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Determination of Arabidopsis Floral Meristem Identity by *AGAMOUS*

Yukiko Mizukami[1] and Hong Ma[2]

Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, New York 11724

Determinate growth of floral meristems in Arabidopsis requires the function of the floral regulatory gene *AGAMOUS* (*AG*). Expression of *AG* mRNA in the central region of floral meristems relies on the partially overlapping functions of the *LEAFY* (*LFY*) and *APETALA1* (*AP1*) genes, which promote initial floral meristem identity. Here, we provide evidence that *AG* function is required for the final definition of floral meristem identity and that constitutive *AG* function can promote, independent of *LFY* and *AP1* functions, the determinate floral state in the center of reproductive meristems. Loss-of-function analysis showed that the indeterminate central region of the *ag* mutant floral meristem undergoes conversion to an inflorescence meristem when long-day–dependent flowering stimulus is removed. Furthermore, gain-of-function analysis demonstrated that ectopic *AG* function results in precocious flowering and the formation of terminal flowers at apices of both the primary inflorescence and axillary branches of transgenic Arabidopsis plants in which *AG* expression is under the control of the 35S promoter from cauliflower mosaic virus. Similar phenotypes were also observed in *lfy ap1* double mutants carrying a 35S–*AG* transgene. Together, these results indicate that *AG* is a principal developmental switch that controls the transition of meristem activity from indeterminate to determinate.

INTRODUCTION

Alterations in the activity of the indeterminate shoot apical meristem upon the transition to flowering result in the onset of determinate growth in floral meristems. Determination of floral meristems proceeds through an elaboration of distinctive stages in which successive gene activation may be responsible for sequential differentiation of meristematic cells (reviewed in Steeves and Sussex, 1989).

In Arabidopsis, the initiation of floral lateral meristems in peripheral regions of the apical inflorescence meristem is regulated synergistically by the floral meristem identity genes. Among these genes, *LEAFY* (*LFY*) and *APETALA1* (*AP1*) have been shown to be predominant, encoding putative transcription factors (Mandel et al., 1992; Weigel et al., 1992). Loss-of-function mutations in either *LFY* or *AP1* result in partial conversion of flowers to shoots, and *lfy ap1* double mutants further show transformation of flowers into sterile shoots with leaflike organs (Bowman et al., 1993; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993; Weigel and Meyerowitz, 1993). *LFY* mRNA accumulates initially in peripheral regions of the apical inflorescence meristem, and it is expressed continuously throughout developing flowers (Weigel et al., 1992). *AP1* mRNA expression is first seen throughout emerging young floral meristems, but expression in the central region disappears soon after sepal primordia arise at the periphery (Mandel et al., 1992). Furthermore, it has been shown that *LFY* and *AP1* activate the floral organ identity genes (Weigel and Meyerowitz, 1993). These observations suggest that *LFY* and *AP1* can act as developmental switches that establish floral fate in the lateral meristems. Indeed, it has recently been demonstrated that ectopic expression of *LFY* or *AP1* under the control of the 35S promoter from cauliflower mosaic virus resulted in ectopic flower formation in the inflorescence apices (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995), indicating that these genes can activate the gene(s) that regulates the determination of floral meristems.

AGAMOUS (*AG*) is one of the Arabidopsis floral regulatory genes whose expression in the central region of the floral meristem is regulated synergistically by *LFY* and *AP1* (Weigel and Meyerowitz, 1993). *AG* encodes a putative transcription factor of the MADS domain family (Yanofsky et al., 1990) and functions in determination of floral meristem growth and specification of reproductive organ identity. Loss-of-function *ag* mutants exhibit indeterminate growth of floral meristems, producing "double flowers" (Bowman et al., 1991). This floral meristem indeterminacy was shown to occur independently of the differentiation of a functionally normal gynoecium in the center of weak 35S–antisense *AG* transgenic flowers (Mizukami and Ma, 1995). The indeterminate

[1] Current address: Department of Plant Biology, 111 Koshland Hall, University of California, Berkeley, CA 94720.

