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RESEARCH ARTICLE

Separate elements of the *TERMINAL FLOWER 1 cis*-regulatory region integrate pathways to control flowering time and shoot meristem identity

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ABSTRACT

TERMINAL FLOWER 1 (*TFL1*) is a key regulator of *Arabidopsis* plant architecture that responds to developmental and environmental signals to control flowering time and the fate of shoot meristems. *TFL1* expression is dynamic, being found in all shoot meristems, but not in floral meristems, with the level and distribution changing throughout development. Using a variety of experimental approaches we have analysed the *TFL1* promoter to elucidate its functional structure. *TFL1* expression is based on distinct *cis*-regulatory regions, the most important being located 3′ of the coding sequence. Our results indicate that *TFL1* expression in the shoot apical versus lateral inflorescence meristems is controlled through distinct *cis*-regulatory elements, suggesting that different signals control expression in these meristem types. Moreover, we identified a *cis*-regulatory region necessary for *TFL1* expression in the vegetative shoot and required for a wild-type flowering time, supporting that *TFL1* expression in the vegetative meristem controls flowering time. Our study provides a model for the functional organisation of *TFL1 cis*-regulatory regions, contributing to our understanding of how developmental pathways are integrated at the genomic level of a key regulator to control plant architecture.

KEY WORDS: *TERMINAL FLOWER 1* (*TFL1*), Plant architecture, Flowering time, Meristem identity, Inflorescence, Promoter, *Arabidopsis thaliana*

INTRODUCTION

Plant architecture reflects the activity of its meristems. Flowering species show great diversity in aerial architectures as a consequence of how meristem identity is controlled (Della Pina et al., 2014; Park et al., 2014). Upon seed germination, the shoot apical meristem (SAM) has a vegetative identity and generates leaves that often harbour meristems in their axils. Such axillary meristems may lie dormant, or acquire shoot or floral identity. Upon receiving appropriate signals, the main shoot converts to an inflorescence that generates floral meristems on its flanks, or itself converts to a floral meristem. Thus, the timing of events, and the integration of both developmental and environmental signals affecting meristem identity, give rise to the vast array of flowering forms seen in nature (Weberling, 1989). Many genes involved in these signalling pathways and meristem identity are known, but little is understood of how any key player integrates these signals at the genomic level.

TERMINAL FLOWER 1 (*TFL1*) is a key regulator of plant architecture in *Arabidopsis thaliana*, controlling flowering time and the fate of shoot meristems. *tfl1* mutants flower early, after producing fewer leaves than wild type. Moreover, *tfl1* shoot meristems convert to flowers (determinate growth), in contrast to the indeterminate growth of wild-type shoots (Fig. 1C,D) (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992; Schultz and Haughn, 1993). Thus, the inflorescence SAM forms a terminal flower after producing cauline leaves and a small number of flowers, and the meristems in the axils of the cauline leaves directly form solitary (axillary) flowers, rather that indeterminate coflorescences, as in the wild type (Fig. 1C,D).

The pattern of *TFL1* expression is complex and dynamic. Unlike general meristem genes, such as *STM*, *WUS* and members of the *CLV* family (Gaillochet et al., 2015), *TFL1* is expressed in all shoot meristems but not in floral meristems. In the SAM, after germination, *TFL1* is weakly expressed in the centre of the meristem during the vegetative phase and its expression is only strongly upregulated at the floral transition, as vegetative identity switches to inflorescence identity, still remaining restricted to the centre of the inflorescence meristem (Bradley et al., 1997). By contrast, in the shoot meristems in the axils of leaves, *TFL1* expression appears strong from the start of emergence, becoming weaker and more restricted to their centre when the axillary shoots elongate (Ratcliffe et al., 1999; Conti and Bradley, 2007). Therefore, *TFL1* expression discriminates between different developmental phases and different types of shoot meristems, which suggests that the *TFL1* promoter responds to, and so integrates, different signals controlling developmental phase transitions and spatial meristem identity.

The expression pattern of *TFL1* is thought to be pivotal for its function in the control of plant architecture. It has been suggested that *TFL1* expression in the vegetative meristem is essential for its role in regulating the timing of the floral transition (Bradley et al., 1997), and the strong late flowering phenotype of plants with increased, ectopic *TFL1* expression (via *35S::TFL1*) agrees with this (Ratcliffe et al., 1998). Moreover, *TFL1* is required in the inflorescence meristems to repress expression of the floral identity genes *LFY* and *AP1* and, therefore, to maintain their indeterminate growth by preventing these meristems becoming floral meristems (Bowman et al., 1993; Bradley et al., 1997; Baumann et al., 2015).

The wild-type expression pattern of *TFL1* is proposed to be essential for its function, but how *TFL1* expression is regulated is

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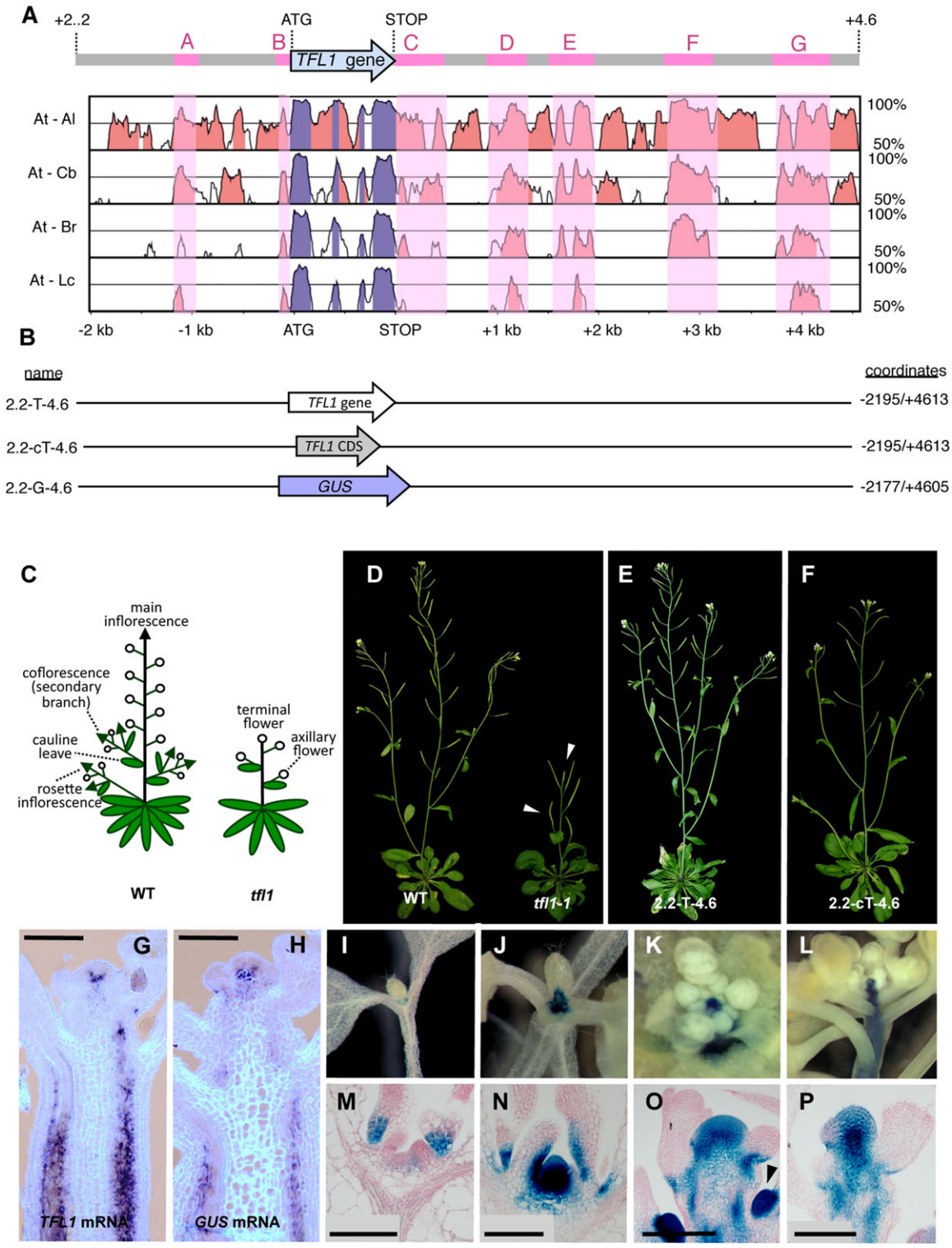

Fig. 1. 2.2 kb of the 5′ region and 4.6 kb of the 3′ region include all essential elements of the *TFL1* promoter. (A) VISTA pairwise alignments of Brassicaceae *TFL1* orthologues. The *TFL1* promoters from *Arabidopsis lyrata* (Al), *Capsella bursa-pastoris* (Cb), *Brassica rapa* (Br) and *Leavenworthia crassa* (Lc) were aligned to the *TFL1* promoter of *Arabidopsis thaliana* (At). Graphical output shows base pair identity (50-100% range) in a sliding window of 100 bp. Non-coding regions with >75% similarity are in red. *TFL1* exons are in blue. Pink areas highlight conserved blocks. The scale indicates coordinates (in kb) in the *A. thaliana TFL1* gene. At the top is shown the *TFL1* genomic region in *A. thaliana*, including the *TFL1* gene and the 5′ and 3′ intergenic regions, with conserved sequence blocks (A-G) in pink. (B) Constructs used in the experiments shown in this figure. (C) Diagrams of the plant architecture of wild-type (WT) and *tfl1* mutant *Arabidopsis* plants. (D) Wild-type (Col) and *tfl1-1* mutant plants grown under long-day conditions. Arrowheads mark terminal and axillary flowers in the *tfl1-1* inflorescence. (E,F) Fully complemented *tfl1-1* mutant plants after transformation with 2.2-T-4.6 or 2.2-cT-4.6 genomic constructs. (G,H) RNA *in situ* hybridisation for *TFL1* or *GUS* in inflorescence apices of wild-type plants transformed with the 2.2-G-4.6 reporter construct. (I-L) GUS staining of 2.2-G-4.6 apices at different developmental stages: vegetative (I), floral transition (J), bolting (K) and 15 days after bolting (L). (M-P) Longitudinal sections of 2.2-G-4.6 apices at vegetative stage (M), floral transition (N), bolting (O) and 15 days after bolting (P). Arrowhead (O) marks GUS signal in axillary meristem. Scale bars: 100 µm.

