The Plant Cell, Vol. 9, 2011–2024, November 1997 © 1997 American Society of Plant Physiologists

EMF Genes Regulate Arabidopsis Inflorescence Development

Lingjing Chen, Jin-Chen Cheng, Linda Castle,[1] and Z. Renee Sung[2]

Department of Plant Biology, University of California, Berkeley, California 94720

Mutations in *EMBRYONIC FLOWER* (*EMF*) genes *EMF1* and *EMF2* abolish rosette development, and the mutants produce either a much reduced inflorescence or a transformed flower. These mutant characteristics suggest a repressive effect of EMF activities on reproductive development. To investigate the role of *EMF* genes in regulating reproductive development, we studied the relationship between *EMF* genes and the genes regulating inflorescence and flower development. We found that *APETALA1* and *AGAMOUS* promoters were activated in germinating *emf* seedlings, suggesting that these genes may normally be suppressed in wild-type seedlings in which EMF activities are high. The phenotype of double mutants combining *emf1-2* and *apetala1*, *apetala2*, *leafy1*, *apetala1 cauliflower*, and *terminal flower1* showed that *emf1-2* is epistatic in all cases, suggesting that *EMF* genes act downstream from these genes in mediating the inflorescence-to-flower transition. Constitutive expression of *LEAFY* in weak *emf1*, but not *emf2*, mutants increased the severity of the *emf* phenotype, indicating an inhibition of EMF activity by *LEAFY*, as was deduced from double mutant analysis. These results suggest that a mechanism involving a reciprocal negative regulation between the *EMF* genes and the floral genes regulates Arabidopsis inflorescence development.

INTRODUCTION

Arabidopsis primary shoots undergo a series of developmental phase changes as they mature and age (Schultz and Haughn, 1993). Their development can be broadly categorized into three major phases based on node morphologies: first, the rosette or vegetative phase, with nodes closely compressed and bearing a petiolated leaf and an axillary bud; second, the early-inflorescence phase, with nodes separated by internode elongation and bearing a sessile leaf and a coflorescence; and third, the late-inflorescence phase, with nodes bearing solitary flowers. Thus, two major phase transitions are involved in Arabidopsis main shoot development: the transition from rosette to early inflorescence when the rosette begins to bolt and the transition from early to late inflorescence (or from inflorescence to flower) when the primary shoot switches from producing cauline leaves and coflorescences to flowers (Schultz and Haughn, 1993). Ultimately, the primary shoot meristem becomes senescent and ceases producing flowers from its flanks (Shannon and Meeks-Wagner, 1991).

The transition from rosette to early inflorescence is considered to be the vegetative-to-reproductive transition. It is regulated by many flowering-time genes, that is, floral repression and floral promotion genes (or early- and late-flowering genes, respectively) (Koornneef et al., 1991; Zagotta et al., 1992). Loss-of-function mutations in floral repression genes, such as *EARLY FLOWER 1 (ELF1)*, cause early flowering, whereas mutations in floral promotion genes, such as *CONSTANS (CO)*, delay transition from the rosette-to-inflorescence stage (Koornneef et al., 1991). In addition, two *EMBRYONIC FLOWER (EMF)* genes, *EMF1* and *EMF2*, are proposed to be involved in this process as floral repressors, suppressing the onset of reproductive development (Sung et al., 1992; Martinez-Zapater et al., 1994; Castle and Sung, 1995; Yang et al., 1995). Based on this floral repressor concept, vegetatively growing plants must decrease EMF1 and EMF2 activities to initiate reproductive growth. It has been proposed that the floral repression genes maintain whereas floral promotion genes inhibit EMF1 and EMF2 activities. A balance of these gene actions would cause a gradual decline in EMF activities and determine the time of vegetative-to-reproductive transition (Castle and Sung, 1995; Yang et al., 1995).

The transition from inflorescence to flower is regulated by flower meristem identity genes, such as *LEAFY (LFY)*, *APETALA1 (AP1)*, *AP2*, and *CAULIFLOWER (CAL)* (Irish and Sussex, 1990; Mandel et al., 1992; Bowman et al., 1993; Jofuku et al., 1994). Mutants with defective *LFY*, *AP1*, *AP2*, or *AP1 CAL* genes are impaired in flower initiation; thus, inflorescence-like or flowerlike shoots, instead of flowers, initiate peripherally from the apical meristem during the late-inflorescence phase. In addition to these genes, the *TERMINAL FLOWER1 (TFL1)* gene is reported to negatively regulate meristem identity gene function in inflorescence development. Both the primary shoot and the lateral shoots in *tfl1*

[1] Current address: Novartis Crop Protection, Inc., Palo Alto Research Center, 975 California Avenue, Palo Alto, CA 94304.

[2] To whom correspondence should be addressed. E-mail zrsung@nature.berkeley.edu; fax 510-642-4995.

mutants terminate in a flower, reflecting a precocious inflorescence-to-flower transition (Alvarez et al., 1992). Molecular data have shown that the *LFY* gene is ectopically expressed in the entire apical meristem of *tfl1* primary and lateral shoots, which is consistent with the *tfl1* phenotype (Bradley et al., 1997). Thus, *TFL1* functions to maintain inflorescence development. Mutants impaired in *EMF1* or *EMF2* produce a reduced inflorescence and a terminal flower, indicating a role for the *EMF* genes in delaying the inflorescence-to-flower transition.

The development of Arabidopsis floral organs also depends on normal *EMF* gene function. As in *ap1* and *ap2* mutants, weak *emf* mutants, such as *emf1-1* and all of the *emf2* mutants, lack petals (Yang et al., 1995). The strong *emf* mutant, *emf1-2*, is impaired in the development of all floral organs: only carpelloid organs form (Yang et al., 1995). The effects of *emf* mutations on inflorescence and flower development suggest that *EMF1* and *EMF2* continue to function during reproductive development.

To understand the role of *EMF1* and *EMF2* during reproductive development, we (1) further characterized the *emf* mutant phenotypes, including that of a new allele *emf1-3*, (2) examined the expression of *AP1* and *AGAMOUS* (*AG*) in *emf* mutants, (3) constructed and analyzed double mutants combining *emf* and mutants defective in inflorescence-to-flower transition, and (4) generated and analyzed *emf* plants harboring a chimeric construct of the cauliflower mosaic virus 35S promoter fused to a *LFY* gene (35S::*LFY*) (Weigel and Nilsson, 1995). Our results show that *EMF* gene activities regulate inflorescence development through their interaction with floral genes. To explain the role of EMF activity in regulating multiple phase transitions, we propose that during rosette and early-inflorescence development, the floral genes, such as *AP1* and *AG*, are suppressed by high *EMF* gene activities, which decline as the plant develops. In late-inflorescence development, the low level of EMF activities allows the expression of floral genes, which subsequently repress EMF activities. Our findings indicate a reciprocal negative regulatory relationship between the *EMF* genes and some of the floral genes during inflorescence development.

RESULTS

Transition from the Inflorescence-to-Flower Phase Is Altered in *emf* Mutants

Reproductive Development in emf *Mutants with Weak Phenotypes*

emf mutants germinated as seedlings lacked petiolated rosette leaves, indicating the possible loss of vegetative growth (Sung et al., 1992; Bai and Sung, 1995; Yang et al., 1995). Besides the defect in rosette leaf development, *emf* mutants were also abnormal in reproductive development. *emf1-1* and the six *emf2* mutants had similar phenotypes, as shown in Figures 1A, 1B, 1G, and 2F. Here, we describe the characteristics of reproductive development in *emf* mutants with weak phenotypes, using *emf1-1* as an example.

Homozygous *emf1-1* seedlings grown under short- or long-day conditions produced a short stem, ~1 to 5 mm in height, with approximately five or six sessile leaves on it. The length of the leaves varied from 0.3 to 1.5 mm. As for the wild-type plants shown in Figure 2A, all of the leaves were flanked by tiny stipules at their base (Figure 2B).

During early-inflorescence development, *emf1-1* plants often produced one to three solitary flowers in the axils of upper cauline leaves (Figure 2F), whereas in wild-type plants, instead of solitary flowers, coflorescences were produced. The ability to form flower buds decreased basipetally. Seldom did a flower develop in the axil of lower leaves before the plant became senescent. During late-inflorescence development, *emf1-1* produced one to four flowers that were not subtended by cauline leaves. In contrast to the wild type, the inflorescence of *emf1-1* terminated in a flower (Figures 1A and 2F) or sometimes a fused floral cluster (Figure 1B).

Flower development in *emf1-1* was also affected, as indicated by the apparent apetalous phenotype (Figure 1A). Both stamens and pistils were sterile. In the homozygous mutant population, some plants did not blossom. Their flower buds turned yellow and ceased to enlarge at early developmental stages as a result of early senescence (Bai and Sung, 1995). *emf2* plants showed the same phenotypes as *emf1-1* (Figure 1G). The accelerated floral formation in leaf axils and shoot apex showed that, like *tfl* mutants grown in long-day conditions (Shannon and Meeks-Wanger, 1991), weak *emf* mutants are affected in the early- to late-inflorescence transition even under short-day conditions.

