The *TERMINAL FLOWER2* (*TFL2*) Gene Controls the Reproductive Transition and Meristem Identity in *Arabidopsis thaliana*

Annika Sundås Larsson,* Katarina Landberg* and D. R. Meeks-Wagner†

**Department of Physiological Botany, Uppsala University, Villav. 6, S-752 36 Uppsala, Sweden and †Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403*

Manuscript received December 31, 1997
Accepted for publication March 16, 1998

ABSTRACT

A new mutant of *Arabidopsis thaliana* that initiates flowering early and terminates the inflorescence with floral structures has been identified and named *terminal flower2* (*tfl2*). While these phenotypes are similar to that of the *terminal flower1* (*tfl1*) mutant, *tfl2* mutant plants are also dwarfed in appearance, have reduced photoperiod sensitivity and have a more variable terminal flower structure. Under long-day and short-day growth conditions *tfl1 tfl2* double mutants terminate the inflorescence without development of lateral flowers; thus, unlike *tfl1* single mutants the double mutant inflorescence morphology is not affected by day length. The enhanced phenotype of the double mutant suggests that *TFL2* acts in a developmental pathway distinct from *TFL1*. The complex nature of the *tfl2* single mutant phenotype suggests that *TFL2* has a regulatory role more global than that of *TFL1*. Double mutant analysis of *tfl2* in combination with mutant alleles of the floral meristem identity genes *LEAFY* and *APETALA1* demonstrates that *TFL2* function influences developmental processes controlled by *APETALA1*, but not those regulated by *LEAFY*. Thus, the *TFL2* gene product appears to have a dual role in regulating meristem activity, one being to regulate the meristem response to light signals affecting the development of the plant and the other being the maintenance of inflorescence meristem identity.

THE transition from vegetative to reproductive development in flowering plants is accompanied by a major change in the elaboration of the plant shoot system. In *Arabidopsis thaliana* this transition is regulated by interactions between the genetic developmental program and environmental signals such as light. The transition to the reproductive phase is delayed in short-day (SD) growth conditions in this species (Napp-Zinn 1985). During normal vegetative development the shoot apical meristem gives rise to rosette leaves and axillary inflorescence meristems in a compact spiral growth pattern (Medford *et al.* 1994). Upon the transition to the reproductive phase the shoot apical meristem acquires inflorescence identity as recognized by the production of flowers instead of leaves (Hempel and Feldman 1994). This transition results in the elongation of the main axis of the shoot where cauline leaves subtend previously initiated axillary meristems that develop as coflorescence shoots. Following the development of 2 to 5 coflorescences, solitary lateral floral meristems arise from the shoot apical meristem, each of which gives rise to floral organs in a compact whorled growth pattern (Smyth *et al.* 1990; Shannon and Meeks-Wagner 1991; Hempel and Feldman 1994). Wild-type Arabidopsis plants have an indeterminate growth pattern, in which the apical meristem produces lateral floral meristems until growth arrests, and is itself never converted into a floral meristem (Shannon and Meeks-Wagner 1991).

Critical regulatory genes that control meristem identity have been identified in Arabidopsis by mutations that disrupt normal inflorescence or flower development. Genes known to be important for meristem identity during the reproductive phase are *LEAFY* (*LFY*), *APETALA 1* (*AP1*), *APETALA 2* (*AP2*), *CAULIFLOWER* (*CAL*) and *TERMINAL FLOWER 1* (*TFL1*) (Irish and Sussex 1990; Kunst *et al.* 1989; Bowman 1992; Schultz and Haughn 1991; Shannon and Meeks-Wagner 1991, 1993). When one or more of the *LFY*, *AP1*, *AP2* and *CAL* genes are impaired, floral meristems display various degrees of inflorescence meristem character indicating that these genes are required for floral identity. These floral meristem identity genes encode putative transcriptional regulators, and thus likely exert their influence on meristem development by directly controlling the activity of genes needed for flower initiation. Disruption of *TFL1* function leads to the opposite phenotype of mutations in the floral meristem identity genes. *tfl1* mutants display a conversion of the shoot apical meristem to a floral meristem. This phenotype suggests a central role of *TFL1* in maintaining inflorescence meristem identity and in the control of the indeterminate meristem state. In addition to the determinate inflorescence phenotype, *tfl1* plants also initiate flowering earlier than wild-type plants. This indicates a dual role for *TFL1* in the apical meristem: maintaining meristem

Corresponding author: D. Ry Meeks-Wagner, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.
E-mail: meeksw@molbio.uoregon.edu

identity of the inflorescence phase and regulating the timing of the transition from the vegetative to the reproductive developmental phase. *TFL1* appears to encode a membrane-associated protein, expressed in the cells of the shoot apex, that may function in signal transduction to regulate the floral meristem identity genes (Bradley *et al.* 1997).

Environmental conditions have prominent effects on different characters of the plant. Genes sensing environmental signals and regulating plant development accordingly are likely to act at early stages in the network of genetic pathways, and such genes have been identified by mutations that give rise to defects in response to known endogenous and exogenous signals such as hormones, light and temperature. Endogenous levels of plant hormones transduce signals that regulate development, and gibberellic acid (GA) in particular has been shown to play a role in the timing of floral transition (Martínez-Zapater *et al.* 1994). Furthermore, the *spindly* (*spy*) mutant, which displays long hypocotyl, pale color, increased internode elongation, early flowering, parthenocarpy and partial male sterility, has a basal level of gibberellin signal transduction, independent of GA (Jacobsen and Olszewski 1993). Recently, the *spy* mutation or exogenously added GA, was shown to affect the identity of floral meristems in several of the floral meristem identity mutants (Okamuro *et al.* 1996a, 1996b). It has been suggested that GA is part of the control mechanism regulating the activity of either the flower or the inflorescence meristem identity genes. In a similar manner, light or photoperiod regulates both the timing of the floral transition and the fate of inflorescence and floral meristems. The photoperiod insensitive *early flowering 3* (*elf3*) mutant displays elongated hypocotyl and early flowering but normal inflorescence and floral meristem identity (Zagotta *et al.* 1996). However, when *elf3* mutants are also rendered defective for phytochrome function due to a mutation in the *ELONGATED HYPOCOTYL2* (*HY2*) gene, the double mutant plants flower early and also form a terminal flower after the production of several solitary lateral flowers. This suggests that pathways controlling photoperiod/light response also can influence meristem identity during the reproductive phase of plant development. Similarly the pleiotropic *curly leaf* (*clf*) mutation that affects leaf morphology, flower morphology and flowering time is sensitive to environmental conditions (Goodrich *et al.* 1997). The structural and functional homology of *CLF* to the Drosophila gene *Enhancer of zeste* (*E*(z)), and the observation that *CLF* functions upstream of the floral organ identity gene *AGAMOUS*, suggests a role for *CLF* in maintaining specific patterns of gene expression throughout development.