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poorly understood. Several regulators of its expression have been identified, most of them involved in the repression of *TFL1* expression in flowers (Kaufmann et al., 2010; Moyroud et al., 2012; Winter et al., 2011; Liu et al., 2013; Pérez-Ruiz et al., 2015); nevertheless, little is known about the structure of the *cis*-regulatory regions that are targeted, apart from the observation that sequences in the 3′ intergenic region are important for *TFL1* function (Ohshima et al., 1997; Kaufmann et al., 2010). Flowering time pathways are responsive to the environment and affect *TFL1* expression, but again no model describes how they integrate *TFL1* expression at the genomic level.

Elucidating the functional structure of the *TFL1* promoter is important for understanding how developmental and environmental pathways are integrated at the genomic level to direct correct plant architecture. For instance, is *TFL1* controlled in the same way in all shoot meristems, through common elements in its promoter, suggesting a common mechanism and signalling pathway? Are the elements responsible for *TFL1* expression in different shoot meristems located in different places in the promoter, which would suggest that different signals control these meristems? In addition, a functional map of the *TFL1* promoter would facilitate identification of the transcription factors that regulate *TFL1* expression, which should help in deciphering the genetic network that controls plant architecture.

Here, through a variety of experimental approaches, including phylogenetic shadowing, phenotyping of T-DNA insertion lines, complementation analysis and promoter::GUS fusions, we have carried out an intensive analysis of the *TFL1* promoter. Our results reveal that *TFL1* expression is based on distinct *cis*-regulatory regions that are required to direct expression to different meristems at the shoot apex to shape the architecture of the *Arabidopsis* plant.

RESULTS

The 3′ and 5′ intergenic regions of *TFL1* contain all the elements required for correct expression

Although the *cis*-regulatory elements of plant gene promoters seem to be most frequently located in the 5′ intergenic region, in the case of *TFL1* existing data indicated that some relevant *cis*-regulatory elements are located in the 3′ intergenic region (Ohshima et al., 1997; Kaufmann et al., 2010). As a first step to analyse the structure of the *TFL1* promoter, we tested whether the 5′ and 3′ intergenic regions encompass all the regulatory elements required for correct expression.

In our study of the *TFL1* promoter, we used three complementary approaches: phenotypic analysis of lines with T-DNA insertions, complementation of the *tfl1* mutant with fragments from the *TFL1* genomic region, and the expression analysis of reporter constructs with the *GUS* gene in wild-type plants. The design of genomic and reporter deletion constructs was based on the location of T-DNA insertions and of conserved regions (see below). *TFL1* genomic constructs, for complementation, were named N1-T-N2 and *GUS* reporter constructs were named N1-G-N2, where N1 and N2 indicate the approximate length of the 5′ and 3′ flanking fragments, respectively (Fig. 1B, Fig. S1B, Fig. S2B).

Our first genomic construct, 2.2-T-4.6, contained the *TFL1* gene sequence [coding sequence (CDS) plus introns] and its complete 3′ and 5′ flanking intergenic regions (2.2 kb and 4.6 kb, respectively) (Fig. 1B, Fig. S1B). The 2.2-T-4.6 construct was able to fully complement the phenotype of *tfl1* mutant plants (Fig. 1E), as previously described (Sohn et al., 2007; Kaufmann et al., 2010). Substituting the *TFL1* gene sequence by the *TFL1* CDS in the construct 2.2-cT-4.6 (Fig. S1B) gave a similar degree of complementation (Fig. 1F).

When an equivalent reporter construct 2.2-G-4.6, in which the *TFL1* gene sequence was replaced by the *GUS* gene (Fig. 1B, Fig. S2B), was transformed into wild-type plants it reproduced the *TFL1* mRNA expression pattern previously reported (Bradley et al., 1997; Conti and Bradley, 2007). Consistent with this, *in situ* hybridisation on young inflorescence apices of these plants showed that the distribution of the mRNAs of the *GUS* gene and of the endogenous *TFL1* gene were very similar, in the inflorescence meristem and the inflorescence stem vasculature (Fig. 1G,H). GUS staining assays in these plants showed that GUS signal was weak in the vegetative meristem (Fig. 1I,M) and was strongly upregulated in the inflorescence meristem with the floral transition (Fig. 1J,N). After bolting, strong GUS signal was maintained in the inflorescence meristem, in young axillary shoot meristems and throughout the inflorescence stem (Fig. 1K,L,O,P).

Taken together, these results indicate that both the 5′ and 3′ intergenic regions contain the elements required for wild-type expression of *TFL1*, and suggest that intron sequences in the gene are not essential for *TFL1* expression.

To identify conserved regions within the *TFL1* promoter we compared the genomic sequences of *TFL1* orthologues from a range of Brassicaceae species. Seven highly conserved sequence blocks (named A to G) were detected in the intergenic 5′ and 3′ regions of *Arabidopsis thaliana*, *Arabidopsis lyrata*, *Capsella bursa-pastoris* and *Brassica rapa* – species with a very similar inflorescence architecture (Fig. 1A). The conserved sequence blocks, two in the 5′ region and five in the 3′ region, were named A (−1.1 to −0.9), B (−0.1 to ATG), C (stop to +0.5), D (+0.9 to +1.3), E (+1.5 to +2.0), F (+2.7 to +3.2) and G (+3.7 to +4.3) (Fig. 1A, Fig. S3). These conserved sequence blocks were used as a basis for the design of constructs for the promoter analysis described below. Note that coordinates indicate the distance from the start (−) or the end (+) of the *TFL1* coding sequence.

Interestingly, the level of sequence conservation was reduced in the *TFL1* orthologue of *Leavenworthia crassa*, a Brassicaceae species with a different plant architecture, known as rosette flowering, where plants lack an inflorescence stem and produce solitary flowers at the axil of rosette leaves (Liu et al., 2011). Noticeably, the conserved block F was missing from the *L. crassa* *TFL1* orthologue (Fig. 1A, Fig. S3).

Both 5′ and 3′ promoter regions are necessary for the transcriptional regulation of *TFL1*

We asked whether both the 5′ and the 3′ intergenic regions are required for the transcriptional regulation of *TFL1*. The genomic construct 2.2-T-0.4, containing the complete 5′ but only 0.4 kb of the 3′ region (Fig. 2A, Fig. S1B), failed to complement the *tfl1* mutation (Fig. 2B) (Kaufmann et al., 2010). Accordingly, wild-type plants transformed with a equivalent reporter construct, 2.2-G-0.4 (Fig. S2B), mostly showed no GUS expression; the expression pattern was diverse among the plants showing expression, with GUS signal being frequently observed in organs where *TFL1* is not normally expressed, such as flowers and leaves (Fig. 2C-F,L).

Similarly, the genomic construct min-T-4.6, with the complete 3′ but lacking the 5′ region, which was replaced by a minimal 35S promoter (Fig. S1B), also failed to complement the *tfl1* mutation (Fig. 2G). Accordingly, most plants transformed with the equivalent reporter construct min-G-4.6 (Fig. S2B) showed no or low GUS signal. However, in the few plants showing GUS signal (~10%; Fig. 3K), the signal was correctly restricted to the centre of the shoot apices and its level increased after the floral transition (Fig. 2H-L),

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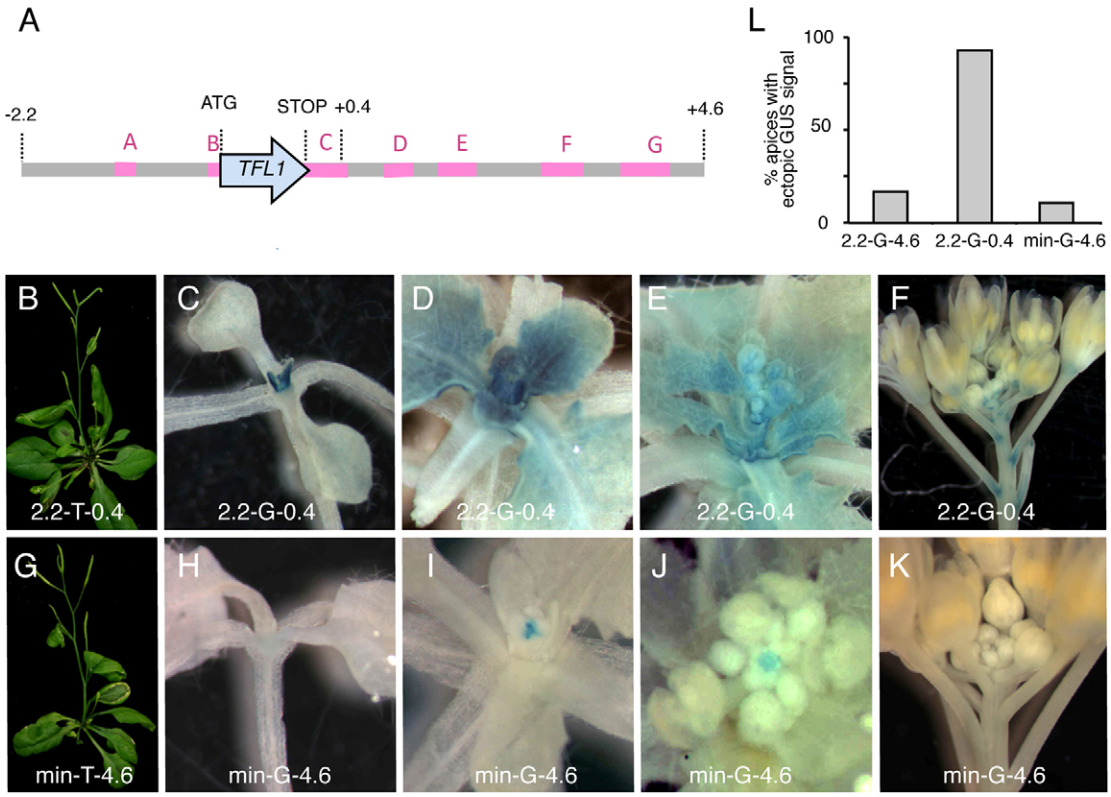

Fig. 2. Both 5′ and 3′ regions are required for proper regulation of *TFL1* expression. (A) The *TFL1* promoter. Dotted lines and coordinates indicate fragments used in constructs employed in this figure. Conserved sequence blocks are in pink. (B) *tfl1-1* mutant plant transformed with 2.2-T-0.4. (C-F) GUS staining of apices from 2.2-G-0.4 plants at different developmental stages: vegetative (C), floral transition (D), bolting (E) and 15 days after bolting (F). (G) *tfl1-1* mutant plant transformed with min-T-4.6. (H-K) GUS staining of apices from min-G-4.6 plants at different developmental stages: vegetative (H), floral transition (I), bolting (J) and 15 days after bolting (K). (L) Percentage of inflorescence bolting apices showing ectopic GUS signal, with respect to the number of apices showing expression, for different reporter constructs. At least 12 independent T1 plants were analysed per construct.

resembling the expression in plants with the full-length reporter construct 2.2-G-4.6 (Fig. 1H-K).

