structures), and flowers (Fs) made by the main shoot were recorded for wild-type (WT) Arabidopsis or tfl1-1 mutants containing pANT::TFL1, pLFY::TFL1, or pAP1::TFL1. WT plants containing p35S::TFL1 and ap1-12 mutants were also analysed. Numbers represent the average of 23–54 plants with standard deviations as shown. The solid black bars in (F) in the tfl1 background represent termination of the main shoot by conversion to a flower.

pLFY, as ectopic TFL1 mRNA appeared in CLs. No I1 expression was observed for pAP1, in agreement with no phenotypic effect in this phase. In contrast, the general expression of p35S must account for its strong I1 phenotype. It is difficult to comment on levels of expression as plant tissues differ; however, in situ hybridizations do reveal the distribution, and this is clearly more extensive in p35S. Thus making more CLs and branching architecture is dependent upon the pattern of TFL1 expression. This must then contribute to maintaining the ‘veg’ character to delay phase transitions.
It suggests that repressors such as TFL1 can contribute to ‘veg’ even when expressed ectopically outside of the shoot meristem. During this I1 phase in tfl1 mutants, endogenous mutant tfl1-1 mRNA was absent from the main shoot as it had already converted to a terminal flower by 12–14 d (Fig. 4E). Signal was restricted to young axillary meristems in the axils of RLs that had not yet converted to terminal flowers (Fig. 4E). For the different promoter lines, TFL1/tfl1-1 mRNA was seen for much longer in the main shoot meristems (Fig. 4F–H). Also, signal was strong in lateral meristems of CLs, correlating with their conversion from axillary flowers to shoots in these lines. It was also seen that TFL1/tfl1-1 signal appeared to be more extensive throughout the shoot meristem, not as restricted to the centre as in the WT. This novel pattern may reflect the partial floral nature of the main meristem to allow these promoters to be expressed beyond the normal central domain of TFL1. How each domain within the meristem contributes to the effect of TFL1 on ‘veg’ and delaying flowering cannot be resolved here.

TFL1 inhibits floral meristem development

After the I1 phase of making CLs, WT plants enter a second inflorescence phase (I2) and the shoot meristem generates floral meristems (FM) from its flanks. These FMs proceed through various stages of development until forming siliques (Fig. 2A; Smyth et al., 1990). Expression of LFY from stage
0, and AP1 from stage 1, reflecting low ‘veg’, ensures suppression of shoot identity and production of flowers.

In all the transgenic lines tested here, expression of TFL1 from pANT, pLFY, or pAP1 inhibited floral meristem development, delaying the production of flowers and causing the production of an I1* phase. This phase was also seen in p35S::TFL1 lines, and consisted of shoot-like structures without any subtending CL, or ap1-like flowers that resulted in multiple siliques arising from a common floral stem, the pedicel (e.g. Fig. 2 insert). Although numbers were variable, these structures were found in all transgenic lines (Fig. 1). In contrast, these structures were rarely seen in control WT or tfl1 plants (Fig. 1; zero in this experiment). Although clear in the WT background, the number of novel structures generated was small (0.1–2) for any of the promoters used (Fig. 1). Stronger effects appeared in the tfl1 mutant background, where both pLFY::TFL1 and pAP1::TFL1 gave up to 4–8 structures.

The length of I1* seen in pAP1::TFL1 and pLFY::TFL1 lines was sometimes the same as in p35S::TFL1 (Fig. 1). Also, pLFY::TFL1 usually gave I1* shoots before I1* ap1-like floral structures, while pAP1::TFL1 rarely gave I1* shoots and more usually gave only ap1-like floral structures. ap1 mutants were examined, and it was found that, as in previous reports, an early, strong phenotype could be distinguished where mutant plants made structures similar to the ap1-like floral structures recorded in the transgenic lines, with flowers within flowers (Figs 1, 2I insert; Bowman et al., 1993). Later, weak phenotypes were observed where flowers were abnormal (e.g. reduced petals), but did not have flowers within flowers. These later weak phenotypes were called ap1-like ‘flowers’ for this comparative analysis, as the final siliques were singular, as in the WT, rather than having multiple siliques on one pedicel. Thus the strongest pAP1::TFL1 lines could have an I1* phase similar to ap1 mutants (Fig. 1).