[2] To whom correspondence should be addressed. E-mail mah@cshl.org; fax 516-367-8369.

growth that is typical of shoot meristems demonstrates that *ag* floral meristems fail to acquire determinacy, a distinct characteristic of the floral meristem, and suggests that lateral meristems of *ag* mutants are not completely committed to the floral fate. Furthermore, *LFY* and *AP1* functions together are unlikely to be able to substitute for *AG* function in specifying the determinate floral fate, because constitutive *AP1* expression in the central region of *ag* floral meristems, in the presence of *LFY* expression, cannot promote floral meristem determinacy (Gustafson-Brown et al., 1994). Therefore, *AG* most likely plays an important and distinct role in the definition of floral meristem identity.

One previous observation that strongly supports this hypothesis is that *ag* mutants grown under short-day (SD) photoperiods, which are unfavorable for floral induction, produce an ectopic inflorescence shoot in the center of flowers; that is, they undergo floral reversion (Okamuro et al., 1993). This suggests that the identity of floral meristems cannot be defined in the absence of *AG* function. However, this phenomenon was not examined in detail; therefore, the origin of the secondary inflorescence remains uncertain. Phenotypic analysis of *ag ap1* and *ag lfy* double mutants and of the *ag ap1 ap2* triple mutant also suggested that *AG* might have a distinct function independent of *LFY* and *AP1* functions in the definition of floral meristem identity (Bowman et al., 1993; Schultz and Haughn, 1993).

How does *AG* define the floral meristem? In particular, what functional relationship does *AG* have with the primary floral meristem identity genes, such as *LFY* and *AP1*? The observation that accumulation of *AG* mRNA in the central region of floral meristems is dependent on the overlapping functions of *LFY* and *AP1* (Weigel and Meyerowitz, 1993) suggests two possibilities for AG action in floral meristem determination. First, not only *AG* expression but also *AG* function may rely on the activities of LFY and AP1. If this is the case, *AG* function as well as *LFY* and *AP1* functions are required to determine floral meristem identity. The second possibility is that *AG* function alone may be sufficient to determine floral meristem fate. That is, constitutive *AG* function can promote a determinate fate for floral meristems in the absence of *LFY* and *AP1* functions. One way to evaluate the role of *AG* and the interaction between *AG* and the floral

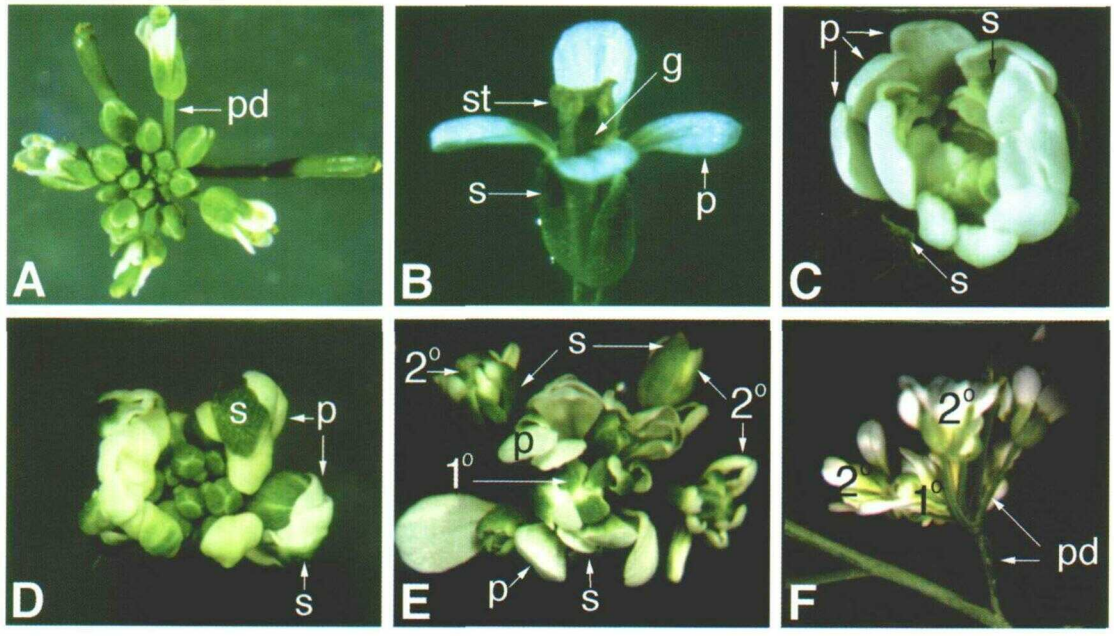

Figure 1. Phenotypes of Arabidopsis Inflorescences and Flowers.