Characteristics of the Strong emf *Mutant Phenotype*

emf1-2 displayed strong mutant phenotypes. No *emf2* mutants with strong phenotypes as *emf1-2* were found. Bhatt et al. (1996) described an *emf*-like (*leafless*) mutation from a transposon mutagenesis experiment using a modified autonomous *Activator* (*Ac*) element. Our complementation result showed that it was allelic to *emf1* mutants (data not shown). Thus, it was named *emf1-3*. The phenotype of *emf1-3* (Figures 1E, 1F, 2D, and 2E) was similar to that of *emf1-2* (Figures 1C and 1D) (Yang et al., 1995). Like *emf1-2*, *emf1-3* seedlings required more time to germinate than did *emf1-1* or *emf2* mutants. As was the case with all *emf* mutants, *emf1-3* had sessile cotyledons, lacked rosette leaves, had a short hypocotyl, and had normal roots. Like *emf1-2*,

stigmatic papillae developed at the base of cotyledons and all sessile leaf primordia, indicating the carpelloid nature of the primordia. Additional characteristics of the two strong *emf* alleles are described below.

Upon germination, cotyledons of the mutants underwent reproductive differentiation. Stigmatic papillae appeared at the lower margin of the cotyledons as early as 4 days after germination. Ovulelike structures might develop from the adaxial surface at the base of the cotyledons. Those ovulelike structures sometimes developed into tubular structures, with a clear junction connecting the integument and the elongated nucellus (Figure 2C).

Phenotypic variations existed among *emf1-2* and *emf1-3* plants. Three types of shoot were observed: (1) a much-reduced inflorescence (Figures 1E and 2D), (2) a transformed flower (Figure 1D), and (3) a pistil (Figures 1C and 1F). In the first type, the shoot was composed of several conspicuous carpelloid structures that sometimes surrounded a transformed terminal flower. The carpelloid structures were sessile and developed stigmatic papillae cells on the top and ovulelike structures on the edges. The ovulelike structures sometimes developed into a tubular structure, with the differentiation of stigmatic papillae cells at the tips. In the axils of the carpelloid structures, one small, poorly developed and highly carpelloid flower occasionally developed. In addition to the carpelloidy feature, no trichomes formed on the organs and no stipules flanked their bases (Figure 2C). The transformed terminal flower was composed of one whorl of carpelloid sepals, one whorl of reduced stamens, which sometimes became carpelloid, and a pistil that was often an open carpel. Pedicels were not always present below the terminal flowers.

In the second type, the shoot consisted of a single transformed flower with one whorl of carpelloid sepals, one whorl of reduced stamen, and one prominent pistil (Figure 1D). The carpelloid sepals developed stigmatic papillae cells at their tips. In the third type, the shoot was composed of only one pistil: no other organs formed between the pistil and cotyledons (Figures 1C and 2E). Variations in the pistil phenotype included carpel development inside the pistil, which could burst out of the top of the pistil and form a stack of carpelloid structures (Figure 1F). These features confirmed the absence of vegetative growth and the acceleration of flower development in *emf* mutants.

The absence of stipules and trichomes on *emf1-2* and *emf1-3* lateral organs is consistent with their carpelloid nature and origin as floral organs. Stipules are the tiny organs that flank the base of wild-type rosette and cauline leaves, but they are absent from that of the floral organs (Bowman, 1994; Medford et al., 1994). In mutants that display floral reversion, for example, *ap1-1*, *ap2-1*, and *lfy*, stipules develop at the base of "sepals" as these sepals revert to cauline leaf-like structure (Smyth et al., 1990; Bowman et al., 1991; Huala and Sussex, 1992). Thus, the absence of stipules indicates floral organ identity. Similarly, trichomes are not found on stamens and pistils of wild-type plants.

AP1 and *AG* Genes Are Precociously Expressed in *emf* Mutants

emf mutants showed acceleration in the inflorescence-to-flower transition. The carpelloid lateral organs of *emf* strong mutants suggested that EMF activities suppress the expression of floral genes in the early stages of shoot development. To investigate this possibility, we examined the expression of *AP1* and *AG* in *emf* mutants.

AP1::GUS *Expression in* emf *Mutants*

In wild-type Arabidopsis, *AP1* RNA is first detected in young floral primordia as soon as they are visible on the flanks of the inflorescence meristem (Mandel et al., 1992). As flower development progresses, *AP1* gene expression is localized to the developing sepals and petals (Mandel et al., 1992). To study the temporal and spatial expression of *AP1* in *emf* mutants, we produced transgenic *emf1-1*, *emf1-2*, and *emf2-3* plants harboring a chimeric construct of the *AP1* promoter fused to a β-glucuronidase gene (*AP1::GUS*).

In *emf1-1* and *emf2-3* transgenic plants, *AP1::GUS* activity became detectable as early as 5 days after germination. Both shoot meristem and leaf primordia were GUS-positive, as shown in Figure 3A. As the leaves expanded, GUS activity was lost in the developing leaves. However, the subsequent leaf primordia were again GUS-positive (Figures 3B and 3F). In contrast, the parental *AP1::GUS* seedlings were GUS-negative during this time (Figure 3E). During flower formation, unlike the expression pattern in wild-type plants, *AP1* expression in the mutants was not limited to the perianth whorl (*emf* mutants lack petals) but was seen in the stamen and pistil primordia as well (Figure 3G).

In *emf1-2*, *AP1::GUS* activity was first detected in 4-day-old seedlings (Figure 3C). In addition to the shoot apex, the hypocotyl and cotyledons were also GUS-positive (Figure 3C). With the development of the lateral organs, GUS activity disappeared in the hypocotyl and cotyledons of 10-day-old *emf1-2* seedlings. However, the emerging shoot apical tissues were GUS-positive (Figure 3D). When the lateral organs differentiated into carpelloid structures, GUS activity was lost at their tips where the stigmatic tissue developed. Ectopic *AP1* expression in both weak and strong *emf* mutants suggested that *AP1* expression normally may be suppressed by high levels of EMF1 and EMF2 activities. The temporal difference in *AP1* expression between the strong and weak *emf1* mutants is consistent with the notion of EMF decline during shoot development (Yang et al.,1995). The amount of EMF1 activity in *emf1-1* cotyledons is probably higher than that in leaf primordia, so *AP1* expression is inhibited.

AG *Gene Expression in* emf *Mutants*

AG ectopic expression was found in the carpelloid sepals in *ap2* mutants (Bowman et al., 1991; Weigel and Meyerowitz,

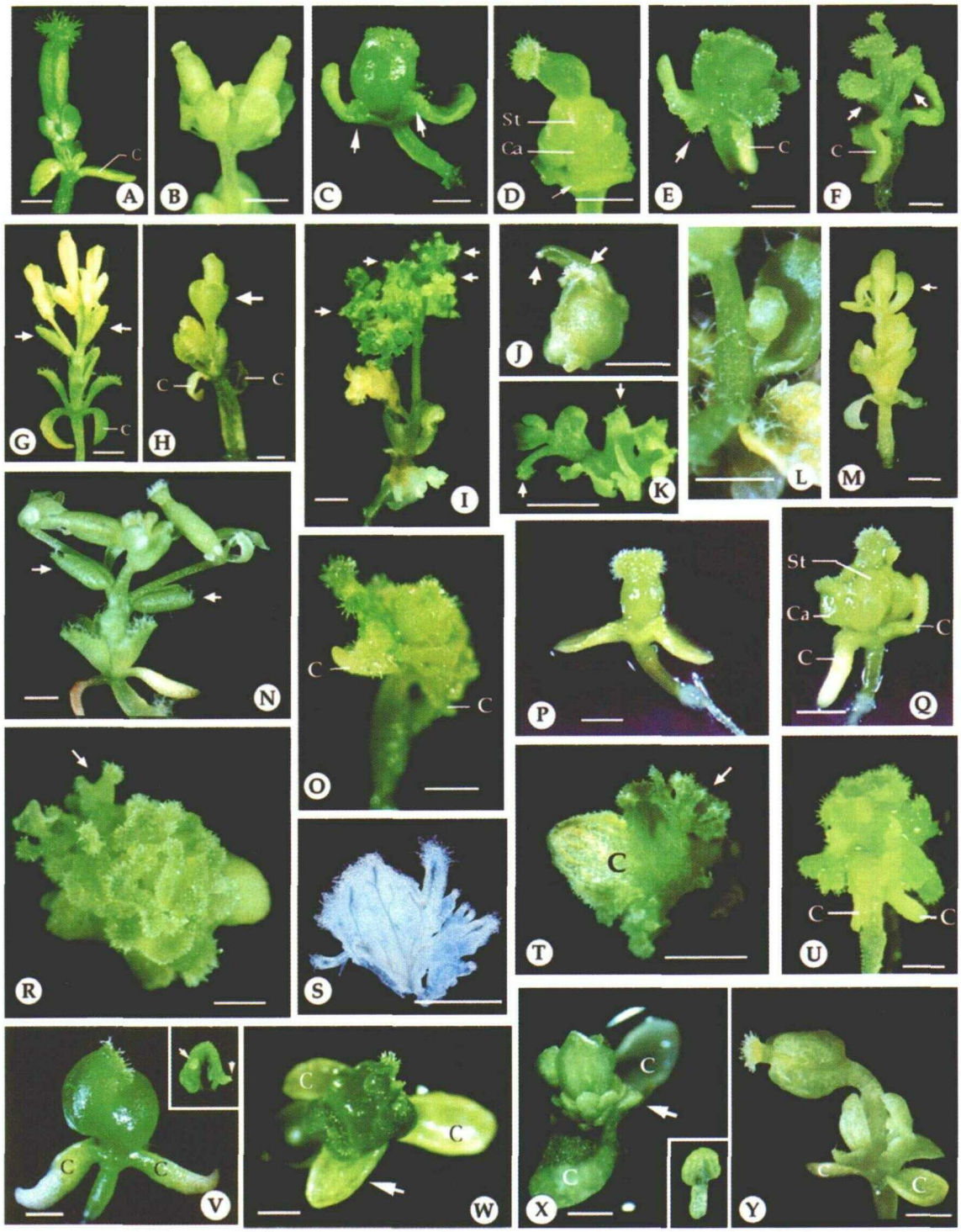

Figure 1. *emf* Single and Double Mutants and Transgenic Plants.