These results show that both 5′ and 3′ intergenic regions are required for correct regulation of *TFL1* expression. However, they also indicate that the 3′ region may be sufficient for correct spatial expression but that the 5′ region directs consistent and stable levels of expression.

The 5′ region of the promoter ensures high expression levels of *TFL1*

To understand the role of the 5′ region in the regulation of *TFL1* and to delimit possible *cis*-regulatory regions, we tested complementation and reporter constructs with a series of deletions of the 5′ region and a set of T-DNA lines with insertions in this region (Fig. 3A, Fig. S1B, Fig. S2B, Fig. S4).

Most *tfl1* plants (more than 70%) transformed with the genomic constructs 0.6-T-4.6 or 0.6-T-3.6, retaining only 0.6 kb from the 5′ region and therefore with conserved sequence block A deleted, but containing most of the 3′ region (Fig. 3A, Fig. S1B), showed full complementation (Fig. 3B). A very similar result was obtained with genomic construct 0.3-T-3.6, in which the 5′ region was deleted to −0.3 kb (Fig. S1A, Fig. 3A,C).

By contrast, only a small proportion of *tfl1* plants (19%) transformed with the 0.2-T-3.6 construct, where the 5′ intergenic region was deleted to −0.2 kb (Fig. 3A, Fig. S1B), showed full complementation, and the most frequent architecture (37%) comprised plants that had determinate coflorescences that ended in terminal flowers, while the main inflorescence showed indeterminate growth (Fig. 3D). A construct in which the 5′ was further deleted to −0.16 kb, 0.16-T-3.6 (Fig. 3A, Fig. S1B), was unable to produce any fully complemented plants and the most frequent architecture (37%) comprised plants where the main inflorescence ended in a terminal flower after producing several determinate coflorescences (Fig. 3E). Finally, construct 0.1-T-3.6, in which the 5′ was deleted to −0.1 kb (Fig. 3A, Fig. S1B), was unable to rescue any of the *tfl1* defects, and most plants showed a *tfl1* phenotype, in which coflorescences were replaced by axillary flowers (Fig. 3F), as described for min-T-4.6 plants (Fig. 2G).

As a complementary approach to the genomic deletions, we studied the effect of T-DNA insertions in the 5′ and 3′ intergenic regions on the regulation of *TFL1* expression. For the 5′ region, we analysed the phenotype of lines homozygous for T-DNA insertions at −0.3, −0.2 and −0.1 kb upstream of the ATG of the *TFL1* gene (Fig. 3A, Fig. S1A). Unexpectedly, although *tfl1* plants transformed with genomic constructs carrying 5′ deletions at −0.2, −0.16 and −0.1 kb showed a progressively simplified architecture, plants with T-DNA insertions at −0.3, −0.2 and −0.1 kb in the 5′ region showed a wild-type phenotype (Fig. S4).

Finally, wild-type plants transformed with a set of reporter constructs containing 5′ deletions, namely 0.6-G-4.6, 0.3-G-4.6, 0.2-G-4.6, 0.16-G-4.6, 0.1-G-4.6 and min-G-4.6 (Fig. S2B), showed a similar GUS pattern (Fig. 3G-K), strongly resembling that of the 2.2-G-4.6 reporter with the complete 3′ and 5′ regions (Fig. 1O). However, the intensity of the GUS signal gradually decreased in the deletion series (Fig. 3G-K). The decrease in GUS signal apparently started earlier in the lateral coflorescences before

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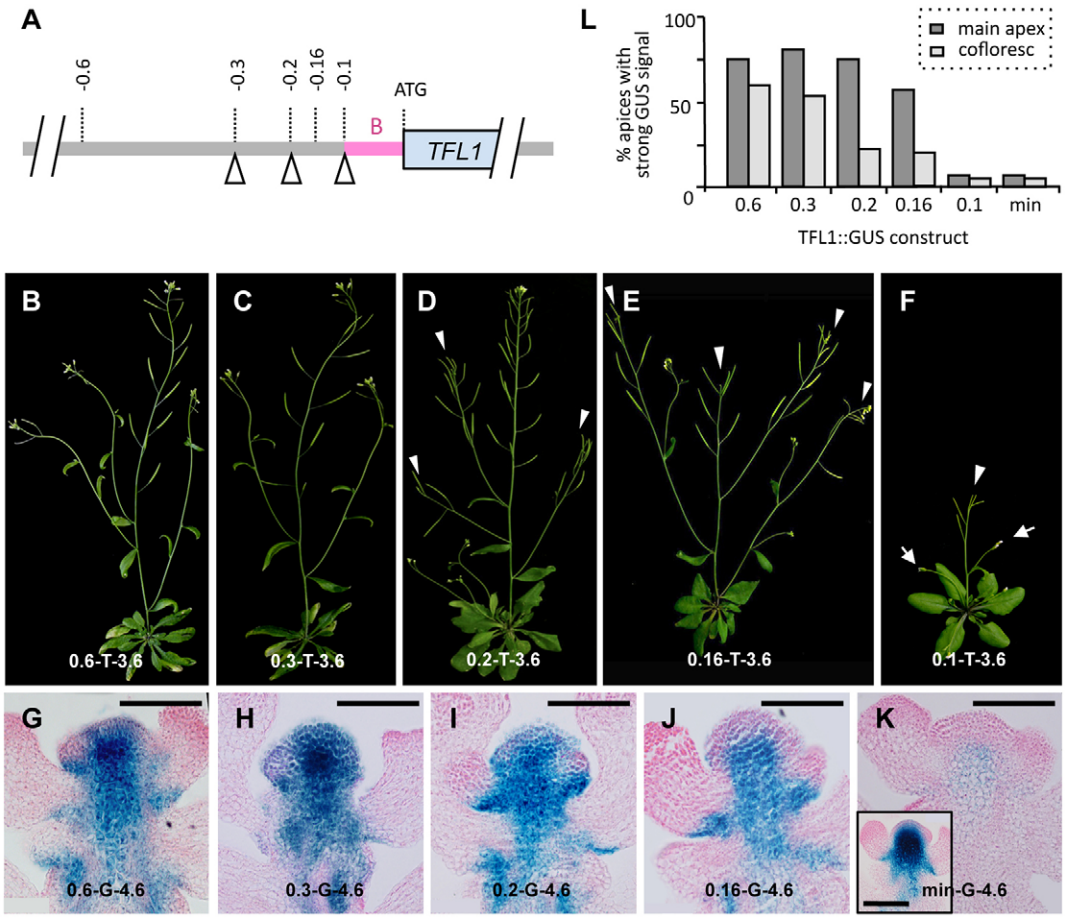

Fig. 3. Deletion analysis of the 5′ promoter region of *TFL1*. (A) The 5′ *TFL1* promoter region. Coordinates indicate the constructs (dotted lines) and T-DNA insertions (triangles) used in this figure. Conserved sequence block B is in pink. (B-F) *tfl1-1* mutant plants transformed with different genomic constructs, as indicated. Arrowheads point to terminal flowers and arrows indicate axillary flowers. (G-K) GUS signal in longitudinal sections of inflorescence apices from wild-type plants transformed with the reporter constructs indicated. Inset (K) shows a min-G-4.6 apex with strong GUS signal. (L) Percentage of inflorescence apices with strong GUS signal from plants transformed with reporter constructs containing deletions of the 5′ region (*y*-axis). The *x*-axis indicates the length in kb of the corresponding 5′ fragments in the constructs. The apex of the main inflorescence (main apex) and of the most apical secondary branch (cofloresc) were analysed from a minimum of 12 independent T1 lines per construct. Scale bars: 100 µm.

decreasing in the main inflorescence (Fig. 3L), correlating with the coflorescences forming terminal flowers before the main shoot.

Together, these results indicate that the 0.3 kb 5′ region upstream of the *TFL1* CDS contains *cis*-regulatory elements required for *TFL1* to reach a suitable level of expression. This functionally defined region was named region I.

The 3′ region contains *cis*-regulatory elements for spatiotemporal expression of *TFL1*

To understand the role of the 3′ region in the regulation of *TFL1* and to delimit possible *cis*-regulatory regions, we tested complementation and reporter constructs with a series of deletions and a set of T-DNA lines with insertions in this region (Fig. 4A, Fig. 5A, Fig. S1B, Fig. S2B).

As shown above, the genomic construct 0.6-T-3.6, in which the conserved region G was deleted (Fig. 4A, Fig. S1B), fully complemented the *tfl1* mutant phenotype (Fig. 3B). Similarly, a genomic construct with a further deletion of the 3′ region, 0.6-T-3.3 (Fig. 4A, Fig. S1A), fully rescued the defects of *tfl1* plants (Fig. 4B), although the percentage of fully complemented plants was markedly reduced (27%). Correlating with the deletion results, plants with a T-DNA insertion at +3.3 kb in the 3′ region (Fig. 4A, Fig. S1A) showed a wild-type phenotype (Fig. 4C). The expression patterns of equivalent reporter constructs 0.6-G-3.6 and 0.6-G-3.3 (Fig. S2) were very similar to that of the construct with the complete 5′ and 3′ intergenic regions, 2.2-G-4.6 (Fig. 4F-M, compare with Fig. 1M), although signal intensity was generally weaker in 0.6-G-3.3 plants, especially in the inflorescence SAM (Fig. 4L). These data indicate that the 3.6 kb fragment includes all of the 3′ regulatory elements required for *TFL1* expression and suggest that the sequence between +3.3 and +3.6 kb (region V) contains elements required for an appropriate transcription level, mainly in the main inflorescence apex.