This paper describes the isolation of a new determinate mutant, *terminal flower 2* (*tfl2*). We present data suggesting that in addition to its role in maintaining inflorescence meristem identity *TFL2* is active in the signal perception pathway by which photoperiod regulates the transition to reproductive growth, and that *TFL2* is likely to function more globally than *TFL1* in regulating shoot development.

MATERIALS AND METHODS

Plant materials and growth conditions: A screen was performed on greenhouse-grown M_2 generation plants derived from fast neutron mutagenized seed of *A. thaliana* ecotype Columbia obtained from Lehle Seeds, Tucson, AZ. Seeds from individual mutant plants were collected and back-crossed to wild-type Columbia.

For EMS mutagenesis of *tfl1-1*, seeds were imbibed overnight in water, mutagenized in 0.4% EMS for 8 hr and subsequently washed eight times in water before planting. One plant with an enhanced terminal flower phenotype was isolated from the M_1 population.

For all analyses seeds were sown in a 3:1 mix of soil:vermiculite after 2 days of vernalization at 4°. For measurements all plants were grown in growth cabinets at 20° under cool-white fluorescent lights during either an 18-hr light/6-hr dark photoperiod (long day; LD) or a 9-hr light/15-hr dark photoperiod (SD).

Chromosomal mapping of the *tfl2* mutation: The *tfl2-2* mutation was found to be linked to *tfl1-1* on chromosome *5*, and thus *tfl2* was mapped relative to the *tfl1* mutation as well as the simple sequence length polymorphism markers nga225, nga151 and nga106. Genetic distance between *tfl1* and *tfl2* was calculated by the product ratio method using an F_2 population of 270 plants generated by back-crossing the double mutant to wild type (the mutations were in coupling phase). The conversion to map units was made using the Kosambi mapping function (Koornneef *et al.* 1983).

F_2 plants from a cross between the Columbia ecotype carrying the *tfl2* mutation and the wild-type Landsberg ecotype were scored for their genotype with respect to the production of terminal floral structures. Total DNA was prepared from F_3 families according to Martienssen and Springer (1997), and PCR reactions using primer pairs nga225, nga151 and nga106 were carried out as described (Bell and Ecker 1994). 350 chromosomes were analyzed and the conversion to map units was made using the Kosambi mapping function (Koornneef *et al.* 1983).

Construction of double and triple mutant lines: The *tfl1 tfl2* double mutant was constructed with plants homozygous for *tfl2-1* and *tfl1-1* alleles, respectively, both in the Columbia background. The resulting F_2 families segregated for *tfl1* and *tfl2* in a ratio deviating from the 9:3:3:1 ratio as expected from the mapping data. The novel *tfl1 tfl2* phenotype was confirmed by crossing individuals to Columbia wild-type plants and scoring both *tfl1* and *tfl2* segregating in the progeny.

The *tfl2 ap1* double mutant was constructed with plants homozygous for *tfl2-1* (Columbia background) and *ap1-1* (Landsberg background), respectively. The progeny of resulting F_1 plants segregated both mutations and the double mutants in the expected 9:3:3:1 ratio. Secondary flowers were scored along the main axis of the F_2 plants. A total of 502 flowers of 24 plants and 666 flowers of 20 plants were scored for *tfl2 ap1* and *ap1*, respectively.

The *tfl1 tfl2 lfy* triple mutant and the *tfl2 lfy* double mutant was constructed by crossing *tfl1-1 tfl2-1* and *tfl1-10 fy-16* double mutants, all alleles in the Columbia background. *tfl1-1 fl2-1* was used to pollinate late developing pistils of *tfl1-10 lfy16* plants. Five resulting F_2 families were analyzed and segregated

the expected 9:3:3:1 regarding *tfl1, tfl1 lfy, tfl1 tfl2* and *tfl1 tfl2 lfy*.

Specimen preparation and photography: For scanning electron microscopy tissues were fixed in FAA (3.7% formaldehyde, 5% acetic acid, 50% ethanol) and dehydrated in a graded ethanol series. Specimens were critical point dried, mounted on stubs, and coated with gold before examination in an XL 30 scanning electron microscope (Philips Technologies, Cheshire, CT) at an accelerating voltage of 10 kV.

Cell size determination: For measurements of leaf epidermal cell size and leaf area the fifth rosette leaf was examined. Epidermal cells from the middle part of the leaves were examined by scanning electron microscopy and leaf area samples were scanned using an AGFA Studioscan II scanner (Gevaert N.V., Mortsel, Belgium). Epidermal cell areas and leaf areas were measured using the public domain NIH Image 1.59 program (National Institutes of Health, Bethesda, MD), which was also used to adjust contrast and brightness. Measurements were tested with the Student's *z*-test for unmatched samples using Microsoft Excel 4.0.

GA-treatment: GA-treated plants were sprayed once a week, from day 17 after planting, with 0.1 mm GA3 (Sigma Chemical Co., St. Louis, MO) and 0.02% Tween-20. Control plants were sprayed with only 0.02% Tween-20.

RESULTS

Isolation of *tfl2*, a new determinate mutant of Arabidopsis: To search for genes involved in inflorescence meristem function two genetic screens were carried out. In a general greenhouse screen of 17,000 M_2 plants of the Columbia ecotype derived from fast neutron mutagenesis, one early-flowering determinate plant was identified. This mutant complemented the *tfl1-1* mutant and thus represented a new genetic locus. This locus was named *TERMINAL FLOWER 2* (*TFL2*). A second screen was carried out with EMS-mutagenized *tfl1-1* seed. One enhanced determinate plant isolated in the M_1 generation was shown to be due to a second-site mutation. This mutation caused a determinate inflorescence phenotype independent of the presence of the *tfl1* mutation, and was found to be allelic with the fast neutron derived allele described above. Based on our further characterization of this allele we believe it was a spontaneous mutation that occurred in the *tfl1* line prior to EMS mutagensis. These alleles were designated *tfl2-1* (fast neutron derived) and *tfl2-2*. Both *tfl2* mutations are inherited as nuclear recessive mutations, and thus we suspect that the *tlf2-2* mutation may have resulted from a spontaneous mutation in the *tlf1-1* line used for EMS mutagenesis. Preliminary examination of the two alleles did not reveal any difference in the severity of the mutant phenotypes, and thus the *tfl2-1* allele was used for further experiments.