(A) A 25-day-old *emf1-1* seedling showing that the primary shoot terminated in an apetalous flower.
(B) A fused flower on shoot apex of a 22-day-old *emf1-1* plant.
(C) A 25-day-old *emf1-2* plant with a pistil developed upon germination and stigmatic tissue (arrows) developed at the base of the cotyledons.
(D) A 25-day-old *emf1-2* plant with a pistil surrounded by carpelloid structures and reduced stamens. The arrow indicates a cotyledon.
(E) A 25-day-old *emf1-3* plant showing carpelloid structures. The arrow indicates the stigmatic tissue developed at the base of cotyledons.

1994). To investigate the molecular basis of the *emf* mutant phenotype, we examined *AG* expression in *emf* mutants by using in situ RNA hybridization.

In wild-type plants, *AG* transcripts were first detectable in the floral meristem after the emergence of sepal primordia. As organogenesis progressed, *AG* gene expression was restricted to developing stamens and carpels, as shown in Figures 4A and 4B. Later, *AG* transcripts decreased in the ovary wall and remained in the ovules. In *emf1-1* and *emf2-3* plants grown under short-day conditions, flower buds appeared 10 days after germination. *AG* was expressed in the developing stamen and pistil as in wild-type plants. But in general, the signals were weaker than that in wild-type flowers (Figures 4I and 4J). No expression of *AG* was detected in cotyledons, hypocotyls, stems, and sessile leaves.

In *emf1-2*, there were no detectable *AG* transcripts in the shoot apical meristem of 5-day-old plants (Figures 4C and 4D). *AG* transcripts became detectable after the primordia of the carpelloid structures were formed. With the differentiation of the primordia into carpelloid structures, the transcription of *AG* continued (Figures 4E and 4F). The region of cotyledon that would develop papillae or ovulelike protrusions also showed *AG* expression (Figures 4G and 4H). As the plants aged, *AG* expression subsided from the carpelloid structures. The temporal pattern of *AG* expression in the carpelloid structures of *emf1-2* was similar to that in wild-type pistils (Drews et al., 1991). The precocious and ectopic *AG* expression in the lateral organs was consistent with their carpelloid features and suggests negative regulation of *AG* by *EMF* directly or indirectly. Expression of both *AP1* and *AG* was affected in *emf* mutation, suggesting that the wild-type level of EMF activity in early developmental stages suppressed multiple aspects of the floral program.

Relationship between *EMF* and Genes Regulating the Inflorescence-to-Flower Transition

As Arabidopsis undergoes transition from early- to late-inflorescence development, the shoot produces flowers instead of another shoot, the coflorescence. As a result, solitary flowers form during the late-inflorescence stage. The levels of *LFY*, *AP1*, *AP2*, and *CAL* RNA are increased at this time to promote flower initiation (Mandel et al., 1992; Weigel et al., 1992; Okamuro et al., 1993; Yanofsky, 1995). In contrast, *EMF* gene activities seem to retard flower initiation.

Figure 1. (continued).

(F) A 25-day-old *emf1-3* plant. Arrows indicate the primary pistil, which is split at the base because of the emergence of a secondary pistil.
(G) A 25-day-old *emf2-3* plant showing a terminal flower and two solitary flowers in the axils of cauline leaves (arrows).
(H) A 22-day-old *emf1-1 ap2-1* seedling. Sepals of the terminal flower (arrow) were similar to that of *emf1-1* but not of *ap2-1*.
(I) A 30-day-old *emf1-1 ap2-1* plant. Arrows point to the sepals that were severely lobed and tipped with stigmatic tissue.
(J) An upper cauline leaf of a 30-day-old *emf1-1 ap2-1* plant. Arrows point to the stigmatic tissue developed at the tips of the lobes.
(K) A sepal of an *emf1-1 ap2-1* flower. Arrows point to the stigmatic tissue developed at the tips of the lobes.
(L) A 30-day-old *emf1-1 ap2-1* plant with a solitary flower developed from the axil of a lower sessile leaf. The sepals were not bractlike, as with *ap2-1*.
(M) A 22-day-old *emf1-1 ap1-1* plant showing a flower (arrow) terminating the inflorescence. No secondary flower developed in the axils of the sepals.
(N) A 30-day-old *emf1-1 ap1-1* plant showing solitary flowers in the axils of cauline leaves and no secondary flowers developed in the axils of sepals. Arrows indicate the cauline leaves.
(O) A 30-day-old *emf1-2 tfl 1-1* plant showing that only carpelloid structures developed after germination.
(P) A 22-day-old *emf1-2 ap1-1* plant showing that only one pistil developed after germination, as was the case for *emf1-2* shown in **(C)**.
(Q) A 22-day-old *emf1-2 ap1-1* plant. A flowerlike shoot developed after germination. The central pistil of the flowerlike shoot was surrounded by reduced stamens and carpelloid structures, similar to that of *emf1-2* shown in **(D)**.
(R) Top view of a 30-day-old *emf1-2 ap2-1* plant. The lower lateral carpelloid organs (arrow) became repeatedly lobed.
(S) A lateral carpelloid organ of the *emf1-2 ap2-1* plant. The carpelloid structrure was cut off from the plant and entirely stained with toluidine blue O (0.05% in benzoate buffer).
(T) A cotyledon of a 30-day-old *emf1-2 ap2-1* plant. The arrow points to the lobed basal edge.
(U) A 22-day-old *emf1-2 ap1-1 cal* plant. Only carpelloid structures were developed after germination.
(V) A 25-day-old 35S::*LFY emf1-1* plant differentiated stigmatic tissues on the sessile leaves but not cotyledons. At upper right, a side view of one of the carpelloid structures is shown. Arrows point to two ovules borne on the edges of the carpelloid structure. The ovule on the right developed stigmatic papillae cells at the tip (arrow).
(W) A 25-day-old 35S::*LFY emf1-1* plant with two cotyledons, one sessile leaf (arrow), and several carpelloid organs.
(X) A 25-day-old 35S::*LFY emf1-1* plant with 16 reduced stamens and a central pistil. The arrow and box at bottom right indicate the first reduced stamen produced after germination.
(Y) A 25-day-old 35S::*LFY emf2-3* plant. All of the sessile leaves were like those of *emf2-3* single mutants and were not transformed. The ovary with a long gynophore below was shorter and thicker than that of *emf2-3* plants.
C, cotyledon; Ca, carpelloid sepal; St, reduced stamen. Bars = 0.5 mm.

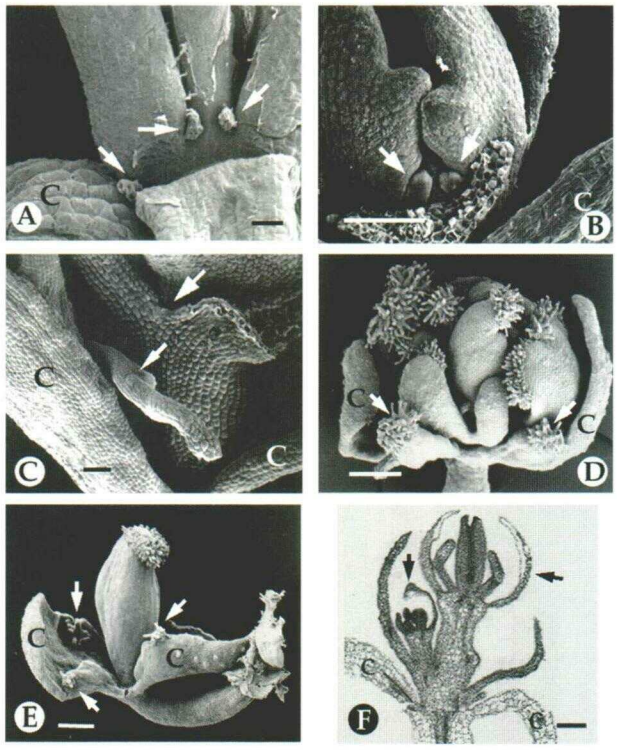

Figure 2. Scanning Electron Microscopy and Light Microscopy of the Wild-Type Columbia and *emf* Mutants.

(A) Scanning electron microscopy (SEM) of a 12-day-old Columbia wild-type plant. The arrows indicate stipules flanking the petiole bases.
(B) SEM of a 12-day-old *emf1-1* seedling. The arrows point to stipules flanking the base of sessile leaves.
(C) SEM of a 30-day-old *emf1-2* seedling. The large arrow points to the stipuleless base of a carpelloid structure. A transformed ovule showing an elongated funicle and nucellus developed from the base of a cotyledon. The small arrow indicates the junction between the nucellus and integument.
(D) and **(E)** SEM of 25-day-old *emf1-3* mutants. The arrows indicate the stigmatic tissue developed at the base of the cotyledon.
(F) Longitudinal section of a 15-day-old *emf1-1* seedling. The arrows indicate a terminal (right) and an axillary flower (left), respectively.
C, cotyledon. Bars = 100 μm.