The I1* phase of pANT::TFL1 lines consisted of both I1* shoots and ap1-like flowers, but this phase in tfl1 was short as in the WT (Fig. 1). Therefore, unlike pLFY or pAP1, the pANT::TFL1 lines never appeared to have a strong effect on flower development.

Since all lines had significant delays in making the transition from CL production (I1) to normal flowers (I2) and made a I1* phase, TFL1 expression was analysed in comparison with the floral genes LFY and AP1.

In the WT, TFL1 was expressed in the centre of the shoot meristem (and weakly in inflorescence stems), but not in lateral primordia or floral meristems (Fig. 5A). In a complementary manner, LFY and AP1 were not expressed in the shoot meristem of the WT, but only in the lateral meristems of I2, the floral meristems (Fig. 5B).

In WT lines with pLFY::TFL1, TFL1 was expressed ectopically and overlapping with LFY (Fig. 5C, D). Note that although LFY signal appeared stronger than TFL1, it could not be concluded that TFL1 was expressed less or was less stable than LFY, as the probes were different. WT lines containing pAP1::TFL1 also had clear ectopic expression of TFL1 in lateral meristems (Fig. 5E). Endogenous AP1 expression overlapped with TFL1 (Fig. 5F). These patterns for pLFY and pAP1 lines were consistent for many different lines, while lines with the weakest phenotypes had undetectable ectopic TFL1 expression. Also, the patterns were generally consistent over 16–22 d of growth, during which time some I1* shoot or ap1-like structures would have been made, as well as normal flowers.

WT lines containing pANT::TFL1 showed ectopic TFL1 in the young developing lateral meristems and their primordia (Fig. 5G). Comparison of TFL1 with AP1 in these lines showed that ectopic TFL1 occurred in lateral meristems that probably gave rise to flowers (Fig. 5H, I).

The TFL1/tfl1-1 expression patterns were compared with those of LFY and AP1. Due to having common promoters, the pattern of LFY mRNA reflected the pattern of TFL1 in pLFY::TFL1 expression. In contrast, tfl1-1 mRNA reflected its own promoter and this was highest in shoot-like structures.
As tfl1 mutant plants were just bolting, tfl1-1 expression was seen in the main shoot (in the centre below the dome) at the same time as LFY also became ectopically expressed there (Fig. 6A, B). In pLFY::TFL1, the TFL1/tfl1-1 RNA was also seen at bolting, but no clear expression was seen of LFY at this time, reflecting the delay in flowering (Fig. 6C, D). After bolting, pLFY::TFL1 lines had TFL1/tfl1-1 expression that partially overlapped with that of LFY (Fig. 6E, F). Ectopic LFY in the shoot meristem was weak compared with LFY in lateral meristems (Fig. 6F). Ectopic expression of TFL1/tfl1-1 in the shoot meristem, beyond the central cells, was again seen. Some structures appeared ap1-like in tissue sections, and had ectopic TFL1/tfl1-1 (Fig. 6E left insert). In contrast, in ap1 mutant plants, ap1 mutant flowers did not appear to have strong ectopic TFL1 expression (Fig. 6E right insert).

For pAPI::TFL1 lines in tfl1, ectopic expression of TFL1 often appeared as clear as endogenous tfl1-1 seen in the tfl1 mutant itself (Fig. 6A, G). This expression was found at different time points, even when the shoot was making apparently normal flowers (Fig. 6G, H). Expression of API partially overlapped with ectopic TFL1 in flowers in these lines (Fig. 6H). Interestingly, API was often undetectable in the shoot meristem, compared with lateral API (Fig. 6H). This suggested that pAPI::TFL1 was also undetectable in the shoot meristem, yet no terminal flower was evident at any of these time points (13–21 d).

**Flowers are produced despite TFL1 expression**

For all of the lines in the WT background, normal-looking, fertile flowers (Fs) were produced after the I1* phase, typical of a wild-type I2 phase (Fig. 2A–D). Occasionally, ap1-like structures were found later on the shoot in I2. The inflorescences of WT and transgenic lines generated a similar number of flowers before normal senescence (Fig. 1). However, in one pLFY::TFL1 line and one pANT::TFL1 line, both of which had very weak V-I1* phenotypes, terminal flowers were made in I2 after ~25–30 flowers. This suggested problems in late TFL1 function in these lines.