Genetic linkage analysis showed that the *TFL2* locus maps to the top of chromosome *5*, 22.9 ± 3.6 map units away from *TFL1*. Further mapping experiments placed *TFL2* 1.3 ± 0.8 cM away from simple sequence length polymorphism-marker nga106 (Bell and Ecker 1994). No other *TFL*-like mutations map to this region of chromosome *5*, and thus *TFL2* is a newly defined locus. *embryonic flower 1* (*emf1*), another mutation that causes a dramatic inflorescence phenotype, also maps to this region of chromosome *5* (Sung *et al.* 1992); however, a complementation test showed that the *emf1* and *tfl2* mutations define separate loci.

tfl2 mutations disrupt normal photoperiodic flowering response and plant size, as well as inflorescence meristem development; apart from the development of terminal floral structures (see below) *tfl2* mutations also affect flowering time and overall plant size. In general *tfl2* plants flower earlier and produce smaller shoots than the wild type.

Figure 1A shows wild-type, *tfl1* and *tfl2* plants at 15 days after sowing, a time when *tfl1* plants have just initiated inflorescence stem elongation. Considerable development of the *tfl2* inflorescences has already taken place. Flowering time was measured both as the number of rosette leaves produced at the time of visible floral buds, and as the number of days from sowing until appearance of the floral buds. Figure 2 shows that *tfl2* plants flower even earlier than *tfl1* plants, and that the dramatic effect of the *tfl2* mutation on flowering time is evident under both LD (18 hr of light, 6 hr of dark) and SD (9 hr of light, 15 hr of dark) growth conditions. Flowering is delayed less than twofold in *tfl2* mutants under SD conditions as compared to LD conditions while *tfl1* and wild type both are delayed 3- to 5-fold (Shannon and Meeks-Wagner 1991). Thus, photoperiod sensitivity in the *tfl2* mutant is strongly reduced as compared to wild type and *tfl1*, although the *tfl2* mutant is not completely photoperiod insensitive.

While the overall size of *tfl2* plants is reduced as compared to the size of wild-type shoots, *tfl2* plants still show the wild-type proportions of the shoot architecture. This is unlike many characterized dwarf mutants that show a reduced height due to lack of internode elongation but wild-type size of all plant organs (Koornneef and van der Veen 1980; Koornneef *et al.* 1985). Figure 1B shows a comparison of wild-type and *tfl2-1* plants grown for four weeks in LD conditions. Relative to wild type, internode length is shortened and the termination of the main axis by the terminal flower causes the early release of axillary shoots in *tfl2* mutants (Figure 1B). As shown in Figure 1C, *tfl2* rosette leaves are smaller than wild-type or *tfl1* mutant leaves. To determine the cause of the dwarf phenotype, the vegetative shoot meristem was examined by light microscopy of longitudinal meristem sections. No apparent differences in cell size, cell number or cellular organization were detected between mature vegetative meristems of wild-type and *tfl2* plants (data not shown).

To investigate whether the dwarf phenotype was due to disturbances at the cellular level, such as defects in cell division or cell elongation during organogenesis, we compared the epidermal cells of both abaxial and adaxial sides of the fifth rosette leaf from wild-type and *tfl2* plants. As shown in Figure 3, there were no apparent

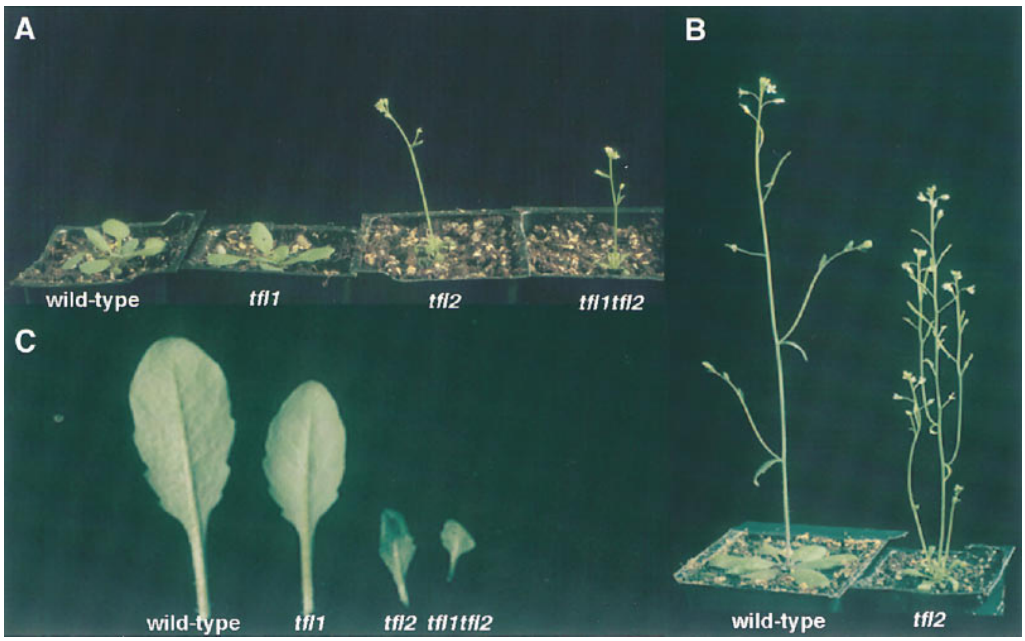

Figure 1.—Shoot phenotypes of *tfl1-1* and *tfl2-1* single mutants and the *tfl1-1 tfl2-1* double mutant. (A) Wild-type, *tfl1-1*, *tfl2-1* and *tfl1-1 tfl2-1* plants photographed 15 days after sowing. Flower buds were visible in the *tfl1-1* rosette. (B) Wild-type and *tfl2-1* plants photographed 4 wk after sowing. (C) The fifth rosette leaf from wild-type, *tfl1-1*, *tfl2-1* and *tfl1-1 tfl2-1* plants. Leaves were photographed at the time when 1-cm bolt had developed. All plants were grown under cool fluorescent light in LD photoperiods.

differences in cell shape or the distribution of stomata between *tfl2* and wild-type epidermal cells. Adaxial epidermal cells of *tfl2* leaves were measured and shown to be nearly five times smaller than the corresponding cells from wild type. The size of the fifth rosette leaf was measured and found to be approximately 20 times smaller in *tfl2* compared to wild type. Using average cell size and average leaf size measurements, the number of cells in wild-type and *tfl2* leaves was estimated to be 4.9×10^4 and 1.3×10^4, respectively. Because these estimates are within the same order of magnitude, we suggest that cell expansion rather than cell division is the main cause of the dwarf character of *tfl2* mutants.