To study the relationship between *EMF* and these genes in regulating inflorescence and flower development, we performed double mutant analysis.

Double Mutants with Weak emf *Alleles*

In this study, three weak phenotype mutants, *emf1-1*, *emf2-2*, and *emf2-3*, were crossed with *ap1-1*, *ap1-1 cal*, and *ap2-1* plants, and double mutants were obtained as described in Methods.

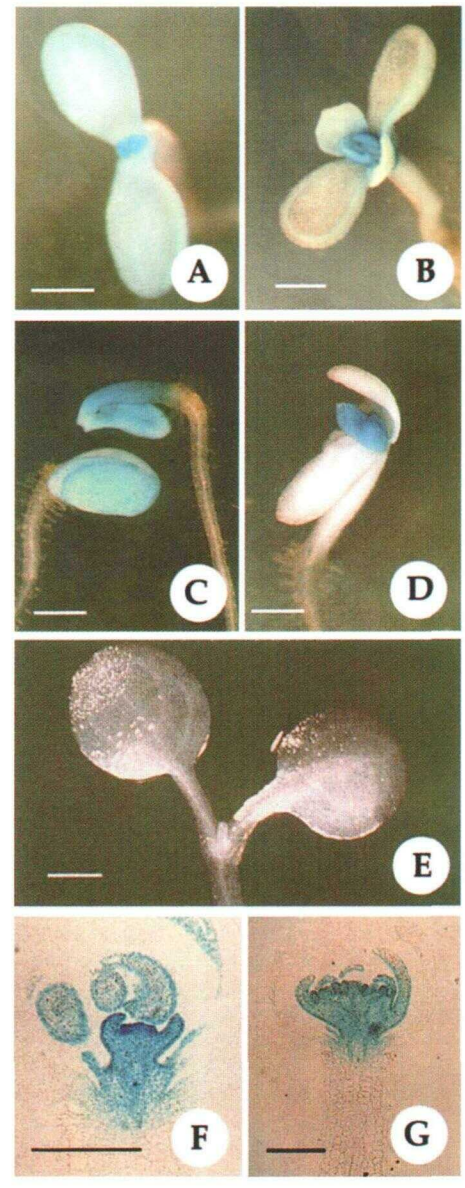

Figure 3. GUS Activity in *emf1-1 AP1*::*GUS*, *emf1-2 AP1*::*GUS*, and *AP1*::*GUS* Plants.

(A) A 5-day-old *emf1-1 AP1*::*GUS* seedling showing GUS-positive shoot apex and GUS-negative cotyledons.
(B) A 10-day-old *emf1-1 AP1*::*GUS* seedling showing the GUS-positive shoot apex and GUS-negative leaves as they expanded and matured.
(C) Two 4-day-old *emf1-2 AP1*::*GUS* seedlings showing GUS-positive cotyledons and hypocotyl.
(D) A 10-day-old *emf1-2 AP1*::*GUS* seedling. GUS activity was lost in expanding cotyledons, whereas the shoot apex, including the developing carpelloid organs, showed GUS activity.
(E) A 5-day-old *AP1*::*GUS* plant without detectable GUS activity.
(F) and **(G)** Longitudinal sections of a GUS-positive inflorescence meristem **(F)** and three floral meristems **(G)** of *emf1-1 AP1*::*GUS* plants.
Bars = 0.5 mm in **(A)** to **(E)** and 100 μm in **(F)** and **(G)**.

In general, double mutants resembled the *emf* parent, as indicated in Figures 1H, 1M, 1N, 5, and Table 1. There was little difference between double mutants constructed with *emf1-1* and with *emf2-2* or *emf2-3*. Like the *emf* parents, the double mutant seedlings displayed oval-shaped, sessile cotyledons and short hypocotyls. The inflorescence of double mutants also displayed the *emf* phenotype in producing solitary flowers in the leaf axils and terminal flowers (Figures 1L and 1N). Unlike the *ap1-1* parent plants (Irish and Sussex, 1990; Bowman et al., 1993), no secondary flowers developed inside of the flowers of the *emf ap1-1* double mutants, and no extra coflorescences without subtending cauline leaves were found on the *emf ap1-1* inflorescences. Unlike the *ap1-1 cal* parent (Bowman, 1992), no massive proliferation of meristems was found to replace the flower of *emf1-1 ap1-1 cal* or *emf2-2 ap1-1 cal* mutants. Unlike *ap2-1* parents (Bowman et al., 1991), no bractlike sepals were found on the *emf1-1 ap2-1* or *emf2-3 ap2-1* double mutants. The effect of *ap1-1* and *cal* in floral reversion may be seen only in the form of internode elongation between sepals in some flowers of *emf1-1 ap1-1* and *emf1-1 ap1-1 cal* double mutants. These cases were found in axillary flowers in the early-inflorescence phase. No internode elongation was found between sepals of *emf2-2 ap1-1 cal* and *emf2-3 ap1-1* flowers. The floral organs of double mutants were generally similar to that of *emf* parents. For example, both stamens and pistils were sterile. However, the role of *AP1* and *AP2* in specifying sepal identity was visible in sepals, which became carpelloid in some flowers (Table 1).

In the population of *emf1-1 ap2-1* double mutants, there was a fraction, <5%, of the plants (Figure 1I) that produced an inflorescence twice the length of the *emf* parent and cauline leaves, which were larger than average *emf* leaves. Sepals on this 5% of double mutant flowers were severely carpelloid. Their margins lobed repeatedly, and filamentous structures sometimes differentiated from them (Figure 1K). Both lobes and filamentous structures developed stigmatic papillae at their tips. No petals formed on the flowers. Stamens also became carpelloid. The pistil frequently developed as an open carpelloid structure with many small carpelloid ovules. In this small population of plants, the *ap2-1* mutation caused sepals and cauline leaves (Figure 1J) of weak *emf* mutants to become more carpelloid. The reason for the appearance of the extreme phenotype in the *emf1-1 ap2-1* population is not clear. The appearance of this phenotype may be due to the multiple roles *AP2* plays in development and the weak allele used in the experiment.

Double Mutants with emf1-2

To further investigate *emf* epistasis, double mutants combining a strong *emf* mutant, *emf1-2*, and *lfy1-1*, *ap1-1*, *ap1-1 cal*, *ap2-1*, or *tfl1-1* were constructed as described in Methods. The double mutants of *emf1-2 lfy-1*, *emf1-2 ap1-1*, *emf1-2 ap1-1 cal*, *emf1-2 ap2-1*, and *emf1-2 tfl1-1* were

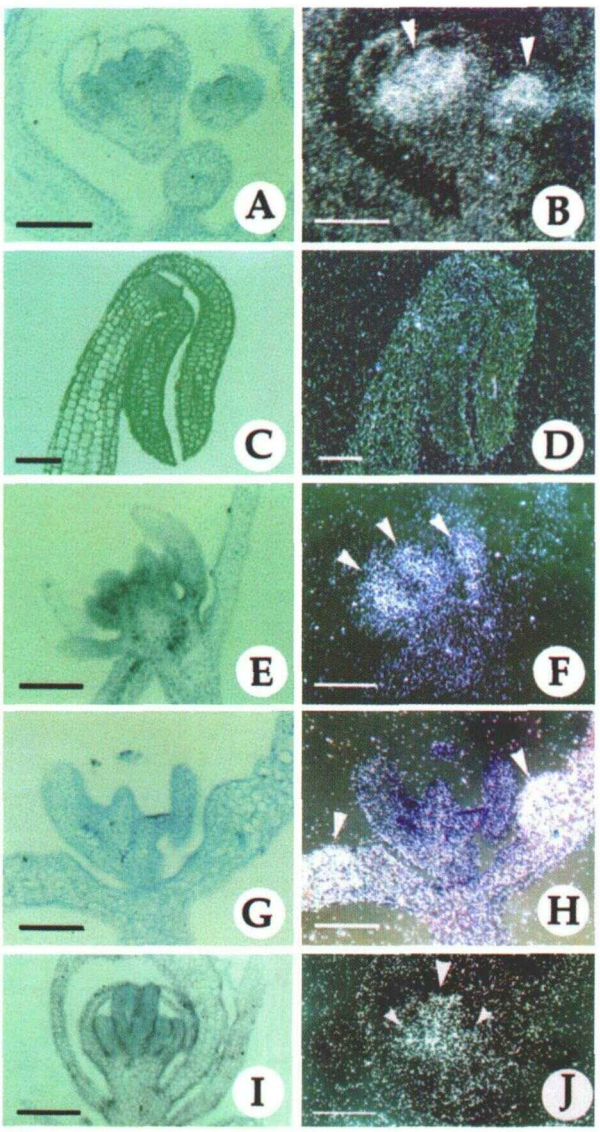

Figure 4. Expression of the *AG* Gene in Wild-Type Columbia Plants and *emf1* Mutants.

Sections in **(A)**, **(C)**, **(E)**, **(G)**, and **(I)** were photographed using bright-field optics, and **(B)**, **(D)**, **(F)**, **(H)**, and **(J)** were photographed using dark-field optics. Bars = 100 μm.