(A) Wild-type inflorescence.

(B) Mature wild-type flower consisting of four sepals, four white petals, six stamens, and a gynoecium with two fused carpels, topped with stigmatic papillae.

(C) Mature, indeterminate *ag-1* flower with a repeated pattern of floral organs of sepal–petals–petals from the outermost whorl to the center.

(D) *ag-1 co-2* flower developing an inflorescence shoot at the center.

(E) *ag-1* flower developed after a shift to darkness for 24 hr. The primary flower consists of sepals, petals, and secondary *ag* flowers.

(F) Shifted *ag-1* flower exhibiting an inflorescence branchlike feature later in development.

g, gynoecium; p, petal; pd, pedicel; s, sepal; st, stamen; 1°, primary flower; 2°, secondary flower.

meristem identity genes in specifying floral meristem identity is to examine the effects of ectopic *AG* expression on the fate of apical and lateral meristems in the presence and absence of *LFY* or *AP1* functions.

In the experiments described here, we analyze the effects of the loss-of-function *ag* mutation and the gain-of-function 35S–*AG* transgene on apical and lateral meristem fates in different genetic backgrounds and/or under different photoperiods. We show that when the flowering stimulus that is provided by long-day photoperiods is removed, *ag* floral meristems but not wild-type floral meristems are converted to inflorescence meristems during flower development. We also show that ectopic *AG* function results in an accelerated transition to flowering and differentiation of apical inflorescence meristems into ectopic terminal flowers. Furthermore, ectopic *AG* function can promote floral fate even in early-arising lateral meristems of *lfy ap1* double mutants. Therefore, *AG* plays an indispensable role in specifying determinate floral fate throughout reproductive development.

RESULTS

ag-1 Mutant Floral Meristems Are Converted to Inflorescence Shoot Meristems upon Floral Reversion

As shown in Figure 1A, a wild-type Arabidopsis inflorescence shoot continuously produced flowers, each of which consists of four types of organs: sepals, petals, stamens, and a gynoecium (Figure 1B). Figure 2A shows the wild-type inflorescence apex, in which the inflorescence shoot meristem produces floral meristems in a spiral phyllotaxy, whereas floral meristems give rise to floral organ primordia in a whorled phyllotaxy. The *ag-1* mutation affects floral organ identity as well as determinate floral structure. Therefore, *ag-1* flowers continue to produce sepals and petals centripetally (Figures 1C and 2C).

To examine the role of *AG* in specifying floral meristem identity, we analyzed the identity of the *ag* mutant lateral meristem upon floral reversion and the origin of the secondary inflorescence. It is known that mutations in genes, such as *CONSTANS* (*CO*), affect the inductive photoperiod-dependent floral promotion pathway, causing a phenocopy of plants grown under SD conditions (Koornneef et al., 1991; Martinez-Zapater et al., 1994; Putterill et al., 1995). Thus, instead of using SD conditions to evoke floral reversion, we generated the *ag-1 co-2* double mutant and examined the morphology of floral meristems under inductive photoperiods. Initially, additive effects of two mutations were observed in the *ag-1 co-2* double mutant; that is, the flowering time was delayed as it was for *co-2* single mutants (data not shown), and early-arising flowers were initially indistinguishable from those of *ag-1* single mutants (Figures 2B and 2C). Scanning electron microscopy of early-arising *ag-1 co-2* flowers revealed that the lateral meristem failed to give rise