By contrast, genomic constructs 0.6-T-2.8 and 2.2-T-2.8, in which the 3′ region was further deleted to +2.8 kb thereby removing also conserved region F (Fig. 4A, Fig. S1B), were unable to complement the *tfl1* mutant, and these plants showed determination of all inflorescence shoots (Fig. 4D, Fig. S4). Both the main inflorescence and the coflorescence apices produced cauline leaves with associated branches, like wild-type plants, but afterwards they only produced a few lateral flowers and then a terminal flower (Fig. 4D, Fig. S4D). In plants with equivalent reporter constructs 0.6-G-2.8 and 2.2-G-2.8 (Fig. S2B) the GUS signal was weak in the vegetative meristem, upregulated with the floral transition and

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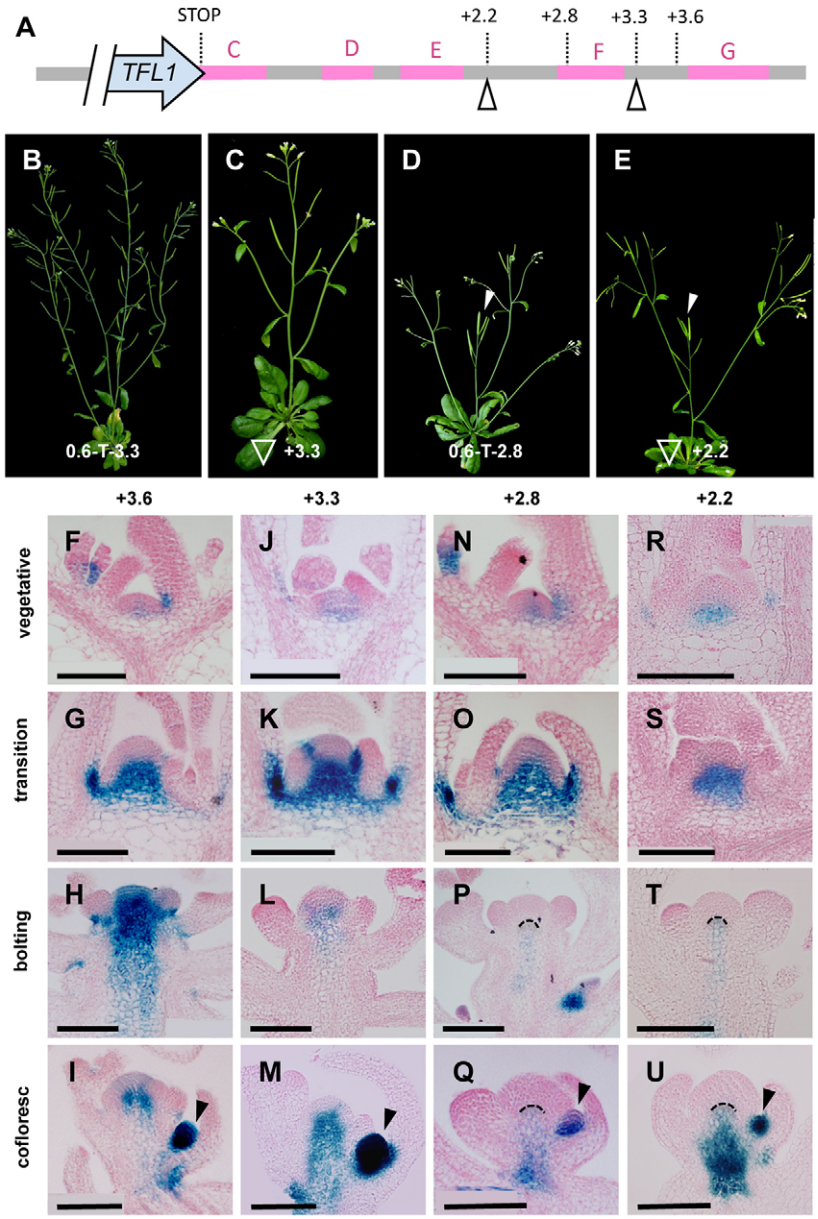

Fig. 4. Deletion analysis of the 3′ promoter region of *TFL1* from +2.2 to +4.6 kb. (A) The 3′ *TFL1* promoter region. Coordinates indicate the constructs (dotted lines) and T-DNA insertions (triangles) used in the experiments in this figure. Conserved sequence blocks are in pink. (B) *tfl1-1* mutant plant transformed with the 0.6-T-3.3 genomic construct. (C) Homozygous plant with a +3.3 T-DNA insertion line. (D) *tfl1-1* mutant plant transformed with the 0.6-T-2.8 genomic construct. (E) Homozygous plant with a +2.2 T-DNA insertion. Arrowheads (D,E) point to terminal flowers in the main inflorescence. (F-U) GUS signal in longitudinal sections of apices from wild-type plants transformed with reporter constructs containing various 3′ genomic fragments. Numbers above indicate the length in kb of the corresponding 3′ fragments. Developmental stages are indicated on the left: vegetative, (floral) transition and bolting refer to the apex of the main inflorescence; cofloresc (coflorescence) refers to the inflorescence apex of a secondary branch. Dashed lines (P,T,Q,U) mark the upper limit of the GUS signal. Arrowheads (I,M,Q,U) mark GUS signal in axillary meristems. Scale bars: 100 µm.

strong in axillary meristems (Fig. 4N-P, Fig. S4E,F), as in the full-length reporter (Fig. 4F-M). However, for 0.6-G-2.8 and 2.2-G-2.8, expression was absent from the meristem of the main and axillary inflorescence (coflorescence) shoots after they started elongating, being observed only in the inflorescence stem (Fig. 4P,Q, Fig. S4G). These results indicate that the 3′ region between +2.8 and +3.3 kb (region IV) is essential to maintain *TFL1* expression in the inflorescence meristem, so preventing the determination of inflorescence shoots. A reporter construct with a further deletion, 2.2-G-2.2 (Fig. 4A, Fig. S2B), had the same expression pattern as 2.2-G-2.8 (Fig. 4R-U), and plants with a T-DNA insertion at +2.2 kb (Fig. 4A, Fig. S1A) had essentially the same phenotype as plants complemented with 0.6-G-2.8 or 2.2-G-2.8 (Fig. 4D,E). This suggests that the region between +2.2 and +2.8 kb does not contain any significant regulatory elements.

Plants containing genomic construct 0.6-T-1.6 with a 3′ deletion that also removed conserved region E and plants with T-DNA insertions at +1.6 kb or +1.3 kb (Fig. 5A, Fig. S1) essentially showed the same phenotype. The main inflorescence was similar to that of plants with a T-DNA insertion at +2.2 kb, where a few flowers were made before a terminal flower. However, coflorescences were notably simplified (Fig. 5B-D, compare with Fig. 4D,E): they had only a few flowers, and eventually cauline leaves with solitary flowers, and a terminal flower (Fig. 5B-D). In plants with equivalent reporter constructs 0.6-G-1.6 and 2.2-G-1.3 (Fig. S2) GUS signal was observed in the vegetative meristem and was upregulated in the floral transition, but it was very weak or absent from axillary meristems (Fig. 5G-N). These results suggest that the region between +2.2 and +1.6 kb (region III) is required for strong expression in axillary meristems and, therefore, to form complex coflorescences. They also suggest that the region between +1.6 and +1.3 kb does not contain any significant regulatory elements.

Finally, plants with T-DNA insertions at +1.0 kb or +0.4 kb and plants containing genomic construct 0.6-T-0.4 with a deletion that removed conserved region D (Fig. 5A, Fig. S1) had a *tfl1* mutant phenotype, with coflorescences converted into solitary axillary flowers (Fig. 5E,F). Most of the plants with equivalent reporter constructs 2.2-G-1.0 and 0.6-G-0.4 (Fig. S2B) did not show GUS

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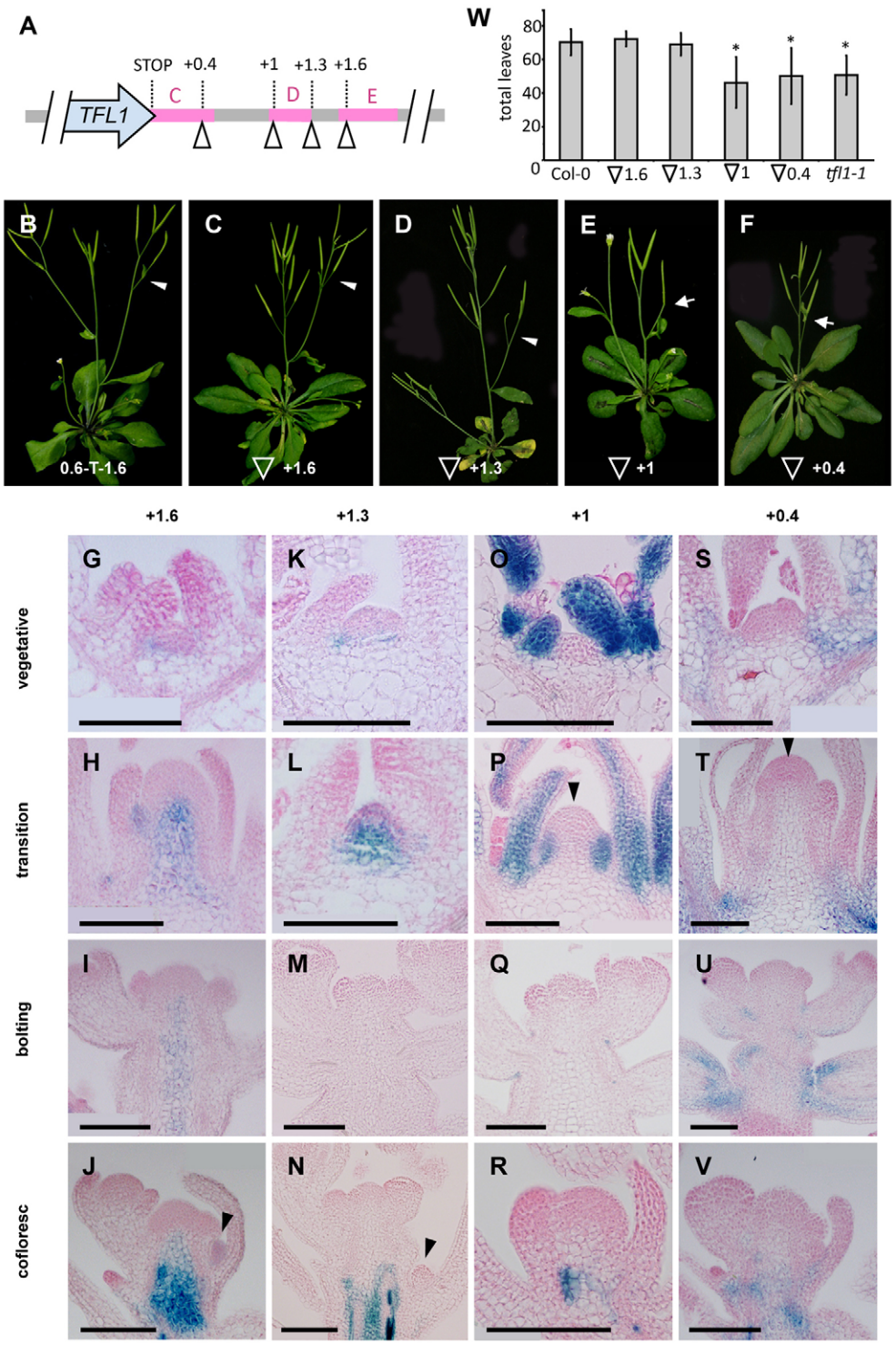