The dwarf phenotype of *tfl2* plants appears to be confined to the shoot, as there was no statistical difference in primary root length between wild-type and *tfl2* plants, and there were no obvious differences in the development of lateral roots (data not shown). While the dwarf phenotype of *tfl2* mutants is not typical for mutations in GA metabolism and signaling, *tfl2* plants were tested for their response to exogenous GA_3. To investigate internode elongation in response to exogenous GA application, wild-type, *tfl2*, and *ga1* mutants were sprayed with GA_3 (see Wilson *et al.* 1992). The typical short internode phenotype of the *ga1* mutant was reversed by GA_3 application, while *tfl2* plants did not respond to the GA treatment (data not shown).

Trichome formation on the abaxial side of the rosette leaves is also known to be influenced by GA. For example, the production of abaxial trichomes is accelerated in the *spy* mutation (constitutive GA response) and delayed in the *ga* mutations (GA deficient) (Chien and Sussex 1996; Telfer *et al.* 1997). No significant difference was observed in the number of leaves without abaxial trichomes in wild-type and *tfl2* plants grown under LD conditions. Wild-type and *tfl2* plants both developed approximately 6 leaves prior to the appearance of abaxial trichomes (data not shown). This observation, together with the failure of *tfl2* mutants to respond to GA application, suggests that the *tfl2* mutation does not disrupt normal GA synthesis pathways.

The terminal structure of *tfl2* plants was analyzed under the dissection microscope and scanning electron microscope, and representative samples are shown in Figure 4. As shown in Figure 4, B and C, terminal flowers formed by *tfl2* mutant plants commonly develop as separate flowers with pedicels and are only occasionally sessile and fused with the last initiated lateral flower(s) (Figure 4A), as is common in *tfl1* mutants (Shannon and Meeks-Wagner 1991). Similar to *tfl1* mutants, the terminal flowers formed by *tfl2* mutants frequently show mixed organ identity, such as carpelloid sepals (Figure 4, C and H) and stamens (Figure 4D), and sometimes chimeric organs are produced (Figure 4E). In some cases the last lateral flowers show carpelloid sepals (Figure 4F) and in plants with a high degree of carpelloid organ formation in the terminal flower the structures lack petals (Figure 4, G and H). While most characteristics of *tfl2* terminal flowers occur in *tfl1* mutants, the severity and variation of floral defects is greater in the *tfl2* mutant. When grown under LD conditions, 80% of *tfl2* plants displayed well developed terminal floral

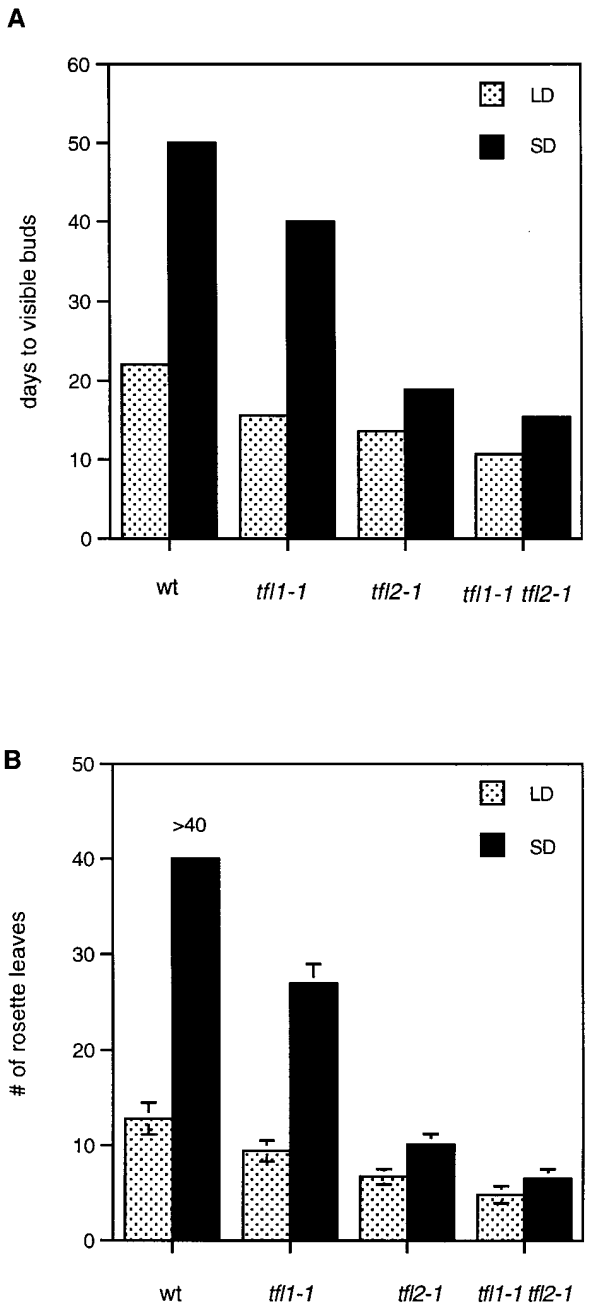

Figure 2.—Flowering time of wild-type, *tfl1-1*, *tfl2-1* and *tfl1-1 tfl2-1* plants. (A) Histogram of the number of days to visible buds. Days to visible buds is defined as the time at which 50% of the population possessed flower primordia visible to the unaided eye. (B) Histogram of number of rosette leaves at flowering. The number of true rosette leaves was determined on the day flower primordia were first observed. A statistical difference was shown between all populations using Student's *z*-test at 0.1%. Error bars represents the standard deviation from the mean. The population size varied between 35 and 43 plants for the different populations. All plants were grown under cool white fluorescent light in LD and SD photoperiods.