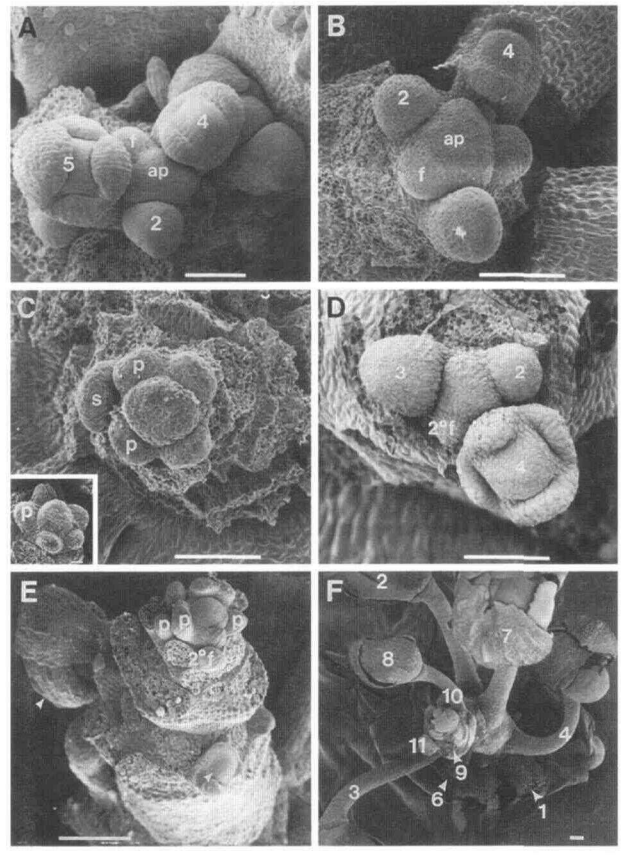

Figure 2. Scanning Electron Microscopy of the Center of Inflorescences and Flowers.

(A) Wild-type inflorescence apex with young floral buds arranged in a spiral phyllotaxy. Numbers indicate floral stages (Smyth et al., 1990).
(B) *ag-1 co-2* inflorescence apex. Numbers indicate floral stages.
(C) Central region of a young *ag-1 co-2* flower. The first and second sets of floral organs were removed. The inset shows the *ag-1* floral center at the same stage.
(D) Central region of a mature *ag-1 co-2* flower. Numbers indicate floral stages.
(E) Center of a shifted *ag-1* flower. Arrowheads indicate secondary floral buds.
(F) Mature, shifted *ag-1* flower. All floral organs in the primary flower were removed. Numbers indicate the order of secondary flowers.
ap, inflorescence apex; f, floral bud; p, petal primordium; s, sepal primordium; 2°f, secondary floral bud. In **(A)** to **(D)** and **(F)**, bars = 50 μm; in **(E)**, bar = 100 μm.

to a gynoecium and continued to produce perianth organ primordia in a whorled phyllotaxy (Figure 2C). This observation indicates that *ag-1 co-2* lateral meristems are initially indeterminate floral meristems. After generating two or three repetitive sets of perianth organs, however, each early-arising *ag-1 co-2* floral meristem produced an inflorescence shoot in the central region instead of floral organs, exhibiting floral reversion (Figure 1D). The central region of *ag-1 co-2*

floral meristems became morphologically indistinguishable from the inflorescence apex, giving rise to floral meristems in a spiral phyllotaxy (Figure 2D). These observations reveal that floral reversion in the *ag-1 co-2* plants is due to transformation of the preexisting, indeterminate floral meristem into an inflorescence shoot meristem.

Whereas early-arising *ag-1 co-2* flowers (approximately five to 10 first formed) underwent reversion, late-arising flowers did not (data not shown), suggesting that *AG* may be required for determination of floral meristem identity only during early stages of reproductive development in the absence of *CO* function or flowering stimulus provided by inductive photoperiods. To test whether *AG* is involved in specifying floral meristem identity throughout inflorescence development, we performed a noninductive shift experiment and examined the identity of late-arising lateral meristems that developed during and/or after the shift. Wild-type and *ag-1* plants were grown initially under inductive photoperiods until they had produced >10 mature flowers and then shifted to darkness for 24 to 48 hr. Approximately 1 week after the shift, reversion was apparent in all flowers developing in the apical portion of the *ag* inflorescence but not in that of the wild-type inflorescence. The shifted, late-arising *ag* flowers continued to produce both floral organs and lateral secondary flowers (Figure 1E).

While the perianth organs of the primary flowers were detached by senescence and secondary flowers grew, these lateral structures formed an inflorescence branch, although there was apparently no internode elongation between the secondary flowers (Figure 1F). Although these lateral apices retained floral features to give rise to floral organ primordia in concentric whorls (Figure 2E), the apices also produced lateral meristems spirally and acropetally and exhibited traits of the inflorescence shoot meristem (Figures 2E and 2F). This demonstrates that *ag* plants fail to define fully floral meristem identity in late-arising flowers as well as early-arising ones, suggesting that *AG* is required for determination of floral meristem identity throughout reproductive growth.