Fig. 5. Deletion analysis of the 3′ promoter region of *TFL1* from +0.4 to +1.6 kb. (A) The 3′ *TFL1* promoter region. Coordinates indicate the constructs (dotted lines) and T-DNA insertions (triangles) used in this figure. Conserved sequence blocks are marked in pink. (B) *tfl1-1* mutant plant transformed with the 0.6-T-1.6 genomic construct. Arrowhead points to a simplified coflorescence with an axillary flower. (C-F) Homozygous plants for different T-DNA insertion lines in the 3′ region, as indicated. Arrowheads (C,D) point to simplified coflorescences and arrows (E,F) point to axillary flowers. (G-V) GUS signal in longitudinal sections of apices from wild-type plants transformed with reporter constructs containing different 3′ genomic fragments. Numbers above indicate the length in kb of the 3′ fragment in the constructs. Developmental stages are indicated on the left: vegetative, (floral) transition and bolting refer to the apex of the main inflorescence; cofloresc (coflorescence) refers to the inflorescence apex of a secondary branch. Arrowheads mark absence of GUS signal in the SAM (P,T) and axillary meristems (J,N). (W) Flowering time of T-DNA insertion lines in the 3′ promoter region compared with *tfl1-1* and wild-type (Col-0) plants. Plants were grown under short-day conditions (8 h light/16 h dark). Number of leaves is shown as the mean±s.d. At least seven plants were analysed per genotype. **P*<0.05 versus wild type (Col), Student's *t*-test. Scale bars: 100 µm.

expression. In the few plants with expression, signal was absent from all shoot meristems, including vegetative meristems, and it was variable and ectopic in some lateral organs (Fig. 5O-V). These results suggest that the region between +1.3 and +1.0 kb (region II) is required for *TFL1* expression in the vegetative meristem, for its upregulation with floral transition, and to repress its expression in lateral organs.

Notably, whereas plants with T-DNA insertions at either +1.6 or +1.3 kb flowered at the same time as the wild type, the flowering time of plants with T-DNA insertions at either +1.0 or +0.4 kb was early, as in the *tfl1* mutant (Fig. 5W). This indicates that regulatory elements contained in region II (+1.0/+1.3) are required to control flowering time. As deletion of conserved sequence block D (region II) already resulted in no complementation, and plants with insertions at +1.0 kb or +0.4 kb had a *tfl1* mutant phenotype, deletion analysis of the 3′ region was not pursued further from +0.4 kb.

Regulatory regions sufficient for *TFL1* expression

The data presented above define discrete *cis*-regulatory regions 5′ and 3′ of the *TFL1* gene (Figs 6 and 7, regions I -V), overlapping

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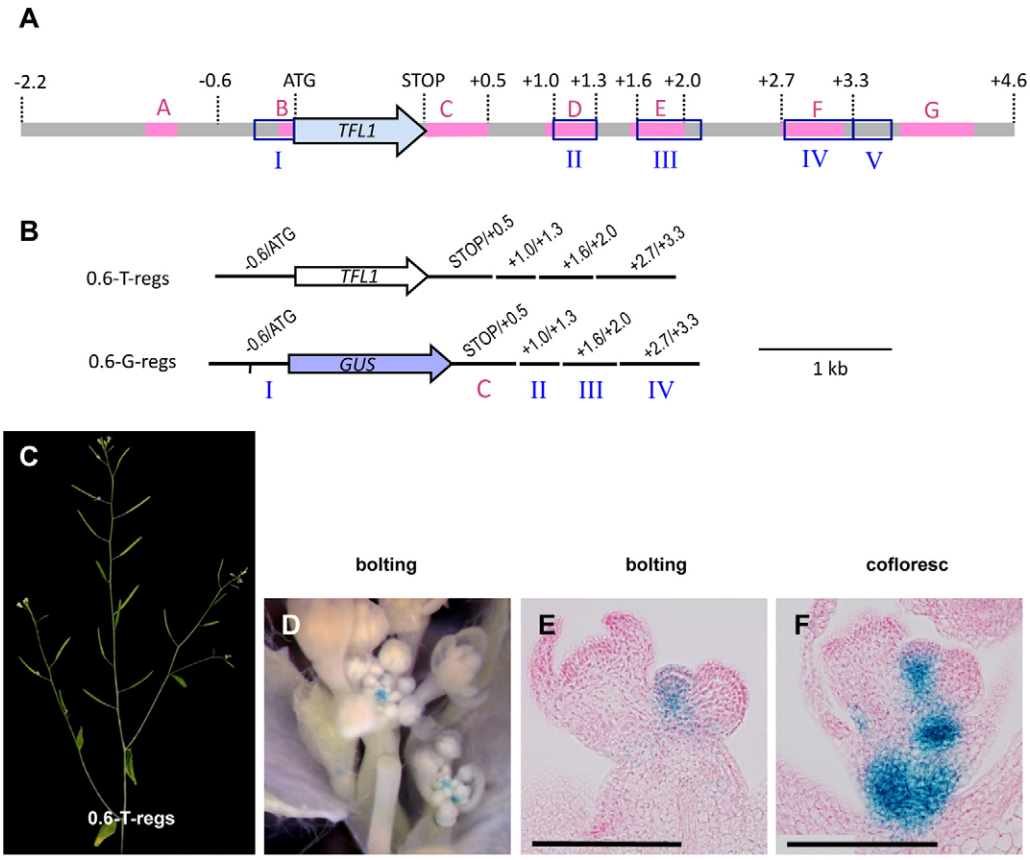

Fig. 6. Conserved *cis*-regulatory regions are sufficient to drive correct *TFL1* expression. (A) The *TFL1* genomic region. Dotted lines indicate the fragments in constructs employed in the experiments in this figure. Conserved sequence blocks (A-G) are in pink. The functional *cis*-regulatory regions identified in this work (I-V) are marked with blue rectangles. Coordinates indicate the distance in kb from the start (–) or end (+) of the *TFL1* coding sequence. (B) The constructs used in the experiments in this figure. (C) Main inflorescence shoot of a *tfl1-1* mutant complemented by transformation with the 0.6-T-regs genomic construct. (D-F) GUS signal in inflorescence shoot apices of wild-type plants transformed with the 0.6-G-regs reporter construct. Whole-mount of a main inflorescence bolting apex (D), and longitudinal sections through the apices of a main inflorescence at bolting (E) or a coflorescence (F). Scale bars: 100 µm.

with conserved sequence blocks B, D, E and F (Fig. 6A), that are necessary to control its expression. To assess whether these regions are also sufficient to direct correct *TFL1* expression, we tested the performance of a genomic and a reporter construct that combined these regions (Fig. 6B).

The genomic construct 0.6-T-regs contains the *TFL1* gene, a 5′ fragment −0.6 kb/ATG, which includes regulatory region I and overlaps conserved sequence block B, regulatory regions II, III and IV, and a 0.5 kb 3′ fragment downstream the *TFL1* stop codon, which overlaps with conserved block C (Fig. 6A,B). Out of nine *tfl1* plants transformed with 0.6-T-reg, two showed full complementation (Fig. 6C). This proportion of fully complemented *tfl1* plants was similar to that obtained when using 0.6-T-3.3 (27%), with the same regulatory regions as 0.6-T-reg but in the context of the full promoter. Also, the expression of the equivalent reporter construct 0.6-G-regs (Fig. 6A,B) was the same as that of 0.6-G-3.3, these being the shortest reporter constructs that gave a wild-type expression pattern (Fig. 6D-F, compare with Fig. 4L,M). These results indicate that a combination of the 5′ and 3′ regulatory regions defined by our analysis plus 0.5 kb downstream of the *TFL1* CDS is sufficient to direct correct *TFL1* expression.

DISCUSSION

Spatial and functional structure of the *TFL1* promoter

Our results define five major functional *cis*-regulatory regions required for proper expression of the *Arabidopsis TFL1* gene, four of which substantially overlap with blocks of high sequence conservation in *TFL1* orthologues from other Brassicacea species (Fig. 7).

The 5′ proximal region mainly contains elements controlling expression level, with region I (−0.3/ATG) absolutely required for *TFL1* expression (Figs 3 and 7). In addition, some non-essential expression level elements seem to be located at the 3′, in region V (+3.3/+3.6). Interestingly, in contrast to most T-DNA insertions in the 3′ region, neither T-DNA insertions at the 5′ proximal region nor at +3.3 in the 3′ region cause a *tfl1*-like phenotype. This seems to fit with the classical definition of transcriptional enhancers, which are considered to be active independently of the distance to their target genes (Shlyueva et al., 2014).

The 3′ intergenic region contains regulatory elements for spatiotemporal expression of *TFL1*. Thus, region IV (+2.8/+3.3) is required for maintenance of *TFL1* expression, although not for its initial expression, in the inflorescence meristems. Its absence causes the main inflorescence and coflorescences to terminate prematurely but remain complex, producing secondary and tertiary branches (Figs 4 and 7). Region III (+1.6/+2.2) is required for strong initial expression in lateral axillary meristems and, consistent with this, plants with deletions to (or insertion at) +1.6 only form very simplified coflorescences (Figs 5 and 7). Finally, region II (+1.0/+1.3) contains elements required for expression in the vegetative meristem and for its upregulation in the inflorescence meristem, as well as for repression in leaves and flowers (Figs 2, 5 and 7).