(A) and **(B)** Wild-type flower buds. The arrowheads point to *AG* transcripts accumulated in stamen and pistil primordia (left) and floral meristem of a stage 4 flower bud (right).

(C) and **(D)** A 5-day-old *emf1-2* seedling with no detectable *AG* transcripts.

(E) and **(F)** A 13-day-old *emf1-2* seedling. The arrowheads indicate *AG* transcripts on the developing carpelloid organs.

(G) and **(H)** A 9-day-old *emf1-2* seedling. The arrowheads indicate *AG* transcripts accumulated at the base of cotyledons where ovule-like structures or stigmatic tissue would develop.

(I) and **(J)** A 15-day-old *emf1-1* seedling. The arrowheads indicate *AG* transcripts accumulated in the developing stamens (small arrowheads) and pistil (large arrowhead).

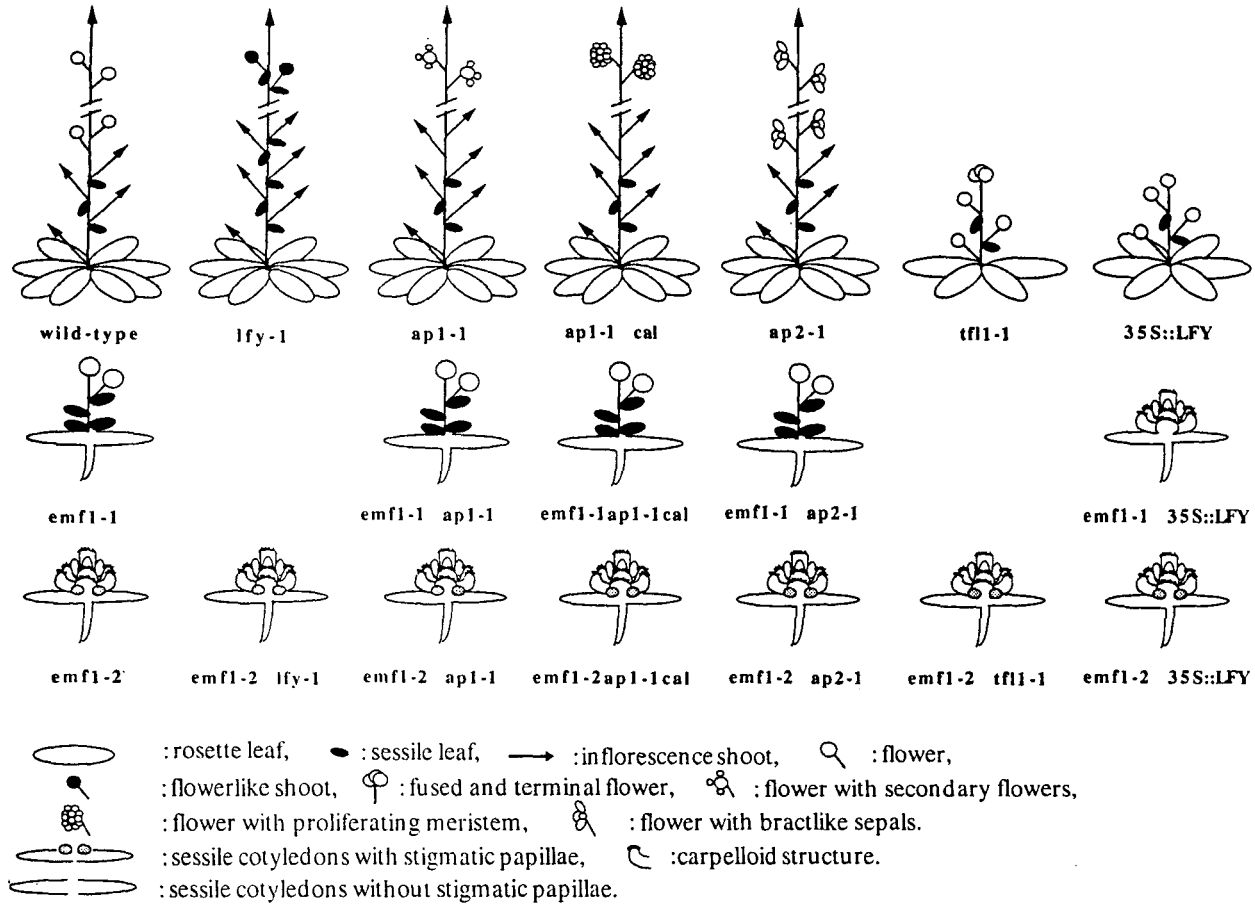

Figure 5. Diagrammatic Representation of *emf* Double Mutant Phenotypes.

similar to the *emf1-2* parent (Figures 1O to 1R, 1U, 5, and Table 1). Like *emf1-2* mutants, all double mutants lacked rosette growth. They developed several carpelloid structures, with or without a transformed terminal flower, and then ceased shoot development. Thus, *emf1-2* was epistatic to *lfy-1*, *ap1-1*, *ap2-1*, and *tfl1-1* with respect to flower initiation, suggesting that *EMF1* acted downstream from these meristem identity genes and was regulated by them in mediating the transition from inflorescence to flower development.

With respect to the lateral organs, a small fraction, ~8%, of the *emf1-2 ap1-1* double mutants had larger carpelloid organs than that of *emf1-2*. But this did not have a profound impact on overall shoot morphology of the double mutants. However, enlarged carpelloid structures occurred in ~70% of the *emf1-2 ap2-1* double mutants. Because of unrestrained marginal growth, the carpelloid structures, especially the basal ones, were enlarged and lobed (Figures 1R and 1S). All of these structures developed stigmatic papillae at their tips, causing the shoot to look like a fuzzy green ball. The cotyledons were affected too. Lobes with stigmatic papillae at the tips were produced at the base of the cotyledons (Figure 1T). These results are similar to those of double mutants with the weak *emf* mutants in that the *ap2-1* mutation had a stronger impact than other mutations on the carpelloidy of *emf* lateral organs. This might be explained by the more ubiquitous role of *AP2* that extends beyond the regulation of flower initiation or by its cadastral role in defining the *AG* expression domain (Jofuku et al., 1994; Weigel and Meyerowitz, 1994).

LFY Negatively Regulates *EMF1* Activity

To seek further evidence supporting the notion of negative regulation of *EMF* by floral genes, we introduced a chimeric construct of a *LFY* gene fused to the 35S cauliflower mosaic virus promoter (35S::*LFY*) into *emf1-1*, *emf1-2*, and *emf2-3* mutants, as described in Methods, and analyzed the impact of 35S::*LFY* on the *emf* phenotype. Transgenic 35S::*LFY* plants flowered earlier than did wild-type plants under short-

day conditions. Their coflorescences were converted to solitary flowers, and primary shoots terminated in terminal flowers (Weigel and Nilsson, 1995).

emf1 *Mutants Harboring 35S::*LFY *Constructs*

35S::*LFY emf1-1* plants germinated like *emf1-1* parents. The seedlings displayed oval-shaped and sessile cotyledons and short hypocotyls. However, the lateral organs produced from the embryonic shoot meristem developed differently from those of the *emf1-1* parents. In contrast to *emf1-1* sessile leaves on which trichomes became visible 6 days after germination, the lateral organs of 35S::*LFY emf1-1* plants did not develop trichomes (Figures 1V to 1X). By 20 days after germination, some of the transgenic plants developed stigmatic tissue on the tips and ovules or ovulelike structures at the lower edges of the lateral organs, resulting in lateral carpelloid structures similar to that of *emf1-2* (Figure 1V). Some plants produced one or more glabrous sessile leaves and then carpelloid structures (Figure 1W). Other plants produced two to 16 staminoid structures followed by a short, thick, pistil-like structure (Figure 1X). These phenotypes showed that 35S::*LFY emf1-1* plants had a stronger tendency toward reproductive growth than did *emf1-1* plants after germination. More significantly, they produced carpelloid organs. The carpelloid nature of 35S::*LFY emf1-1* lateral organs is a characteristic similar to the strong *emf* mutants. On the other hand, the 35S::*LFY emf1-1* plants differed from *emf1-2* plants in that no stigmatic papillae developed at the base of the cotyledons (Figures 1V to 1X).

The production of staminoid and carpelloid organs on the primary shoot after germination had not been seen in any other single or double *emf* mutants. Promotion of stamen formation in 35S::*LFY emf1-1* plants was consistent with one of the *LFY* functions deduced from the *lfy* phenotype: *lfy* "flowers" always lack stamens (Huala and Sussex, 1992). Like *emf* single mutants, all stamens and pistils were sterile in the transgenic plants.