The I2 flowering phase of tfl1 mutants is very short, with very few lateral Fs being made before the shoot meristem itself is converted to a terminal flower (Fig. 1). This gave tfl1 mutant plants their characteristic short stature (Fig. 2E, G). In the tfl1 mutant background, pLFY::TFL1 and pAPI::TFL1 lines made many more lateral Fs (up to 30 times) compared with tfl1 (Fig. 1). These Fs also generally appeared normal, as in the WT, indicating that LFY and API were largely unaffected by co-expression of TFL1 (Fig. 2H, I). However, during this same growth phase the conversion of the shoot meristem to terminal flowers was strongly delayed, indicating that TFL1 strongly inhibited LFY and API action in the main shoot meristem, promoting the ‘veg’ character of these plants.

The conversion of the shoot meristem to a terminal flower results in a typical architecture of siliques clustered at the apex, with the central sique either normal or a bit smaller and distorted (Shannon and Meeks-Wagner, 1991; Schultz and Haughn, 1993). However, in ~5% of pANT and 20–25% of pLFY or pAPI lines, the apex appeared fasciated and bent as it terminated. This phenotype can often be seen in lfy mutants when they terminate in a carpel-like structure (Weigel et al., 1992). This may reflect TFL1 inhibiting LFY even at very late stages, still promoting ‘veg’.

**Discussion**

Use of three independent promoters revealed how the timing and level of TFL1 expression is important in affecting plant architecture. It was shown that TFL1 is able to act outside of the shoot meristem. Ectopic expression of TFL1 is sufficient
to convert lateral meristems to leaves and shoots, by delaying the activity of co-expressed floral meristem identity genes. By expressing TFL1 in different domains (using WT and tfl1 backgrounds), it was also revealed that the main shoot appears more responsive to TFL1 than lateral meristems, as more extensive phase effects were seen in the tfl1 background where the promoters used became active not just in the lateral meristems but also in the shoot meristem. Therefore, the underlying spatiotemporal patterns of interactors for TFL1 probably differ between shoot and lateral meristems. Thus TFL1 promotes ‘veg’ most probably through the shoot meristem. These data should help in understanding which elements of the TFL1 expression pattern are important in establishing and maintaining particular plant architectures.

**TFL1 can function outside of the shoot meristem**

Ectopic TFL1 prevents lateral meristems from undergoing a floral fate. This change in pattern leads to increased branching and plant size, resulting in an altered architecture. In the WT, ectopic expression of TFL1 (via pANT or pLFY) in lateral meristems resulted in more cauline leaves being made. The normal actions of LFY, and probably other factors such as LIM1, were inhibited by TFL1 and so these factors were unable to act in their normal role to suppress leaf and shoot formation (Weigel et al., 1992; Huala and Sussex, 1992; Saddic et al., 2006). Leaf and shoot development occurred despite LFY being expressed at the same time and in the same place as TFL1. Further, when LFY action appeared to be partially restored (as cauline leaves were suppressed), TFL1 still inhibited flower development in lateral meristems. The strongest effects gave rise to abnormal shoots similar to ap1;lfy double mutants, but more often to ap1-like structures (Bowman et al., 1993; Weigel and Meyerowitz, 1993; Mandel and Yanofsky, 1995; Parcy et al., 1998; Wagner et al., 1999). Thus the action of both LFY and AP1, and probably other genes such as FUL or CAL, was inhibited by TFL1 when co-expressed in the same primordia or meristems (Mandel and Yanofsky, 1995; Liljegren et al., 1999; Ratcliffe et al., 1999; Ferrandiz et al., 2000).

TFL1 may also act ectopically in the vegetative phase of Arabidopsis development. Of the promoters used, only p35S had a significant effect on RL number. As the other promoters are known to have only weak expression in early phases, which aspect of p35S, its high expression or expression in the shoot meristem and in leaves, was critical in affecting V phase, cannot be resolved.

A clear effect on the vegetative phase was found when TFL1 was ectopic in both the lateral primordia and shoot meristem. In the tfl1 mutant, both pLFY::TFL1 and pAP1::TFL1...
increased the number of rosette leaves and delayed bolting. Both complemented the *tfl1* flowering time effect, and restored the number of RLs to WT, but not greater. There are two possibilities to explain why *TFL1* was now active in the vegetative phase. Either earlier expression of *TFL1* in the shoot is more effective in the vegetative phase, or a few lateral primordia are more sensitive to *TFL1* action, and so *TFL1* can act in lateral primordia which may be a characteristic of different subphases (Kersetter and Poethig, 1998; Hempel et al., 1998; Suh et al., 2003). The first possibility is suggested to be more likely. First, the complementation of RL number in these lines was the same as when *TFL1* was only expressed in the shoot (as in WT plants). Secondly, *TFL1* has stronger effects (to inhibit floral genes) when expressed in the shoot meristem compared with lateral meristems (see below).