Previous studies have shown that LFY and different MADS-box transcription factors directly bind to the *cis*-regulatory regions identified in this work (Fig. S5). This allows us to speculate as to how the different *TFL1* expression phases could be established. MADS-box factors SOC1, AGL24, SVP and SEP4, which bind through interaction with AP1 to sequences that are present in *cis*-regulatory regions II and III, have been shown to contribute to *TFL1* repression in flowers (Kaufmann et al., 2010; Liu et al., 2013). This would explain why ectopic *TFL1* expression in flowers is observed when these regions are deleted. XAANTAL2 (XAL2, or AGL14), a

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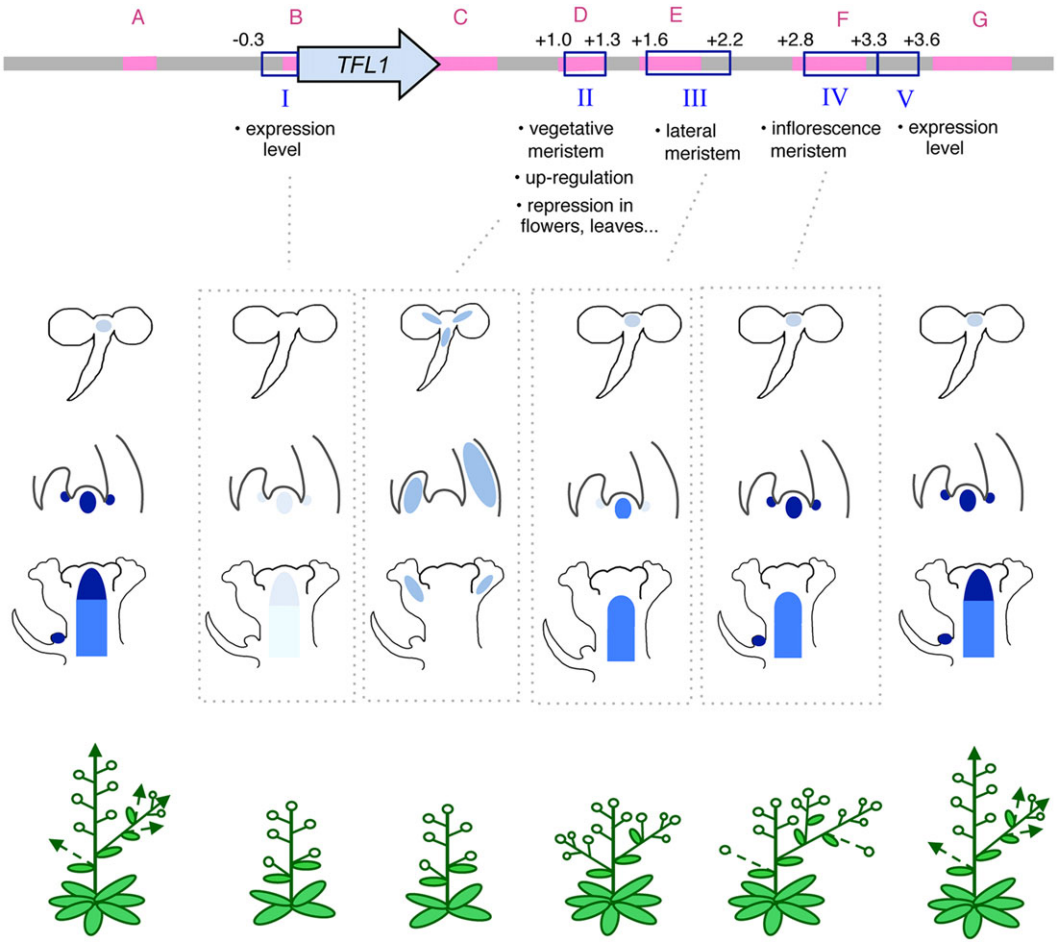

Fig. 7. Regulatory structure of the *TFL1* promoter. The *TFL1* genomic region (top). Phylogenetically conserved sequence blocks are in pink. Blue rectangles (I-V) mark the *cis*-regulatory regions identified in this work, with coordinates indicating the distance in kb from the start (–) or end (+) of the *TFL1* coding sequence. The defects in the *TFL1* expression pattern caused by the deletion of each *cis*-regulatory region are indicated beneath. The diagrams in the first three rows illustrate the GUS expression pattern (blue) in shoot apices during development (from top to bottom per column: vegetative, floral transition and inflorescence bolting), observed in wild-type plants transformed with reporter constructs with deletions of the *cis*-regulatory regions (indicated with a dotted line). The bottom row illustrates the phenotypes observed for *Arabidopsis tfl1-1* mutant plants transformed with genomic constructs with deletions of the corresponding *cis*-regulatory regions. The green dashed arrows and lines indicate indeterminate or determinate branches not represented in detail.

MADS-box protein with sequence similarity to SOC1, has been proposed to act as an activator of *TFL1* expression (Pérez-Ruiz et al., 2015). XAL2 has also been shown to bind to *TFL1* genomic regions (Pérez-Ruiz et al., 2015), to sequences that locate to *cis*-regulatory regions I, II and III, which are required for *TFL1* expression level (I) and the upregulation of *TFL1* expression in the SAM (II) and lateral inflorescence meristems (III), therefore supporting the proposed role of XAL2 as a *TFL1* activator. Finally, only LFY has thus far been shown to bind to sequences that locate to *cis*-regulatory region IV, which is required for maintenance of *TFL1* expression in inflorescence meristems. Although LFY is considered to repress *TFL1* expression in flowers (Ratcliffe et al., 1999), this does not preclude a role as a positive regulator of *TFL1* expression in the inflorescence meristems. In fact, although *LFY* expression is mainly detected in flowers, LFY protein has been shown to move and act non-autonomously, and immunolocalisation results might indicate the presence of LFY protein at low levels in the SAM (Sessions et al., 2000); moreover, although the dual role of LFY as an activator and a repressor of *TFL1* expression in different domains might appear contradictory, such context-dependent activity of transcription factors is in fact very common (for example, see Kaufmann et al., 2010). In addition, it is very likely that other, as yet unidentified factors also play crucial roles in the establishment of the *TFL1* dynamic expression pattern.

An interesting conclusion of our study is that most of the important *cis*-regulatory elements of the *TFL1* promoter are located in the 3′ intergenic region of the gene, coinciding with blocks of sequence conserved among *TFL1* orthologues. Although examples exist of essential *cis*-regulatory elements located at the 3′ end of plant genes, they are relatively scarce (Larkin et al., 1993; Brand et al., 2002; Konishi and Yanagisawa, 2011; Raatz et al., 2011). As discussed by Raatz et al. (2011), this could reflect the fact that not enough attention has been paid to possible 3′ *cis*-regulatory elements in studies on plant promoters. Other studies (Castaings et al., 2014), however, indicate that sequence conservation in the 3′ end of orthologous plant genes is not so common, which might suggest that relevant *cis*-regulatory elements are not frequently located in their 3′ intergenic regions.

The *TFL1* promoter has a functional modular structure

Our results indicate that distinct *cis*-regulatory regions of the *TFL1* promoter are required for expression in different spatiotemporal domains at the *Arabidopsis* shoot apex. A similar promoter structure, with distinct *cis*-regulatory regions controlling

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expression in different domains, has also been observed for other developmental regulators, such as the *Arabidopsis CRABS CLAW* (*CRC*) or *SPATULA* (*SPT*) genes, which, like *TFL1*, show expression in different domains that changes during development (Lee et al., 2005; Groszmann et al., 2010). This suggests that this type of promoter structure might help achieve a dynamic expression pattern. For the *CRC* promoter the identified *cis*-regulatory regions act, to some extent, as independent regulatory modules (Yuh et al., 1998; Lee et al., 2005). Elucidating whether this is also the case for the *cis*-regulatory regions in the *TFL1* promoter requires further analysis.

A functional modular structure to the promoters of developmental regulators might facilitate gene evolution to generate a diversity of plant forms. Thus, different combinations of *cis*-regulatory modules in different species could lead to different expression patterns of *TFL1*-like genes, as has been predicted to contribute to different inflorescence architectures (Prusinkiewicz et al., 2007). For instance, it is interesting that in the *Arabidopsis* relative *L. crassa*, in which changes in *TFL1* have been proposed to contribute to its distinct inflorescence architecture (Liu et al., 2011), the conserved block F (region IV) is absent from the 3′ of its *TFL1* gene (Fig. 1A).

Different promoter regions control *TFL1* expression in different shoot meristems

Our results suggest that regulation of *TFL1* in the SAM and lateral shoot meristems is controlled through the activity of different *cis*-regulatory regions in the *TFL1* promoter. On the one hand, *TFL1* expression in the main inflorescence specially requires region IV. In plants with genomic deletions or insertions that remove region IV, the main inflorescences only produce a few flowers and end in a terminal flower, as in *tfl1* mutants. This agrees with the absence of expression of equivalent *TFL1::GUS* deletions in the inflorescence meristem in elongating inflorescences. By contrast, lateral inflorescences of these plants (without region IV) are much more complex than in *tfl1* mutants (where they are replaced by solitary flowers), and expression of *TFL1::GUS* with deletions of region IV is strong in emerging lateral shoot meristems (Figs 4 and 7). On the other hand, *TFL1* in lateral inflorescences seems to particularly require region III. Deletion of region III causes strong simplification of coflorescences, without further affecting the main inflorescence, and this correlates with a substantial decrease in the expression of *TFL1::GUS* with deletions of region III in lateral shoot meristems (Figs 5 and 7).

Different signalling appears to control *TFL1* in the SAM and lateral meristems, suggesting two non-exclusive models. In one model, *TFL1* expression in lateral shoot meristems requires activation by different effectors than in the main shoot. Alternatively, *TFL1* expression might be regulated by the same effectors in SAM and lateral meristems; for instance, if they are at a higher level in the lateral meristems than in the SAM. In this second model, activation in only region III would be sufficient for expression in lateral meristems, whereas expression in the SAM would require activation in both region III and region IV. That main and lateral inflorescences are differently regulated is supported by the specific expression of genes such as *BRANCHED1* in shoot axillary meristems but not in the SAM (Aguilar-Martínez et al., 2007).

A 3′ *cis*-regulatory region is required for control of flowering time by *TFL1*

A remarkable finding of our study is that the +1/+1.3 region of the *TFL1* promoter (region II) is strictly required for a wild-type flowering time. The +1/+1.3 region is required for *TFL1* expression in the vegetative SAM and its upregulation at the floral transition. One hypothesis proposes that *TFL1* expression in the vegetative meristem controls (represses) flowering. This was based on the observation that the main difference between *Arabidopsis TFL1* and the *Antirrhinum majus* homologue *CENTRORADIALIS* (*CEN*) gene is that whereas *TFL1*, which controls flowering time and determination of the inflorescence, is expressed both in the vegetative and the inflorescence SAM, *CEN*, which only controls determination, is expressed only in the inflorescence SAM (Bradley et al., 1993). Our result links *TFL1* expression to the vegetative meristem and floral transition and provides support for this hypothesis.

TFL1 function in pea (*Pisum sativum*) is split between two *TFL1* homologues: *PsTFL1c*/*LATE FLOWERING* (*LF*), which controls flowering but not determination, and *PsTFL1a*/*DETERMINATE* (*DET*), which controls determination of the inflorescence but not flowering (Foucher et al., 2003). Interestingly, *DET*, like *CEN*, is expressed in the inflorescence but not in the vegetative apex, while *LF* is expressed in the inflorescence and in the vegetative apices (Foucher et al., 2003; Berbel et al., 2012). Although knowledge of the *LF* expression pattern is scarce (only from RT-PCR data), the data also suggest that regulation of flowering by *TFL1*-like genes is related to their expression in the vegetative apex. It would be interesting to elucidate whether the subfunctionalisation of the pea *TFL1* genes derives from divergence in their expression patterns.