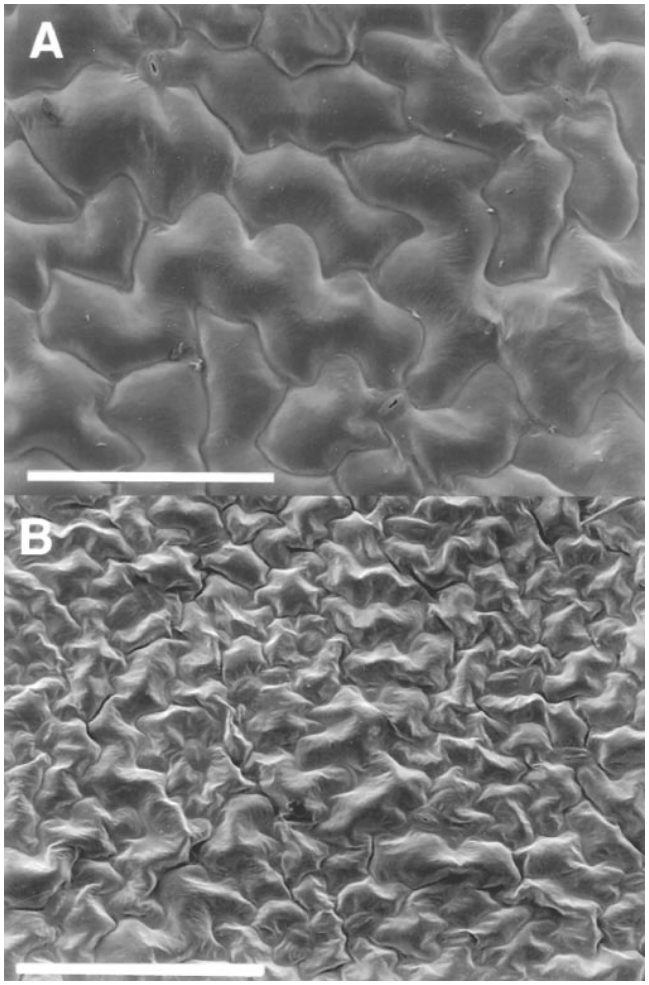

Figure 3.—Scanning electron micrographs of leaf epidermal cells. (A) The adaxial side of the fifth rosette leaf of a wild-type plant. (B) The adaxial side of the fifth rosette leaf of a *tfl2-1* plant. Leaves were fixed when the plants had developed a 1-cm bolt. Plants were grown under cool white fluorescent light in LD conditions. Bar, 100 μm.

structures. The apical region of the remaining plants underwent senescence, displaying arrested terminal floral structures (Figure 4I).

The *tfl2* mutation enhances the *tfl1* phenotype: When plants are grown under LD conditions, the *tfl1* mutation causes early flowering and premature termination of the primary shoot axis with the formation of a terminal flower. The *tfl1 tfl2* double mutant exhibits a phenotype that is more severe than either of the single mutants (Figure 1A). This is true with regard to both the timing of the floral transition and the termination of the primary shoot. Considering flowering time (expressed as either day from sowing to the development of floral primordia or the number of rosette leaves developed during the same interval of growth), the *tfl1 tfl2* double mutant shows an enhancement as compared to either single mutant (Figure 2, A and B). The extremely early termination of the main axis in plants mutant for both *tfl1* and *tfl2* is evident by examining the production of lateral floral meristems. Table 1 compares the number of lateral inflorescence meristems and lateral floral meristems produced by wild-type, *tfl1*, *tfl2* and *tfl1 tfl2* plants grown in LD conditions. On average, wild-type and *tfl2*

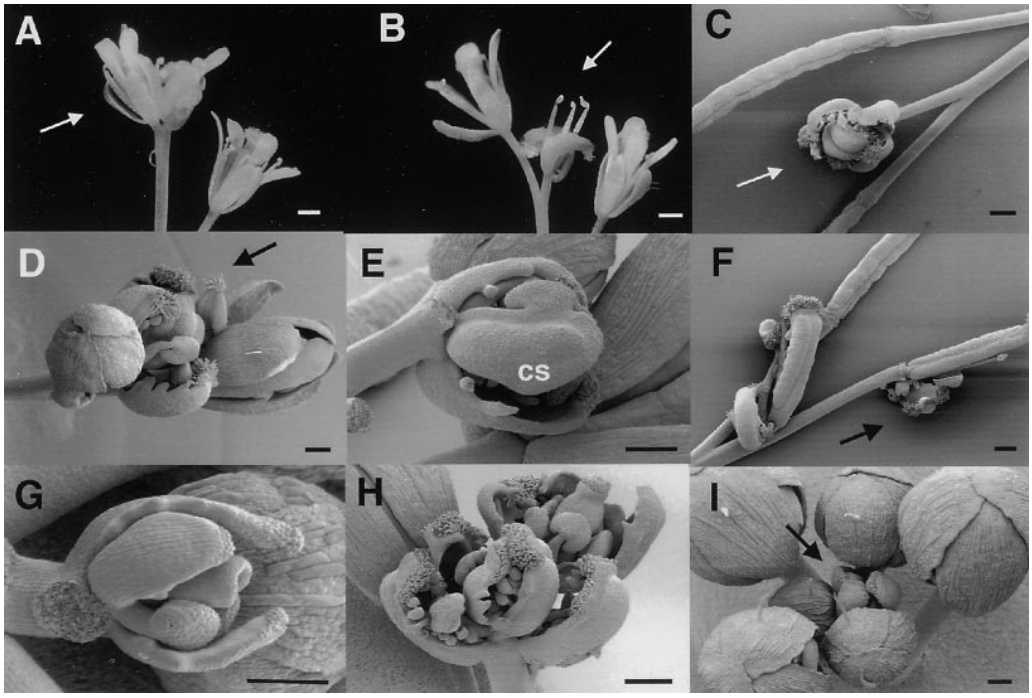

Figure 4.—Variation of the terminal structure of *tfl2-1* plants. (A) Termination without pedicel elongation resulting in a complex structure consisting of the last lateral flowers and the terminal flower (arrow). Bar, 300 μm. (B) Terminal flower with a short pedicel (arrow). The flower developed carpelloid sepals, incomplete whorls of organs and lacks carpels. Bar, 300 μm. (C) Terminal flower with a short pedicel (arrow). The terminal organs developed as carpelloid sepals. Bar, 200 μm. (D) Terminal flower with carpelloid sepals and stigmatic papillae (arrow) developing on an anther. Bar, 100 μm. (E) Terminal flower with a chimeric stamen (cs). Bar, 100 μm. (F) Last lateral flower developed carpelloid sepals (arrow). Bar, 200 μm. (G) Terminal flower lacking petals. Bar, 100 μm. (H) Terminal flower lacking petals. Bar, 100 μm. (I) Inflorescence terminus shows an arrested terminal flower (arrow). Bar, 100 μm. Plants were grown under cool white fluorescent light in LD conditions.

plants develop three lateral inflorescence meristems, whereas *tfl1* and *tfl1 tfl2* plants produce only one or two lateral inflorescences; all inflorescence meristems in *tfl1 tfl2* plants produce a single terminal flower and fail to form any lateral flowers except for those associated with the terminal structure. In addition, the terminal floral structure of *tfl1 tfl2* double mutants showed less morphological variation than in *tfl2* single mutants: regions of the terminal flower never showed pedicel elongation as seen with *tfl2* single mutants.