**Shoot and lateral meristems have different responses to TFL1**

*TFL1* is more effective in inhibiting floral meristem genes when expressed in the main shoot. By introducing *pLFY::TFL1* or *pAP1::TFL1* into *tfl1*, *TFL1* became expressed in both lateral meristems and the shoot meristem, in direct competition with *LFY* and *AP1*. In the *tfl1* mutant, only one or two lateral flowers are made before the shoot itself is converted to a terminal flower. In *tfl1* carrying *pLFY::TFL1* or *pAP1::TFL1*, up to 30 lateral flowers were generated before *LFY* and *AP1* could finally overcome inhibition by *TFL1* and convert the shoot meristem into a terminal flower. Therefore, the competence of the shoot and lateral meristems differs for *TFL1* and *LFY/AP1* action, as, in these lines, all three genes are expressed together at the same level and with the same timing in the two types of meristem, lateral or shoot. This competence may reflect an underlying pattern of interactors needed for *TFL1*, or *LFY* and *AP1* action, to specify shoot or floral meristem identity. Potential interactors include bZIP transcription factors, one of which, *FD*, is expressed both in the shoot meristem and on its flanks in leaf and floral anlage (Abe et al., 2005; Wigge et al., 2005; Jaeger et al., 2006).

The effects of ectopic *TFL1* on lateral meristem development were enhanced in the *tfl1* background. More 11*lap1*-like structures were made when *pLFY::TFL1* or *pAP1::TFL1* were active in *tfl1* compared with the WT. This may reflect earlier *TFL1* expression from these promoters in the shoot meristem so that *TFL1* affects the fate of the earliest cells (anlagen) destined to form the primordia. By establishing some *TFL1* in these cells, this might lead to greater inhibition of *LFY* and *AP1* when these cells emerge on the flanks of the shoot meristem. Therefore, even if *TFL1* is not expressed any more strongly than *LFY* or *AP1* in these lateral meristems, earlier expression (overlapping with key interactors) may be an important factor. The movement of *TFL1* protein throughout the shoot meristem (which includes the anlagen) could restrict early floral gene effects (Conti and Bradley, 2007).

The present study also raises the important question of where or when *TFL1* cannot act. If *TFL1* was expressed later than the floral genes, what would happen? For example, *TFL1* expressed only in the later stages of FM development (via *pAG*) did not promote shoot development (Parcy et al., 2002). Thus expression at the same time as *LFY* or *AP1* may be required to affect meristem identity. This is supported by studies on *ATC*, a *TFL1* homologue. *ATC* is expressed in the hypocotyl, and an *atc* mutant has no effects on meristem identity (Mimida et al., 2001). However, if expressed via *p35S*, *ATC* can act as *TFL1*.

One idea of this work was to test if the indeterminate shoot architecture of *Arabidopsis* (a raceme) would be altered significantly. If the main shoot terminated in *tfl1*, but *TFL1* was expressed in the lateral meristem by *pANT*, for example, then maybe the lateral meristem would have grown and generated a new lateral meristem before terminating. If this occurred, then a branching determinate architecture could be formed, equivalent to the other major form of architecture, a determinate cyme. However, this did not happen by simply placing *TFL1* under the control of lateral/floral promoters in a *tfl1* mutant background. Rather, the data support models that predict it to be necessary to change both the pattern of *TFL1* and floral genes reciprocally (Prusinkiewicz et al., 2007; Koes, 2008; Jaeger et al., 2013). By changing promoters and thus gene regulation, interactions necessary to generate cyme architectures may result (Souer et al., 2008; Kurokura et al., 2013; Park et al., 2014).

Studies in tomato on *TFL1* and *FT* homologues have used *p35S* and mutant backgrounds to highlight the differences in primary and axillary meristems in this species with a cymose architecture (Shalit et al., 2009). In this case, it is suggested that balancing the levels of these factors has key effects on the fate of different meristem types.

**Supplementary data**

Supplementary data are available at *JXB* online.

**Figure S1.** Independent lines show that ectopic *TFL1* affects plant organ numbers.

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