In contrast to *emf1-1*, constitutive expression of *LFY* had little effect on the phenotype of *emf1-2*. The shoot structures of 35S::*LFY emf1-2* were similar to those of *emf1-2*

Table 1. Summary of Double Mutant Phenotypes

		Double Mutant			
Parent 1[a]	Parent 2	Number of Plants Observed	Seedling[b]	Inflorescence[c]	Lateral Organ[d]
ap1-1	*emf1-1*	64	*emf*	*emf*	*emf*, both
ap1-1	*emf1-2*	78	*emf*	*emf*	*emf*
ap1-1	*emf2-3*	53	*emf*	*emf*	*emf*, both
ap1-1 cal	*emf1-1*	92	*emf*	*emf*	*emf*, both
ap1-1 cal	*emf1-2*	68	*emf*	*emf*	*emf*
ap1-1 cal	*emf2-2*	63	*emf*	*emf*	*emf*, both
ap2-1	*emf1-1*	188	*emf*	*emf*	*emf*, novel, both
ap2-1	*emf1-2*	120	*emf*	*emf*	*emf*, novel
ap2-1	*emf2-3*	64	*emf*	*emf*	*emf*, both
lfy-1	*emf1-2*	39[e]	*emf*	*emf*	*emf*
tfl1-1	*emf1-2*	38	*emf*	*emf*	*emf*

[a]Parent 1 plants have wild-type–like seedling and rosette phenotypes, but their reproductive development is altered. *ap1-1* is characterized by the development of secondary flowers, coflorescences without subtending cauline leaves, and bractlike sepals, with the meridian pair being separated from the lateral pair at late floral development; *ap1-1 cal* is characterized by the proliferation of floral meristems; *ap2-1* by the development of bractlike sepals, stigmatic tissue on tips of sepals, and secondary flowers; *lfy-1* by the development of coflorescence-like and flower-like shoots; and *tfl1-1* by the development of terminal flowers and solitary flowers in leaf axils.

[b]The seedling traits of *emf* mutants include short hypocotyls and oval-shaped and petiole-less cotyledons, with a lack of petiolated rosette leaves.

[c]The inflorescence trait of *emf* means that the double mutants showed a reduced inflorescence composed of one to seven flowers, with a terminal flower and solitary flowers in the axils of cauline leaves, as in *emf1-1*, or several carpelloid structures with stigmatic papillae at the tips, as in *emf1-2*.

[d]The lateral organ trait of *emf* mutants means that the cauline leaves and sepals are similar to those of *emf1-1*, *emf2-2*, or *emf2-3*, or that the carpelloid structures are similar to those of *emf1-2*; both means that the sepals became carpelloid or separated by an internode (specific in *emf1-1 ap1-1* and *emf1-1 ap1-1 cal*); novel means that the sepals lobed repeatedly and cauline leaves developed stigmatic papillae at the tips (specific in *emf1-1 ap2-1*) or that the carpelloid structures became lobed and enlarged (in *emf1-2 ap2-1*).

[e]Because of the sterility of *lfy-1* plants, double mutants segregated from plants heterozygous for both *lfy-1* and *emf1-2*. In total, 156 *emf1-2*–like plants were observed, with 39 theoretically expected to be double mutants.

single mutants. After germination, carpelloid lateral organs were produced in 35S::*LFY emf1-2* plants. No staminoid structures were observed. In short, ectopic *LFY* expression in young *emf1-1* seedlings increased the severity of the *emf* phenotype. The conversion of *emf1-1* to an *emf1-2*–like phenotype by 35S::*LFY* suggested negative regulation of *EMF1* by *LFY*.

emf2-3 *Mutants Harboring 35S::*LFY *Constructs*

Because *emf2* single and double mutants shared shoot phenotypes similar to *emf1-1*, we might expect constitutive *LFY* expression to alter the *emf2-3* phenotype as it did in *emf1-1*. However, we found that the introduction of 35S::*LFY* had limited effect on the *emf2-3* phenotype. The shoot phenotype of 35S::*LFY emf2-3* was quite different from that of 35S::*LFY emf1-1*. Upon germination, the shoot apical meristem produced normal sessile leaves that were similar to those in *emf2-3*. No carpelloid or staminoid transformation occurred. After producing the same number of sessile leaves as in *emf2-3*, plants produced a terminal flower (Figure 1Y). The effect of constitutive expression of *LFY* seemed only to be the shortening and thickening of the ovary (Figure 1Y). Although *EMF1* and *EMF2* play similar roles in rosette and inflorescence development, the differences in phenotype between 35S::*LFY emf2-3* and 35S::*LFY emf1-1* suggested that *EMF1* plays a stronger repressive role than *EMF2* in flower initiation and floral organ differentiation. This is consistent with the fact that two-thirds of the known *emf1* alleles are strong, but no strong *emf2* allele was found among the six *emf2* mutants.

DISCUSSION

The Interaction of *EMF* with *AG* and *AP1*

AP1 and *AG* have been classified as both flower meristem identity and flower organ identity genes (Weigel, 1995; Yanofsky, 1995). Because *emf* mutants are also affected in floral meristem and floral organ development, we analyzed the relationship between *EMF* and these genes. Absence of *EMF1* expression is sufficient for *AP1* and *AG* ectopic expression, suggesting that *EMF1* negatively regulates these two genes. However, the residual EMF activities in a germinating *emf1-1* seedling is enough to cause precocious expression of *AP1* but not of *AG*. Spatial pattern of *AP1* and *AG* expression also differs in *emf1-2* mutants, indicating that the regulatory mechanisms vary and that perhaps the genetic interaction between *EMF1* and *AG* may be indirect and involve other genes.

Our previous study showed that an *ag* mutation rescued petal formation in *emf1-1* but not in *emf1-2* mutants (Yang et al., 1995). To explain this phenomenon, we have proposed that the weak *emf* mutations caused ectopic *AG* expression, which, based on the ABC model (Bowman et al., 1991; Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994), would suppress *AP1* and *AP2*, resulting in the loss of petals. To explain the inability of the *ag* mutation to rescue petal formation in *emf1-2*, we proposed a requirement of minimal EMF1 activity for *AP1* expression (Yang et al., 1995). However, our findings, that is, that *AG* RNA was not detected in the outer whorl of *emf1-1* mutant flowers and that the *AP1*::GUS activity was detected in *emf1-1* sepals, do not support these explanations. Owing to the loss of vegetative and early inflorescence phases in *emf* mutants, there may be a disordered expression of genes involved in the flower program, resulting in the loss of petals in weak and the loss of most of the floral organs in strong *emf* mutants. Therefore, EMF1 activity may be required by other gene activities specifying petal formation; or it may specify the extent of floral competence, and the ectopic *AG* and *AP1* expression may only be indicators of the expression of late floral program (Weigel, 1995).

The *EMF* Genes Encode Floral Repressors That Specify the Vegetative State of Arabidopsis Shoot Development

A genetic model for the control of vegetative-to-reproductive transition has been proposed (Martinez-Zapater et al., 1994; Weigel, 1995). The main scheme of the model is that flowering is a default state and is negatively regulated by floral repressors (Martinez-Zapater et al., 1994; Weigel, 1995). The hypothesis assumes that vegetative development is maintained as a result of the suppression of reproductive development. The *EMF* gene products are regarded as the floral repressors because weak *emf* mutants produce an inflorescence directly after germination (Sung et al., 1992). In this study, the characteristics of *emf1* strong mutants lend further support to this hypothesis. The severe *emf1* alleles cause the shoot to shift further into the reproductive state than do weak alleles, as evidenced by several distinct floral characteristics, including lack of stipules (Figure 2C) and trichomes on lateral organs, carpelloidy of lateral organs (Figure 1E), direct development of a single flower or pistil (Figures 1B, 1C, and 1F), and precocious expression of floral genes (Figures 3C, 4F, and 4H). Both morphological and molecular data showed that floral programs proceeded in *emf* seedlings with little or no vegetative phase, indicating that EMF activity suppresses the reproductive transition in wild-type seedlings and rosettes.

To flower, juvenile plants must acquire floral competence first (McDaniel et al., 1992). Because *LFY* is the first one to express among the flower meristem identity genes and it is expressed before floral commitment (Bradley et al., 1997), *LFY* may potentially encode the floral competence factor. However, evidence suggests that it is not directly responsible for floral competence (Weigel and Nilsson, 1995). There-

fore, a pathway, the floral competence factor, parallel to the flower meristem identity genes was proposed to regulate the floral target genes. It is hypothesized that the floral competence factor causes the floral target genes to undergo a change from a completely repressed to a partially derepressed state, which then can be completely derepressed by high *LFY* activity (Weigel and Nilsson, 1995). The notion of floral repressor is compatible with the suppression of floral competence. In this respect, *EMF* products can be viewed as specifying the level of floral competence, which must be abated to a level to enable the partial derepression of floral target genes for *LFY* to initiate flower development. In the absence of *LFY*, as in *lfy* and *lfy ap1* plants, continued increase of floral competence would still occur, resulting in floral target gene expression and carpelloid organ formation.

EMF Gene Products Maintain Inflorescence Development

Like the *tfl1-1* mutant, the primary shoot meristems of *emf* weak mutants develop terminal flowers, and the axillary shoot meristems develop solitary flowers in leaf axils. Loss of EMF or TFL1 acivities switches the meristem from inflorescence to flower development. EMF and TFL1 seem to have similar effects on maintenance of inflorescence growth. However, major differences exist between TFL1 and EMF. EMF is and TFL1 is not required for the photoperiodic response of Arabidopsis flowering (Sung et al., 1992; Schultz and Haughn, 1993; Martinez-Zapater et al., 1994). *emf* mutants lack and *tfl1* plants possess petiolated rosette leaves. Both *lfy-1* and *ap1* can lessen the *tfl1* phenotype, resulting in a coflorescence-like shoot rather than a solitary flower in the leaf axils of double mutants (Schultz and Haughn, 1993). This implies no absolute requirement of TFL1 activity for maintaining inflorescence development (Schultz and Haughn, 1993). In contrast, neither *lfy-1* nor *ap1* could lessen the strong *emf* phenotype, suggesting that EMF activities are indispensable for inflorescence development. Epistasis of *emf1-2* to *tfl1-1* (Figures 1O and 5) suggests that EMF activities are required for TFL1 function and that TFL1's function in inflorescence development may be mediated via its positive regulation of EMF.