TFL1-like genes have been studied in many plants with different inflorescence architectures and, frequently, as in the examples described above, their expression patterns seem to correlate well with their function in shaping plant architecture (Benlloch et al., 2007; Mcgarry and Ayre, 2012; Lifschitz et al., 2014; Wickland and Hanzawa, 2015). It will be important to understand the extent to which divergence in the expression patterns of *TFL1*-like genes has contributed to different plant and inflorescence architectures and how the *cis*-regulatory regions of these genes have evolved to generate these distinct expression patterns.

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis thaliana plants of Columbia (Col) ecotype were used unless otherwise stated. The *tfl1-1* (Col) and *tfl1-2* (Landsberg *erecta*, L*er*) mutants have been described previously (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992).

The T-DNA insertion lines characterised in this study were obtained from the Nottingham *Arabidopsis* Stock Centre (NASC) (Alonso et al., 2003) and are described in Table S1. The position of the T-DNA insertions in the *TFL1* genomic region was confirmed by PCR. Primers used for genotyping are listed in Table S2.

Plants were grown under controlled temperature (21-23°C) and long-day photoperiods (16 h light/8 h dark), as previously described (Fernández-Nohales et al., 2014). Plants used for flowering-time measurements were grown in the same conditions but under short-day photoperiods (8 h light/16 h dark).

Plasmid construction

Genomic and GUS reporter constructs in this study derive from a *TFL1* clone isolated from an *Arabidopsis* L*er* genomic library (Whitelam et al., 1993). The genomic clone included the *TFL1* gene plus 5.5 kb of the 5′ and 8.7 kb of the 3′ flanking sequences. This 15.3 kb fragment was subcloned as a *Not*I fragment into the pBluescript II SK$^+$ vector (Agilent), giving rise to plasmid pBSKTFL1.

For genomic constructs, different fragments from the *TFL1* genomic region in pBSKTFL1 were subcloned into the binary vector pBIN19 (Bevan, 1984). These fragments were obtained by restriction digestion at sites in the *TFL1* genomic region, or by PCR, or by a combination of both methods.

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For reporter constructs, the *β-glucuronidase* gene (*GUS*) from plasmid pBI101 (Jefferson et al., 1987) was subcloned into the pGEM-T Easy vector (Promega) as a *Hin*dIII/*Eco*RI fragment, giving rise to plasmid pG101. Different fragments of the 5′ and 3′ *TFL1* flanking regions were obtained from pBSKTFL1, as described above, and subcloned into pG101 upstream or downstream of the *GUS* gene. The promoter::GUS fusions generated were subcloned into the binary vector pBIN19.

The minimal −46 bp 35S promoter from cauliflower mosaic virus (Benfey and Chua, 1990) was obtained by PCR using plasmid pDW294 (Busch et al., 1999) as a template.

PCR reactions were carried out with high-fidelity PfuTurbo DNA polymerase (Stratagene) using the primers listed in Table S2.

Plant transformation and analysis of transgenic lines

Constructs based on the pBIN19 plasmid were introduced into *Agrobacterium tumefaciens* strain C58. *Arabidopsis thaliana* plants were transformed by vacuum infiltration as described (Bechtold et al., 1993).

For complementation analyses, *tfl1-1* mutant plants were transformed and T1 plants selected on the basis of kanamycin resistance and transferred to soil. At least seven independent T1 lines were analysed for each construct. *tfl1-1* and Col wild-type plants transformed with an empty pBIN19 vector and with the 2.2-G-4.6 construct, respectively, were used as controls in the complementation analysis. Quantitative data of the range of phenotypes obtained for the different genomic constructs are presented in Table S3.

For analyses of the expression pattern of GUS reporter constructs, Col wild-type plants were transformed. A minimum of 15 kanamycin-resistant T1 lines were selected per construct and transferred to soil. GUS activity was first analysed in T1 plants, in whole-mounts of apices of inflorescences at bolting or 15 days after bolting (mature inflorescences). Quantitative data of the range of expression patterns observed for the different GUS reporter constructs in T1 plants are presented in Table S4. The data in Fig. 3L correspond to an independent experiment in which at least 12 T1 plants for each reporter construct were selected and analysed simultaneously. Bolting apices were stained for GUS (at high astringency, see below) and reporter activity in the shoot apex of both the main inflorescence and the most apical (older) coflorescence was determined.

Representative lines for each GUS reporter construct were identified and subsequently tested in the next generation for single-locus insertion of the transgene, based on a 3:1 segregation on selective medium. In this way, three to four independent T2 lines were selected for each construct and GUS expression pattern in the main shoot was analysed in microscopy sections at different developmental stages: vegetative apices (after 6-8 days under long-day conditions), floral transition (12-day plants; this day was determined as described by Bradley et al., 1997), bolting apices (after 20-25 days) and mature inflorescence apices (35-40 days). Expression patterns observed for the different representative lines for each construct were consistently similar.

GUS assays

Samples were stained for GUS as described (Benlloch et al., 2011) with minor modifications. Two staining buffers with different concentrations of ferrocyanide and ferricyanide were employed: 2 mM ferro-ferricyanide (medium stringency) for samples with weak GUS activity, and 10 mM ferro-ferricyanide (high stringency) for samples with strong GUS activity (Sessions et al., 1999).

Images of GUS-stained whole-mount apices in 70% (v/v) ethanol were taken with a Nikon SMZ800 stereomicroscope. Samples were then stained in Eosin and embedded in paraffin as described (Benlloch et al., 2011). Sections (12 µm) were obtained with a Leica RM-2025 microtome and photographed with a Nikon Eclipse 600 light microscope.

RNA *in situ* hybridisation

Plasmids pJAM2045 (for *TFL1*) and SS018 (for *GUS*) were linearised and antisense probes were transcribed *in vitro* using the DIG RNA Labelling Kit and T7 RNA polymerase (Roche). The RNA probes were then subjected to mild alkali hydrolysis as described (Coen et al., 1990). RNA *in situ* hybridisations were performed on 8 µm sections of inflorescence apices as described (Ratcliffe et al., 1999) and imaged using a Nikon Eclipse 600 microscope.

Determination of *TFL1* transcription initiation site

The transcription start site of *TFL1* was determined by three independent rapid amplification of cDNA ends (RACE) experiments as being 45 bp upstream the start codon. For this purpose, mRNA was extracted from bolting inflorescences with the QuickPrep Micro mRNA Purification Kit (Amersham) and 5′-RACE was carried out using the GeneRacer Kit (Invitrogen) according to the manufacturer's instructions and the *TFL1*-specific primers ASMOL70 and ASMOL71 (Table S2).

Sequence analysis of the *TFL1* promoter

For phylogenetic shadowing of *TFL1 cis*-regulatory sequences (Boffelli et al., 2003; Hong et al., 2003), genomic regions of *TFL1* orthologues from different species were isolated and sequenced. *CsTFL1* was isolated from a *Capsella bursa-pastoris* BAC library obtained from Dr R. Schmidt (Leibniz Institute for Plant Genetics and Crop Plant Research, Gatersleben, Germany) using the *TFL1* cDNA as probe. *BrTFL1* from *Brassica rapa* was identified in the BAC KBrH96B10 using GBrowse [at TAIR (www.arabidopsis.org)]. The sequence of the genomic fragment containing *LcTFL1* from *Leavenworthia crassa* (GenBank accession GU 136396) was provided by Dr D. Baum (University of Wisconsin-Madison, USA). *AlTFL1* from *Arabidopsis lyrata* was obtained from GBrowse.

The alignment of the non-coding sequences from the different *TFL1* orthologues and the identification of the conserved regions were carried out using mVISTA (http://genome.lbl.gov/vista/index.shtml) and CHAOS-DIALIGN (http://dialign.gobics.de/chaos-dialign-submission) (Brudno et al., 2003, 2004; Frazer et al., 2004). Because the results of both algorithms were essentially identical, only mVISTA results are shown in this work. A shuffle-LAGAN alignment with a window of 100 bp and a minimum sequence identity of 75% were used for the mVISTA analysis.

For identification of putative MADS domain (CArG boxes) and LFY transcription factor binding sites, we scanned the conserved regions of the *Arabidopsis TFL1* genomic region in the JASPAR database (Sandelin et al., 2004) using its specific section for plants. For CArG boxes, we selected all the available JASPAR matrix models classified as 'MADS-box factors' using default parameters and we finally selected those with a score higher than 10 and a relative score higher than 0.8 that were conserved in at least two species. For LFY binding sites, we selected the JASPAR matrix model for LFY using default parameters but with a relative score of 0.75, and we finally selected those with a score higher than 7.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

F.M., D.B. and A.S.-M. designed the work; A.S.-M., M.J.D., P.F.-N., F.M. and Y.H. carried out experiments; A.S.-M., F.M., D.B., P.F.-N. and Y.H. analysed and interpreted data; F.M. and A.S.-M. wrote the paper with the help of D.B.

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Data availability

Sequences of the genes used in this study can be found in the NCBI GenBank database under the following accession numbers: *Arabidopsis thaliana* Ler *TFL1* (KX139000), *Capsella bursa-pastoris TFL1* (KX139002) and *Brassica rapa TFL1* (KX139001).

Supplementary information

Supplementary information available online at
http://dev.biologists.org/lookup/doi/10.1242/dev.135269.supplemental

References

Aguilar-Martínez, J. A., Poza-Carrión, C. and Cubas, P. (2007). Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. *Plant Cell* **19**, 458-472.

Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., Stevenson, D. K., Zimmerman, J., Barajas, P., Cheuk, R. et al. (2003). Genome-wide insertional mutagenesis of Arabidopsis thaliana. *Science* **301**, 653-657.

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

Baumann, K., Venail, J., Berbel, A., Domenech, M. J., Money, T., Conti, L., Hanzawa, Y., Madueno, F. and Bradley, D. (2015). Changing the spatial pattern of TFL1 expression reveals its key role in the shoot meristem in controlling Arabidopsis flowering architecture. *J. Exp. Bot.* **66**, 4769-4780.

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

Benfey, P. N. and Chua, N.-H. (1990). The cauliflower mosaic virus 35S promoter: combinatorial regulation of transcription in plants. *Science* **250**, 959-966.

Benlloch, R., Berbel, A., Serrano-Mislata, A. and Madueno, F. (2007). Floral initiation and inflorescence architecture: a comparative view. *Ann. Bot.* **100**, 659-676.