No evidence for genetic interactions between *TFL2* and *LEAFY*: Mutations in the *LFY* gene have a strong effect on meristem identity, causing floral meristems to form shoot-like inflorescence structures instead of floral organs. Eventually some carpelloid identity is attained in the flower-like structures initiated late during development of *lfy* mutants (Huala and Sussex 1992; Weigel *et al.* 1992) (Figure 5B). *tfl1 lfy* plants have an inflorescence architecture intermediate between the two single mutants, and near the end of inflorescence devel-

TABLE 1

A comparison of the number of lateral inflorescence and floral meristems initiated along the main axis in wild-type and mutant plants

Plant population	No. of lateral inflorescence meristems	No. of lateral floral meristems	No. of plants in the population
wild-type	2.8 ± 0.9	>30	39
tfl1–1	1.5 ± 0.5	3.5 ± 2.0	35
tfl2–1	2.9 ± 0.4	19.3 ± 1.8	39
tfl1–1 tfl2–1	1.7 ± 0.6	0	39

Mean value ± standard deviation is given. All plants were grown under cool white fluorescent light in LD conditions. Meristems subtended by a cauline leaf were scored as infloescence meristems, and meristems lacking subtending cauline leaf as floral meristems. Meristems were scored at a time when mutant plants had developed the terminal structure or wild-type plants had ceased growth.

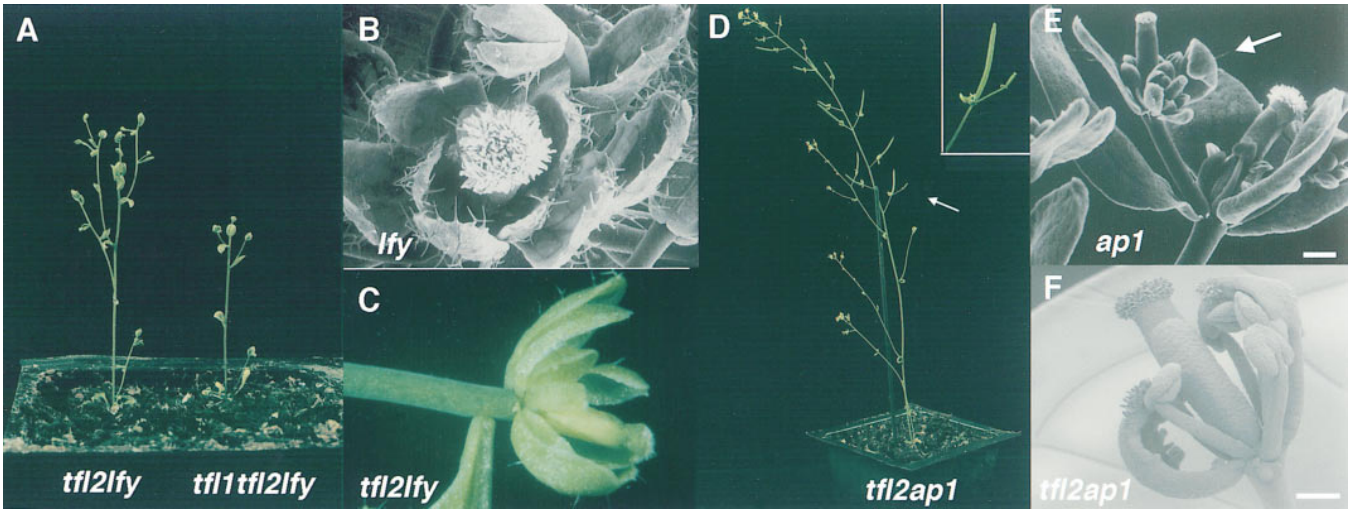

Figure 5.—Phenotypes of double and triple mutants involving *tfl2-1*, *lfy-16*, *tfl1-1* and *ap1-1*. (A) Inflorescence architecture of *tfl2 lfy* and *tfl1 tfl2 lfy* plants. (B) A *lfy* lateral flower. (C) A *tfl2 lfy* lateral flower. (D) Inflorescence architecture of a *tfl2 ap1* plant. Arrow and insert shows the only lateral flower on this plant that developed a secondary flower. (E) An *ap1* lateral flower; arrow indicates a secondary flower. Bar, 200 μm. (F) A *tfl2 ap1* lateral flower. Bar, 200 μm.

opment there is a decrease in internode length, resulting in a cluster of flowers (Shannon and Meeks-Wagner 1993). To investigate possible interactions between *TFL2* and *LFY*, double and triple mutants were constructed with the *tfl2-1*, *lfy-16* and *tfl1-1* alleles (see Figure 5A).

tfl2 lfy double mutants display an additive phenotype without any novel characteristics. The dwarfing, early flowering and terminal differentiation of the apical meristem typical of *tfl2* mutants is combined with the development of a higher number of lateral branches and the conversion of floral organs into bractlike structures or sepal/carpelloid organs as seen in *lfy* single mutants. All branches of *tfl2 lfy* plants terminate with unfused carpels or carpels fused to form a pistil (Figure 5C). Like *tfl2 lfy* mutants, the triple mutant *tfl1 tfl2 lfy* shows an additive phenotype predicted by the *tfl1 tfl2*, *tfl1 lfy* and *tfl2 lfy* double mutant phenotypes.

Interactions between *TFL2* and *AP1*: Because *AP1* is one of the genes involved in the regulation of floral meristem identity, and because the terminal flowers of *tfl2* plants frequently lack petals, a possible interaction between *TFL2* and *AP1* was investigated using the *tfl2-1* and *ap1-1* alleles. In addition to a lack of petals, *ap1* mutants develop secondary flowers arising in the axil of bractlike first whorl organs, giving individual flowers partial inflorescence meristem identity (Irish and Sussex 1990). *tfl1 ap1* or *tfl1 ap2* double mutants did not reveal any interactions between the genes. However, when the *tfl1* mutation was added to the *ap1 ap2* mutant, an interaction with the *AP1*/*AP2* pathway to floral identity was revealed (Shannon and Meeks-Wagner 1991, 1993).

Figure 5D shows an example of a *tfl2 ap1* double mutant. The apparent phenotypic effect of the addition of the *tfl2* mutation to the *ap1* background is suppression of the development of secondary flowers. We determined that the mix of ecotypes used in the construction of the double mutant did not contribute to the novel phenotype of *tfl2 ap1* plants by an analysis of flower development among sibling plants.

tfl2 ap1 plants formed secondary flowers only in 7 ± 6% of the lateral flowers whereas *ap1* single mutants formed secondary flowers in 27 ± 6% of the lateral flowers. The insert in Figure 5D shows the one secondary flower formed in the axil of a first whorl bract in this plant. Figure 5F shows a typical *tfl2 ap1* flower. In all other respects the phenotype of *tfl2 ap1* double mutants was additive, showing severe carpelloidy of first whorl organs, aborted first whorl organs, filamentous organs and lack of petals typical of *ap1* single mutants (Figure 5E) and the terminal floral organs, dwarfing and early flowering typical of *tfl2* single mutants.