Decline of EMF Activity Is Responsible for the Appearance of Floral Character

Many observations indicate the existence of a gradient of "floral character" along the Arabidopsis inflorescence axis. For example, the coflorescence-like shoots of *lfy* plants become more flowerlike as plants develop (Schultz and Haughn, 1991, 1993; Weigel et al., 1992). Ectopic expression of *PISTILLATA* and *AP3* can only cause the upper cauline leaves to be petaloid, whereas the lower leaves remain untransformed (Krizek and Meyerowitz, 1996). The gradient of floral character can also be seen on the shoots of other annual plants, such as tobacco (Tran Than Van, 1973). The common features seen in different plants suggest that the mechanism controlling plant shoot maturation may be a conserved one in angiosperms. This gradient effect may be interpreted as resulting from an increasing amount of floral activators or a decreasing amount of floral repressors during inflorescence development (Krizek and Meyerowitz, 1996).

Our study shows that the decline of floral repressor responsible for the vegetative-to-reproductive transition is also responsible for increasing the floral character during inflorescence development. For example, the differences in weak and strong *emf1* phenotypes suggest that the extent of floral character corresponds with the EMF level. Ectopic expression of *LFY* alone cannot change the vegetative fate of the lateral organs (Weigel and Nilsson, 1995). This can be explained by the relatively high level of EMF activity in the shoot. When EMF is reduced, as in *emf1-1* mutants, the development of lateral organ primordia is more easily shifted into the floral program by constitutive expression of *LFY*, as shown by the cluster of carpelloid and staminoid structures on the 35S::*LFY emf1-1* plants (Figure 1X).

Reciprocal Negative Regulation between *EMF* and Floral Genes

Epistasis of *emf* to floral repression and floral promotion mutations suggests that these floral genes act by modulating EMF activity to cause vegetative-to-inflorescence transition (Yang et al., 1995). Likewise, epistasis of *emf1-2* to *lfy-1*, *ap1-1*, *ap2-1*, and *ap1-1 cal* suggests that EMF acts downstream from those floral genes in mediating the inflorescence-to-flower transition. The phenotype of 35S::*LFY emf1-1* plants confirms the regulation of EMF1 activities by *LFY*. On the other hand, EMF appears to suppress floral genes, as evidenced by the precocious expression of *AP1* and *AG* in *emf* seedlings. Therefore, there seems to be a reciprocal negative interaction between *EMF* and floral genes in controlling the development of Arabidopsis shoots from inflorescence to flower phase. This kind of interaction may seem somewhat paradoxical, but it is consistent with the controllers of phase switching (COPS) hypothesis, which places EMF in the center of the COPS activity (Schultz and Haughn, 1993).

The COPS hypothesis holds that a high level of COPS activity suppresses reproductive development, allowing vegetative growth. If COPS activities continue to decline throughout the life span, the plant can progress from the rosette to inflorescence and to the flower phase. The reciprocal negative regulation between *EMF* and the floral genes provides a plausible mechanism for this hypothesis. During rosette growth, high EMF activity suppresses floral genes. EMF decline, mediated by the flowering-time genes, allows the activation of floral genes, which in turn suppress EMF activity,

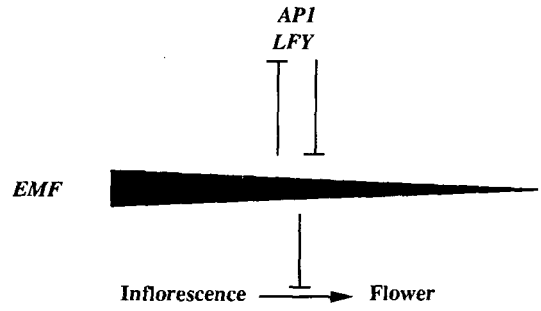

Figure 6. EMF Model for the Inflorescence-to-Flower Transition.

Based on negative interaction between *EMF* and floral genes, the model implies that EMF activities maintain inflorescence growth by suppression of floral gene, for example, *LFY* and *AP1*, expression. However, EMF activities decline gradually. At critical EMF levels, floral genes are activated, and plants switch to floral development. The activities of floral genes in turn suppress EMF activities, promoting further decline of EMF activities. The T-shaped bars indicate suppression, and the gradient indicates the decline of EMF activity during development.

resulting in the sequential activation of other floral genes and the gradual decline of EMF activity during inflorescence and flower development (Castle and Sung, 1995; Yang et al., 1995).

No single floral gene activity can suppress EMF completely, as evidenced by the phenotype of 35S::*LFY emf1-1*, which is more severe than that of *emf1-1* but not as severe as that of *emf1-2* (Figures 1V to 1X). Thus, in addition to *LFY*, other genes may also suppress EMF activity. This is compatible with the fact that ectopic expression of one or two floral genes can shorten the rosette phase but the plant cannot simply skip to floral development, as seen in 35S::*AP1*, 35S::*LFY*, 35S::*AP3* 35S::*PISTILLATA*, and 35S::*AG* transgenic plants (Mandel and Yanofsky, 1995; Weigel and Nilsson,1995; Krizek and Meyerowitz, 1996; Mizukami and Ma, 1997). A possible working model for the *EMF* regulation of shoot development is proposed (Castle and Sung, 1995; Yang et al., 1995). Figure 6 summarizes the relationship between *EMF* and flower meristem identity genes in regulating inflorescence-to-flower development. EMF activities continue to decline in this process, allowing the initiation of floral program. The higher EMF activities in the earlier stage prohibit floral program by directly or indirectly suppressing the activities of floral genes. And in the later stage, the increasing activities of flower meristem identity genes promote the further decline of EMF activities, allowing the late floral program to be activated. To confirm the gradual decline of EMF activities, it is necessary to measure EMF activity directly during Arabidopsis shoot development. To do this, we are now cloning the *EMF* genes.

METHODS

Genetic Materials and Growth Conditions

emf *Alleles*

Nine *emf* mutations, three in the *EMF1* gene (*emf1-1*, *emf1-2*, and *emf1-3*) and six in the *EMF2* gene (*emf2-1*, *emf2-2*, *emf2-3*, *emf2-4*, *emf2-5*, and *emf2-6*), have been characterized previously (Yang et al., 1995; Bhatt et al., 1996). All alleles are in the Columbia background, except for *emf1-3*, *emf2-5*, and *emf2-6*, which are in the Landsberg *erecta* background. The six *emf2* mutants have phenotypes similar to that of *emf1-1*.

emf1-2 and *emf1-3* have the most severe phenotype and are distinct from the other mutants. Based on the phenotypes, the *emf1-1* and six *emf2* alleles are considered weak alleles, whereas *emf 1-2* and *emf1-3* are strong alleles. The *emf1-3* mutant was provided by C. Dean (John Innes Center, Norwich, UK). Although it was derived from an *Ac* transposon tagging experiment, the mutation is not linked to the *Ac* (Bhatt et al., 1996).

Seeds from plants heterozygous for *emf* were surface sterilized, germinated, and grown on Murashige and Skoog medium (Murashige and Skoog, 1962) as described (Yang et al., 1995). Seedlings on agar plates were grown in a growth chamber (Conviron, Winnipeg, Manitoba, Canada), which was maintained under short-day (8 hr of light and 16 hr of dark) conditions at 21°C. The light intensity of the growth chamber was 150 μE. After 10 days, plants showing normal rosette leaves were removed, leaving only homozygous *emf* plants on plates for examination and analysis.

Mutants Affected in Inflorescence and Flower Development and Transgenic Plants

Seeds carrying *ap1-1*, *ap2-1*, and *ap1-1 cal-1* mutations were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus, OH). The *lfy-1* strain was obtained from E. Huala (University of California at Berkeley, Berkeley, CA), and the *tfl1-1* strain was from R. Meeks-Wagner (University of Oregon, Eugene, OR).

Transgenic plants harboring a chimeric construct of the *AP1* promoter fused to a β-glucuronidase gene (*AP1*::*GUS*) were obtained from M. Yanofsky (University of California at San Diego, La Jolla, CA). Transgenic 35S::*LFY* plants were obtained from D. Weigel (Salk Institute for Biological Studies, La Jolla, CA).

Seeds were germinated on agar plates, as described previously (Yang et al., 1995), and transplanted to soil after 10 days. After 1 week under short-day conditions, the plants were moved to long-day conditions (16 hr of light and 8 hr of dark) in the greenhouse.

Genetic Crosses

Three weak *emf* alleles (*emf1-1*, *emf2-2*, and *emf2-3*) and a strong allele (*emf1-2*) were chosen for genetic crosses. Eleven double mutants were constructed and are listed in Table 1. Heterozygous *emf* lines were crossed with plants homozygous for *ap1-1*, *ap1-1 cal*, *ap2-1*, or *tfl1-1*. F_1 plants were selfed. F_2 plants homozygous for *ap1-1*, *ap1-1 cal*, *ap2-1*, and *tfl-1* and heterozygous for *emf* were grown to generate F_3 seed that segregated 25% of the double mutants. For

generation of *emf1-2 lfy-1* double mutants, plants heterozygous for *emf1-2* and *lfy-1* were crossed. F_1 plants that segregated both *lfy-1* and *emf1-2* in the F_2 generation were used for production of double mutants. In these lines, the F_2 population segregated one-sixteenth of the double mutants. Because *ap1-1* and *ap2-1* plants are in the Landsberg background that carries the *erecta* mutation, only F_3 progeny from the homozygous *ERECTA* F_2 population were analyzed to avoid the interference of *erecta* in double mutant analysis. For phenotypic analysis of double *emf* mutants, F_3 seeds were germinated and grown on sterile agar media, as described in the previous section.