Ectopic *AG* Function Mediates Terminal Flower Formation

Given that *AG* is one of the principal regulators of floral meristem identity, *AG* function could be sufficient to promote

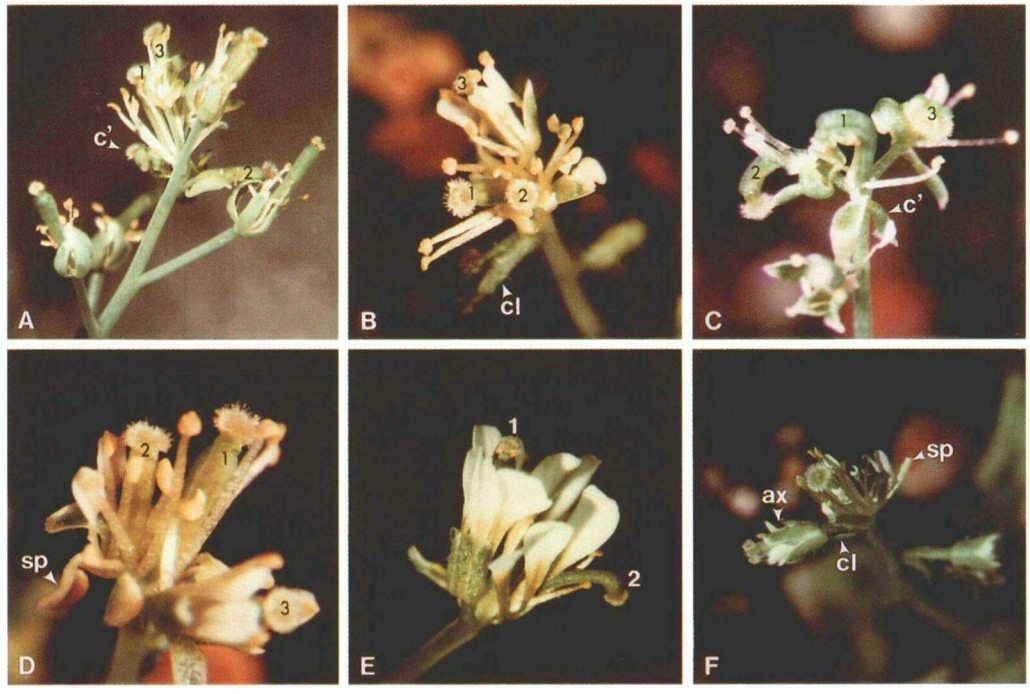

Figure 3. Terminal Flowers in Primary Inflorescences and Inflorescence Branches.

(A) to **(C)** Terminal flowers in the apices of primary inflorescences of 35S–*AG* plants.
(D) Terminal flower in a branch of the primary inflorescence of a 35S–*AG* plant.
(E) Terminal flower in the *tfl1-2* primary inflorescence apex.
(F) Solitary terminal flower with a single pistil in a 35S–*AG tfl1-2* primary inflorescence apex. Each axillary branch is also a solitary flower.
Numbers indicate pistils of the terminal flowers. ax, axillary flower; c′, carpelloid organ; cl, cauline leaf; sp, staminoid petal.

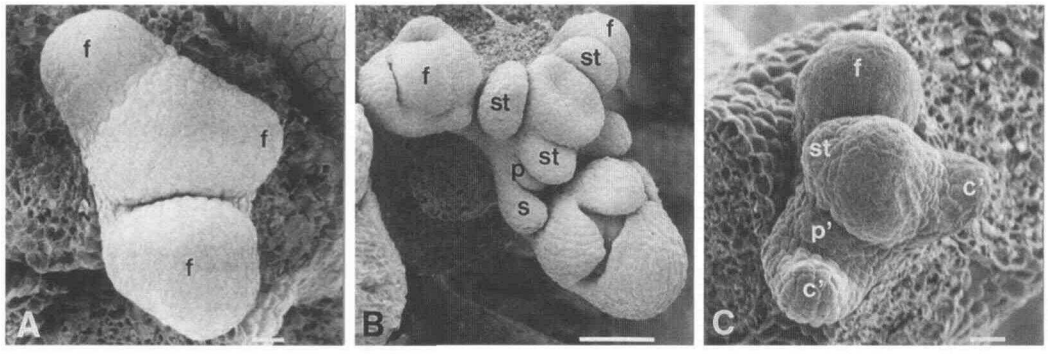

Figure 4. Structures of Primary Inflorescence Apices.