DISCUSSION

The *tfl2* phenotype: Several genes have been identified in Arabidopsis that are necessary for proper development of the shoot. We suggest that *TFL2* belongs to a subset of these genes that control shoot meristem function during the vegetative-to-reproductive transition. The phenotypes of *tfl2* mutants are most similar to those displayed by *tfl1* mutants. These include early flowering, terminal flower formation, normal phyllotaxy and normal root development.

***tfl2* mutants display novel shoot phenotypes:** The similarity between aspects of the *tfl1* and *tfl2* phenotypes indicates that the corresponding gene products are both active in the processes of meristem identity and the timing of the reproductive transition. The *tfl1 tfl2* dou-

ble mutant displays a more severe phenotype than either single mutant for traits common to the two mutants (*i.e.*, timing of reproductive transition and terminal flower formation). Combining the two *tfl* mutations reveals no obvious interactions between the two genes. Therefore, assuming that both *tfl1* and *tfl2* mutations cause a severe reduction in gene function, the double mutant phenotype suggests that the TFL1 and TFL2 gene products are active in two separate pathways involved in meristem development and the transition to the reproductive phase of development. Another possible interpretation, given the pleiotropic nature of *tfl2* mutants, is that *TFL2* is active at a different, more global, regulatory level than *TFL1*, and thus *TFL2* influences several pathways.

***TFL2* mediates photoperiodic responses:** The difference in the response to photoperiod is further evidence that the two *TFL* genes are active at different stages in signaling pathways that regulate the floral transition. The nearly wild-type photoperiod sensitivity of *tfl1* mutants suggests that *TFL1* is involved in endogenous regulation of flowering time, while the decrease in photoperiod sensitivity of *tfl2* mutants suggests that *TFL2* is active in a pathway that greatly delays flowering in SD conditions. It has been suggested that the SD-dependent delay of flowering is controlled via GA biosynthesis and/or perception (Martínez-Zapater *et al.* 1994). The shortened internodes of *tfl2* mutants may indicate that GA metabolism or signaling is affected by the *tfl2* mutation. However, the nature of the dwarf phenotype and the flowering response in *tfl2* mutants is not typical for a mutant affected in GA metabolism or signaling. Furthermore, *tfl2* plants were not responsive to exogenous GA_3 treatment, and the distribution of abaxial trichomes on *tfl2* leaves was not indicative of a disturbance of GA levels. Thus, a direct involvement of *TFL2* in a GA-dependent pathway is unlikely.

The dwarf phenotype of *tfl2* plants apparently reflects the small cell size caused by the mutation, the cells being five times smaller in the mutant than in wild type. As in wild type, the epidermal cells of the leaves in *tfl2* are larger on the adaxial side compared to the abaxial side of the leaf, indicating that there are no major defects in the cellular organization or organogenesis of the vegetative meristem. This is in agreement with the cytological studies of the mature vegetative meristem which show no deviations from wild type regarding cell number, size or organization. The estimate of number of cells per leaf suggests that cell expansion, rather than cell division, is affected by the *tfl2* mutation.

The relation of *TFL2* to the floral meristem identity genes *AP1* and *LFY*: The double mutant analyses suggest that *TFL2* interacts with the pathway controlled by *AP1* but not with that controlled by *LFY*. The formation of secondary flowers in the *ap1* mutant flowers has been interpreted as an indeterminate developmental strategy being expressed by the floral meristem. The suppression of this phenomenon by the introduction of the *tfl2* mutation into the *ap1* mutant background indicates the existence of interactions between developmental pathways regulated by these two genes. In contrast, secondary flowers are still initiated in *tfl1 ap1* double mutants. Thus, mutations in *TFL2* have effects on the *ap1* phenotype distinct from those of mutations in *TFL1*, supporting that they are active in different pathways or at different levels in a cascade of gene activities.

Pleiotropic nature of *tfl2* mutants: The pleiotropy caused by mutations in the *tfl2* locus is evident in alterations in flowering time, meristem development, sensing photoperiod as well as cell expansion, and suggests that *TFL2* has a global role early in the network of regulatory pathways. Impaired response to photoperiod indicates that *TFL2* is active close to the perception of light signals. It has been suggested that pathways identified by the early flowering phytochrome chromophore mutant *hy2*, and/or the early flowering mutant *elf3*, influence the function or activity of inflorescence and/or floral meristem identity genes. This is based on the production of a terminal flower in the *elf3 hy2* double mutant (Zagotta *et al.* 1996). Mutations in the *CLF* locus also result in pleiotropic effects involving timing to floral transition as well as plant size, and it has been shown that the floral organ identity gene *AGAMOUS* is a target gene for *CLF*. It is possible that *TFL2* is active in either *ELF3* or *CLF* pathways, or in a functionally redundant pathway influencing the genes involved in making the flower.

A model for the functional relationships of *TFL1*, *LFY*, *AP1* and *AP2* has been proposed (Huala and Sussex 1992; Weigel *et al.* 1992; Shannon and Meeks-Wagner 1993). Floral meristem identity is regulated by two pathways defined by *LFY* and *AP1/AP2*, respectively. The data presented here make these genes potential targets also for *TFL2* activity with a greater impact on the *AP1/AP2* regulation. The shoot apical meristem acquires competence to respond to flowering stimuli since plants do not flower immediately upon germination in inductive conditions nor when floral meristem identity genes are overexpressed (Mandel and Yanofsky 1995; Weigel and Nilsson 1995). It has been suggested that *TFL1* is one of the components in such a process (Weigel and Nilsson 1995). Further evidence for such an interaction comes from recent work showing that ectopic expression of *AP1* and *LFY* results in a phenotype mimicking the early flowering and termination of the *tfl* mutants (Mandel and Yanofsky 1995; Weigel and Nilsson 1995). This implies that *AP1* and *LFY* activity is inhibited by one or a combination of the *TFL* genes in the wild-type plants. Furthermore, *AP1* overexpressing plants are very similar to the *tfl1 tfl2* double mutant with regard to flowering time as well as identity of lateral meristems, which supports the idea that *TFL1* and *TFL2* have distinct interactions with the *AP1* pathway in the wild-type situation.