To generate *AP1*::*GUS* homozygous plants, plants showing GUS activity in whorl 1 and whorl 2 of a flower were selfed. The plants that showed GUS activity in all offspring were selected as homozygous parental plants. To cross 35S::*LFY* and *AP1*::*GUS* into the three *emf* backgrounds, heterozygous *emf* lines were crossed with lines homozygous for 35S::*LFY* or *AP1*::*GUS*. F_1 plants were selfed. F_2 plants homozygous for 35S::*LFY* or *AP1*::*GUS* and heterozygous for *emf* were grown to generate F_3 plants, of which one-fourth were the desired strains.

Light and Scanning Electron Microscopy

For light microscopy, samples were fixed in FAA (10% formaldehyde, 50% ethanol, and 5% acetic acid) for 1 day. The samples were dehydrated and embedded in paraffin. Sections were cut 10 mm thick and stained with toluidine blue O (0.05% in benzoate buffer). Finished specimens were examined and photographed with a microscope (Axiophot; Carl Zeiss, Inc., Heidelberg, Germany) under bright-field illumination.

For scanning electron microscopy, samples were fixed in 4% formaldehyde, 50% ethanol, and 5% acetic acid overnight and dehydrated in a graded ethanol series. Specimens were critical point dried in liquid CO_2. The dried materials were mounted and coated with palladium in a sputtercoater (Technics Hummerr I; Polaron, Inc., Watford, UK). Specimens were examined with a scanning electron microscope (model ISI-DS-130; Topcon Technologies, Inc., Paramus, NJ) with an accelerating voltage of 10 kV.

In Situ Hybridization

In situ hybridization was performed mainly according to the method of Drews et al. (1991). Two probes were used in these experiments. The *AG* antisense probe was synthesized with T7 RNA polymerase from a HindIII-digested pCIT565 template and contained nucleotides 240 to 977 of the *AG* sequence published in Yanofsky et al. (1990). The control probe that was used for the measurement of the background hybridization was synthesized in the opposite orientation of the antisense *AG* RNA probe with SP6 RNA polymerase from a XhoI-digested pCIT565 template. Both probes were hydrolyzed to an average size of 0.07 to 0.15 kb. The probes were used at a final concentration of 200 to 300 ng/mL/kb with a specific radioacitivity of 1.3×10^9 cpm/μg. A probe solution (70 μL) was applied to each prepared slide. Fixation of tissue, preparation of sections, hybridization, and washes were performed as described by Drews et al. (1991). Slides were coated with emulsion of NTB-2, according to the instructions of the manufacturer (Kodak), and kept in darkness at 4°C for exposure for 2 to 3 weeks.

GUS Activity Assays

Four- to 25-day-old seedlings of *emf1-1 AP1*::*GUS*, *emf1-2 AP1*::*GUS*, and *emf2-3 AP1*::*GUS* plants were prepared for GUS assays. GUS activity in these plants was detected histochemically by using a protocol adapted from the method of Jefferson et al. (1987). The tissue was fixed for 15 min on ice with cold 90% acetone, rinsed with 50 mM phosphate buffer, pH 7.2, containing 0.5 mM $K_3Fe(CN)_6$ and 0.5 mM $K_4Fe(CN)_6$, incubated in 2 mM X-gluc (5-bromo-4-chloro-3-indolyl β-D-glucuronic acid) in the same solution for several hours at 37°C, and observed by using a stereomicroscope.

Image Processing

Slides and negatives were scanned and digitized with a UMAX scanner (UMAX Data Systems Inc., Hsinchu, Taiwan). Images were adjusted for brightness, contrast, and color and assembled for figures with Adobe Photoshop (version 3.0.4; Adobe Systems Inc., Mountain View, CA). All of these figures were printed with a color printer (Tektronix, Inc., Wilsonville, OR).

ACKNOWLEDGMENTS

We thank Drs. Robert Wilson, Jim Mattsson, Dominique Aubert, Dave Martin, and Regina McClinton for critical reading of the manuscript and for suggestions. We also thank Dr. Caroline Dean for providing *emf1-3*; Dr. Eva Huala for *lfy-1*; Dr. Ry Meeks-Wagner for *tfl1-1*; Dr. Martin Yanofsky for *AP1*::*GUS* plants; Dr. Detlef Weigel for 35S::*LFY* plants; the Arabidopsis Biological Resource Center (Ohio State University) for *ap1-1*, *ap2-1*, and *ap1-1 cal*; Dr. Hong Ma for plasmid pCIT565; and Dr. Allen Sessions for help with scanning electron microscopy. This work was supported by U.S. Department of Agriculture Competitive Grant No. 93-37301-8704 to Z.R.S. and in part by Chinese National Science Foundation Grant No. 39570046 to L. Chen.

Received June 11, 1997; accepted September 15, 1997.

REFERENCES

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

Bai, B., and Sung, Z.R. (1995). The role of *EMF1* in regulating the vegetative and reproductive transition in *Arabidopsis thaliana* (Brassicaceae). Am. J. Bot. **82,** 1095–1103.

Bhatt, A.M., Page, T., Lawson, E.J., Lister, C., and Dean, C. (1996). Use of *Ac* as an insertional mutagen in *Arabidopsis*. Plant J. **6,** 935–945.

Bowman, J. (1992). Making cauliflower out of *Arabidopsis*: The specification of floral meristem identity. Flowering Newslet. **14,** 7–19.

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

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

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

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

Castle, L.A., and Sung, Z.R. (1995). The *EMBRYONIC FLOWER* genes of *Arabidopsis* control shoot maturation. Flowering Newslet. **19,** 12–19.

Coen, E.S., and Meyerowitz, E.M. (1991). The war of the whorls: Genetic interactions controlling flower development. Nature **353,** 31–37.

Drews, G.N., Bowman, J.L., and Meyerowitz, E.M. (1991). Negative regulation of the Arabidopsis homeotic gene *AGAMOUS* by the *APETALA2* product. Cell **65,** 991–1002.

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

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

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

Jofuku, K.D., den Boer, B.G.W., Van Montagu, M., and Okamuro, J.K. (1994). Control of Arabidopsis flower and seed development by the homeotic gene *APETALA2*. Plant Cell **6,** 1211–1225.

Koornneef, M., Hanhart, C.J., and Van der Veen, J.H. (1991). A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol. Gen. Genet. **229,** 57–66.

Krizek, B.A., and Meyerowitz, E.M. (1996). The *Arabidopsis* homeotic genes *APETALA3* and *PISTILLATA* are sufficient to provide the B class organ identity function. Development **122,** 11–22.

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

Mandel, M.A., Gustafson-Brown, C., Savidge, B., and Yanofsky, M.F. (1992). Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. Nature **360,** 273–277.

Martinez-Zapater, J.M., Coupland, G., Dean, C., and Koornneef, M. (1994). The transition to flowering in *Arabidopsis*. In *Arabidopsis*, E.M. Meyerowitz and C.R. Somerville, eds (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 403–433.

McDaniel, C.N., Singer, S.R., and Smith, S.M.E. (1992). Development states associated with the floral transition. Dev. Biol. **153,** 59–69.

Medford, J., Callos, J.D., Behringer, F.J., and Link, B.M. (1994). Development of the vegetative shoot apical meristem. In *Arabidopsis*, E.M. Meyerowitz and C.R. Somerville, eds (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 355–378.

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

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15,** 473–497.

Okamuro, J.K., den Boer, B.G.W., and Jofuku, K.D. (1993). Regulation of Arabidopsis flower development. Plant Cell **5,** 1183–1193.

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

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

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

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

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

Tran Thanh Van, M. (1973). Direct flower neoformation from superficial tissue of small explants of *Nicotiana tabacum* L. Planta **115,** 87–92.

Weigel, D. (1995). The genetics of flower development: From floral induction to ovule morphogenesis. Annu. Rev. Genet. **29,** 19–39.

Weigel, D., and Meyerowitz, E.M. (1994). The ABCs of floral homeotic genes. Cell **78,** 203–209.

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

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

Yang, C.H., Chen, L.J., and Sung, Z.R. (1995). Genetic regulation of shoot development in *Arabidopsis*: Role of the *EMF* genes. Dev. Biol. **169,** 421–435.

Yanofsky, M.F. (1995). Floral meristems to floral organs: Genes controlling early events in *Arabidopsis* flower development. Annu. Rev. Plant Physiol. Plant Mol. Biol. **46,** 167–188.

Yanofsky, M.F., Ma, H., Bowman, J.L., Drews, G.N., Feldmann, K.A., and Meyerowitz, E.M. (1990). The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. Nature **346,** 35–39.

Zagotta, M.T., Shannon, S., Jacobs, C., and Meeks-Wagner, R. (1992). Early-flowering mutants of *Arabidopsis thaliana*. Aust. J. Plant Physiol. **19,** 411–418.