Benlloch, R., Kim, M. C., Sayou, C., Thévenon, E., Parcy, F. and Nilsson, O. (2011). Integrating long-day flowering signals: a LEAFY binding site is essential for proper photoperiodic activation of APETALA1. *Plant J.* **67**, 1094-1102.

Berbel, A., Ferrándiz, C., Hecht, V., Dalmais, M., Lund, O. S., Sussmilch, F. C., Taylor, S. A., Bendahmane, A., Noel Ellis, T. H. N., Beltrán, J. P. et al. (2012). *VEGETATIVE1* is essential for development of the compound inflorescence in pea. *Nat. Commun.* **3**, 797.

Bevan, M. (1984). Binary Agrobacterium vectors for plant transformation. *Nucleic Acids Res.* **12**, 8711-8721.

Boffelli, D., McAuliffe, J., Ovcharenko, D., Lewis, K. D., Ovcharenko, I., Pachter, L. and Rubin, E. M. (2003). Phylogenetic shadowing of primate sequences to find functional regions of the human genome. *Science* **299**, 1391-1394.

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

Bradley, D., Carpenter, R., Sommer, H., Hartley, N. and Coen, E. (1993). Complementary floral homeotic phenotypes result from opposite orientations of a transposon at the plena locus of Antirrhinum. *Cell* **72**, 85-95.

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

Brand, U., Grünewald, M., Hobe, M. and Simon, R. (2002). Regulation of CLV3 expression by two homeobox genes in Arabidopsis. *Plant Physiol.* **129**, 565-575.

Brudno, M., Malde, S., Poliakov, A., Do, C. B., Couronne, O., Dubchak, I. and Batzoglou, S. (2003). Glocal alignment: finding rearrangements during alignment. *Bioinformatics* **19** Suppl. 1, i54-i62.

Brudno, M., Steinkamp, R. and Morgenstern, B. (2004). The CHAOS/DIALIGN WWW server for multiple alignment of genomic sequences. *Nucleic Acids Res.* **32**, W41-W44.

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

Castaings, L., Bergonzi, S., Albani, M. C., Kemi, U., Savolainen, O. and Coupland, G. (2014). Evolutionary conservation of cold-induced antisense RNAs of FLOWERING LOCUS C in Arabidopsis thaliana perennial relatives. *Nat. Commun.* **5**, 4457.

Coen, E. S., Romero, J. M., Doyle, S., Elliott, R., Murphy, G. and Carpenter, R. (1990). Floricaula: a homeotic gene required for flower development in Antirrhinum majus. *Cell* **63**, 1311-1322.

Conti, L. and Bradley, D. (2007). TERMINAL FLOWER1 is a mobile signal controlling Arabidopsis architecture. *Plant Cell* **19**, 767-778.

Della Pina, S., Souer, E. and Koes, R. (2014). Arguments in the evo-devo debate: say it with flowers! *J. Exp. Bot.* **65**, 2231-2242.

Fernández-Nohales, P., Domenech, M. J., Martínez de Alba, A. E., Micol, J. L., Ponce, M. R. and Madueño, F. (2014). AGO1 controls arabidopsis inflorescence architecture possibly by regulating *TFL1* expression. *Ann. Bot.* **114**, 1471-1481.

Foucher, F., Morin, J., Courtiade, J., Cadioux, S., Ellis, N., Banfield, M. J. and Rameau, C. (2003). DETERMINATE and LATE FLOWERING are two TERMINAL FLOWER1/CENTRORADIALIS homologs that control two distinct phases of flowering initiation and development in pea. *Plant Cell* **15**, 2742-2754.

Frazer, K. A., Pachter, L., Poliakov, A., Rubin, E. M. and Dubchak, I. (2004). VISTA: computational tools for comparative genomics. *Nucleic Acids Res.* **32**, W273-W279.

Gaillochet, C., Daum, G. and Lohmann, J. U. (2015). O cell, where art thou? The mechanisms of shoot meristem patterning. *Curr. Opin. Plant Biol.* **23**, 91-97.

Groszmann, M., Bylstra, Y., Lampugnani, E. R. and Smyth, D. R. (2010). Regulation of tissue-specific expression of SPATULA, a bHLH gene involved in carpel development, seedling germination, and lateral organ growth in Arabidopsis. *J. Exp. Bot.* **61**, 1495-1508.

Hong, R. L., Hamaguchi, L., Busch, M. A. and Weigel, D. (2003). Regulatory elements of the floral homeotic gene AGAMOUS identified by phylogenetic footprinting and shadowing. *Plant Cell* **15**, 1296-1309.

Jefferson, R. A., Kavanagh, T. A. and Bevan, M. W. (1987). GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **6**, 3901-3907.

Kaufmann, K., Wellmer, F., Muino, J. M., Ferrier, T., Wuest, S. E., Kumar, V., Serrano-Mislata, A., Madueno, F., Krajewski, P., Meyerowitz, E. M. et al. (2010). Orchestration of floral initiation by APETALA1. *Science* **328**, 85-89.

Konishi, M. and Yanagisawa, S. (2011). The regulatory region controlling the nitrate-responsive expression of a nitrate reductase gene, NIA1, in Arabidopsis. *Plant Cell Physiol.* **52**, 824-836.

Larkin, J. C., Oppenheimer, D. G., Pollock, S. and Marks, M. D. (1993). Arabidopsis GLABROUS1 gene requires downstream sequences for function. *Plant Cell* **5**, 1739-1748.

Lee, J.-Y., Baum, S. F., Alvarez, J., Patel, A., Chitwood, D. H. and Bowman, J. L. (2005). Activation of CRABS CLAW in the nectaries and carpels of Arabidopsis. *Plant Cell* **17**, 25-36.

Lifschitz, E., Ayre, B. G. and Eshed, Y. (2014). Florigen and anti-florigen - a systemic mechanism for coordinating growth and termination in flowering plants. *Front. Plant Sci.* **5**, 465.

Liu, N., Sliwinski, M. K., Correa, R. and Baum, D. A. (2011). Possible contributions of TERMINAL FLOWER 1 to the evolution of rosette flowering in Leavenworthia (Brassicaceae). *New Phytol.* **189**, 616-628.

Liu, C., Teo, Z. W. N., Bi, Y., Song, S., Xi, W., Yang, X., Yin, Z. and Yu, H. (2013). A conserved genetic pathway determines inflorescence architecture in Arabidopsis and rice. *Dev. Cell* **24**, 612-622.

Mcgarry, R. C. and Ayre, B. G. (2012). Manipulating plant architecture with members of the CETS gene family. *Plant Sci.* **188-189**, 71-81.

Moyroud, E., Minguet, E. G., Ott, F., Yant, L., Posé, D., Monniaux, M., Blanchet, S., Bastien, O., Thévenon, E., Weigel, D. et al. (2012). Prediction of regulatory interactions from genome sequences using a biophysical model for the arabidopsis LEAFY transcription factor. *Plant Cell* **23**, 1293-1306.

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

Park, S. J., Eshed, Y. and Lippman, Z. B. (2014). Meristem maturation and inflorescence architecture—lessons from the Solanaceae. *Curr. Opin. Plant Biol.* **17**, 70-77.

Pérez-Ruiz, R. V., García-Ponce, B., Marsch-Martínez, N., Ugartechea-Chirino, Y., Villajuana-Bonequi, M., de Folter, S., Azpeitia, E., Dávila-Velderrain, J., Cruz-Sánchez, D., Garay-Arroyo, A. et al. (2015). XAANTAL2 (AGL14) is an important component of the complex gene regulatory network that underlies Arabidopsis shoot apical meristem transitions. *Mol. Plant* **8**, 796-813.

Prusinkiewicz, P., Erasmus, Y., Lane, B., Harder, L. D. and Coen, E. (2007). Evolution and development of inflorescence architectures. *Science* **316**, 1452-1456.

Raatz, B., Eicker, A., Schmitz, G., Fuss, E., Müller, D., Rossmann, S. and Theres, K. (2011). Specific expression of LATERAL SUPPRESSOR is controlled by an evolutionarily conserved 3′ enhancer. *Plant J.* **68**, 400-412.

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

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

Sandelin, A., Alkema, W., Engström, P., Wasserman, W. W. and Lenhard, B. (2004). JASPAR: an open-access database for eukaryotic transcription factor binding profiles. *Nucleic Acids Res.* **32** Suppl. 1, D91-D94.

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

Sessions, A., Weigel, D. and Yanofsky, M. F. (1999). The Arabidopsis thaliana MERISTEM LAYER 1 promoter specifies epidermal expression in meristems and young primordia. *Plant J.* **20**, 259-263.

Sessions, A., Yanofsky, M. F. and Weigel, D. (2000). Cell-cell signaling and movement by the floral transcription factors LEAFY and APETALA1. *Science* **289**, 779-781.
Shannon, S. and Meeks-Wagner, D. (1991). A mutation in the Arabidopsis TFL1 gene affects inflorescence meristem development. *Plant Cell* **3**, 877-892.
Shlyueva, D., Stampfel, G. and Stark, A. (2014). Transcriptional enhancers: from properties to genome-wide predictions. *Nat. Rev. Genet.* **15**, 272-286.
Sohn, E. J., Rojas-Pierce, M., Pan, S., Carter, C., Serrano-Mislata, A., Madueno, F., Rojo, E., Surpin, M. and Raikhel, N. V. (2007). The shoot meristem identity gene TFL1 is involved in flower development and trafficking to the protein storage vacuole. *Proc. Natl. Acad. Sci. USA* **104**, 18801-18806.
Weberling, F. (1989). *Morphology of Flowers and Inflorescences*. Cambridge: Cambridge University Press.
Whitelam, G. C., Johnson, E., Peng, J., Carol, P., Anderson, M. L., Cowl, J. S. and Harberd, N. P. (1993). Phytochrome A null mutants of Arabidopsis display a wild-type phenotype in white light. *Plant Cell* **5**, 757-768.
Wickland, D. P. and Hanzawa, Y. (2015). The FLOWERING LOCUS T/TERMINAL FLOWER 1 gene family: functional evolution and molecular mechanisms. *Mol. Plant* **8**, 983-997.
Winter, C. M., Austin, R. S., Blanvillain-Baufumé, S., Reback, M. A., Monniaux, M., Wu, M.-F., Sang, Y., Yamaguchi, A., Yamaguchi, N., Parker, J. E. et al. (2011). LEAFY target genes reveal floral regulatory logic, cis Motifs, and a link to biotic stimulus response. *Dev. Cell* **20**, 430-443.
Yuh, C. -H., Bolouri, H. and Davidson, E. H. (1998). Genomic cis-regulatory logic: experimental and computational analysis of a sea urchin gene. *Science* **279**, 1896-1902.