The acceleration of the transition to the reproductive

phase in *tfl2* plants indicates that the gene product could be another factor in the regulation of meristem competence, with a possible role in mediating environmental signals since the mutation results in a severe decrease in photoperiod sensitivity. Further studies of the *tfl2* mutant and cloning of the gene will give insight into the area of meristem development and the interactions between genetic programs and environmental signals during plant development.

We thank Susan Varnum for initiating the *tfl1* suppressor / enhancer screen, Carolyn Jacobs for plant care, Gary Wife and Anette Axén for assistance in preparation for light and scanning electron microscopy, and Eve Siecinski for help in preparation of the manuscript. We also thank Detlef Weigel for helpful comments in the review of manuscript.

This work was supported by a grant from the Swedish Natural Science Research Council (B-AA / BU 10116-301) to A.S.L. and a postdoctoral fellowship from the Swedish Institute and the Sweden-America Foundation to A.S.L., and by grants from the United States Department of Agriculture National Research Initiative Competitive Grants Program (Grant No. 93-37304-9040) and the National Science Foundation (MCB-9507218) to D.R.M.-W.

LITERATURE CITED

Bell, C. J., and J. R. Ecker, 1994 Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis.* Genomics **19:** 137–144.

Bowman, J. L., 1992 Making cauliflower out of *Arabidopsis*: the specification of floral meristem identity. Flowering Newslett. **14:** 7–19.

Bradley, D., O. Ratcliff, C. Vincent, R. Carpenter and E. Coen, 1997 Inflorescence commitment and architecture in *Arabidopsis.* Science **275:** 80–83.

Chien, J. C., and I. M. Sussex, 1996 Differential regulation of trichome formation on the adaxial and abaxial leaf surfaces by gibberellins and photoperiod in *Arabidopsis thaliana* (L.) Heynh. Plant Physiol. **111:** 1321–1328.

Goodrich, J., P. Puangsomlee, M. Martin, D. Long, E. M. Meyerowitz *et al.*, 1997 A polycomb-group gene regulates homeotic gene expression in *Arabidopsis.* Nature **386:** 44–51.

Hempel, F. D., and L. J. Feldman, 1994 Bi-directional inflorescence development in *Arabidopsis thaliana*: Acropetal initiation of flowers and basipetal initiation of paraclades. Planta **192:** 276–286.

Huala, E., and I. Sussex, 1992 *LEAFY* interacts with floral homeotic genes to regulate *Arabidopsis* floral development. Plant Cell **4:** 901–913.

Irish, V. F., and I. M. Sussex, 1990 Function of the *apetala*-1 gene during *Arabidopsis* floral development. Plant Cell **2:** 741–753.

Jacobsen, S. E., and N. E. Olszewski, 1993 Mutations at the *SPINDLY* locus of *Arabidopsis* alter gibberellin signal transduction. Plant Cell **5:** 887–896.

Koornneef, M., and J. H. van der Veen, 1980 Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. Theor. Appl. Genet. **58:** 257–263.

Koornneef, M., J. van Eden, C. J. Hanhart, P. Stam, F. J. Braaksma *et al.*, 1983 Linkage map of *Arabidopsis thaliana.* J. Hered. **74:** 265–272.

Koorneef, M., A. Elgersma, C. J. Hanhart, E. P. van Loenen-Martinet, L. Rijn *et al.*, 1985 A gibberellin insensitive mutant of *Arabidopsis thaliana.* Plant Physiol. **65:** 33–39.

Kunst, L., J. E. Klenz, J. Martínez-Zapater and G. W. Haughn, 1989 *AP2* gene determines the identity of perianth organs in flowers of *Arabidopsis thaliana.* Plant Cell **1:** 1195–1208.

Mandel, M. A., and M. Yanofsky, 1995 A gene triggering flower formation in *Arabidopsis.* Nature **377:** 522–524.

Martienssen, R., and P. Springer, 1997 Enhancer and gene trap transposons in *Arabidopsis*, in *Insertional Mutagenesis: A Practical Approach*, edited by George Coupland. Academic Press, London (in press).

Martínez-Zapater, J. M, G. C. Coupland, C. Dean and M. Koornneef, 1994 The transition to flowering in *Arabidopsis*, in *Arabidopsis*, edited by E. M. Meyerowitz and C. R. Sommerville. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Medford, J. I., J. D. Callos, F. J. Behringer and B. M. Link, 1994 Development of vegetative shoot apical meristem, pp. 355–378 in *Arabidopsis*, edited by E. M. Meyerowitz and C. R. Sommerville. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Napp-Zinn, K., 1985 *Arabidopsis thaliana*, pp. 492–503 in CRC Handbook of Flowering, edited by H. A. Halevy. CRC Press, Boca Raton, FL.

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

Okamuro, J. K., W. Szeto, C. Lotys-Prass and D. Jofuku, 1996b Photo and hormonal control of meristem identity in the *Arabidopsis* flower mutants *apetala2* and *apetela*1. Plant Cell **9:** 37–47.

Schultz, E. A., and G. W. Haughn, 1991 *LEAFY*, a homeotic gene that regulates inflorescence development in *Arabidopsis.* Plant Cell **3:** 771–781.

Shannon, S., and D. R. Meeks-Wagner, 1991 A mutation in the *Arabidopsis TFL1* gene affects inflorescence meristem development. Plant Cell **3:** 877–892.

Shannon, S., and D. R. Meeks-Wagner, 1993 Genetic interactions that regulate inflorescence development in *Arabidopsis.* Plant Cell **5:** 639–655.

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

Sung, Z. R., A. Belachew, R. Shunong and R. Bertrand-Garcia, 1992 EMF, an Arabidopsis gene required for vegetative shoot development. Science **258:** 1645–1647.

Telfer, A., K. M. Bollman and R. S. Poethig, 1997 Phase change and the regulation of trichome distribution in *Arabidopsis.* Development **124:** 645–654.

Weigel, D., J. Alvarez, D. R. Smyth, M. F. Yanofsky and E. M. Meyerowitz, 1992 LEAFY controls floral meristem identity in *Arabidopsis.* Cell **69:** 843–859.

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

Wilson, R. N., J. W. Heckman and C. R. Somerville, 1992 Gibberellin is required for flowering in Arabidopsis thaliana under short days. Plant Physiol. **100:** 403–408.

Zagotta, M. T., K. A. Hicks, C. I. Jacobs, J. C. Young, R. P. Hangarter *et al.*, 1996 The *Arabidopsis ELF3* gene regulates vegetative photomorphogenesis and the photoperiodic induction of flowering. Plant J. **10**(4)**:** 691–702.

Communicating editor: D. Preuss