(A) Wild-type inflorescence apex. Floral meristems emerge from the peripheral regions of the inflorescence meristem.
(B) *tfl1-2* primary inflorescence apex producing terminal floral organ primordia.
(C) Inflorescence apex of a 35S–*AG* plant. The terminal meristem gives rise to floral organ primordia.
c′, carpelloid organ primordium; f, lateral floral meristem or bud; p, petal primordium; p′, petaloid organ primordium; s, carpelloid sepal primordium; st, stamen primordium. In **(A)** and **(C)**, bars = 10 μm; in **(B)**, bar = 50 μm.

floral meristem identity in reproductive shoot meristems. We observed previously that the terminal flowerlike structure was formed in inflorescence apices of several 35S–*AG* transformants in which *AG* was ectopically expressed throughout plants (Mizukami and Ma, 1992). This preliminary observation supports the hypothesis that *AG* may be a key regulator of floral meristem identity. To examine further the function of *AG* in promoting floral meristem identity, we analyzed the fate of apical and lateral meristems and the origin of terminal floral structures of 35S–*AG* plants. As shown in Figures 3A to 3C, the inflorescences of the 35S–*AG* plants produced a small number of lateral flowers (4.8 ± 3.2 from a total of 27 plants examined), and eventually their apices were abnormally terminated with a cluster of flowers. Sepals and petals of all flowers, including the apical terminal flowers, were replaced by carpelloid sepals and staminoid petals, respectively, resulting from ectopic *AG* function in the outer two floral whorls, as described previously (Mizukami and Ma, 1992). Terminal flowers included an apical sessile flower(s) and occasionally unfused carpels at the center (Figure 3B). Branches in primary inflorescences of the 35S–*AG* plants also ended with apical flowers. In severe cases, these branches had only a single terminal flower consisting of more than one pistil (Figure 3D). Secondary inflorescences arising from the axils of rosette leaves had the same phenotype (data not shown).

Ectopic *AG* Function Has an Additive Effect on Determination of the *terminal flower1* Inflorescence

The early termination of the inflorescence apex by an abnormal cluster of flowers was also seen in *terminal flower1* (*tfl1*) mutants (Figure 3E; Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). Although *tfl1-2* mutants as well as 35S–*AG* plants rarely bear apical flowers with a single pistil (2.6 ± 0.9 pistils from a total of 15 *tfl1-2* terminal flowers examined and 1.9 ± 0.9 pistils from a total of 27 35S–*AG* terminal flowers examined), a solitary apical flower consisting of a single pistil and many staminoid organs was commonly observed in the 35S–*AG tfl1-2* plants, the *tfl1-2* mutants carrying the 35S–*AG* transgene (Figure 3F; four of five plants examined had a terminal flower with a single pistil). This demonstrates that ectopic *AG* function has an additive effect on the *tfl1* inflorescence meristems.

Normal inflorescence meristems, shown in Figure 4A, maintained an undifferentiated, indeterminate apex, whereas the apex of the *tfl1-2* inflorescence meristem gave rise to floral organ primordia and became determinate (Figure 4B). As in the *tfl1* inflorescence meristem, the apex of the inflorescence meristem of the 35S–*AG* plant also gave rise to floral organ primordia (Figure 4C), indicating that the apex lost the activity of shoot meristems. Unlike floral lateral meristems, the floral terminal meristems of 35S–*AG* plants as well as those of *tfl1* mutants did not produce normal numbers of primordia for each floral organ; furthermore, the primordia were not arranged in concentric whorls with a single center (Figures 4B and 4C). Consequently, the terminal flowers in 35S–*AG* plants were sessile and had abnormal numbers of floral organs, as was the case for *tfl1* mutants (Figures 3B, 3D, and 3E). These observations reveal that termination of the inflorescence in 35S–*AG* plants was due to production of floral organ primordia from the central portion of inflorescence meristems, indicating that ectopic *AG* function evokes determinate floral meristem fate in the center of indeterminate inflorescence meristems.

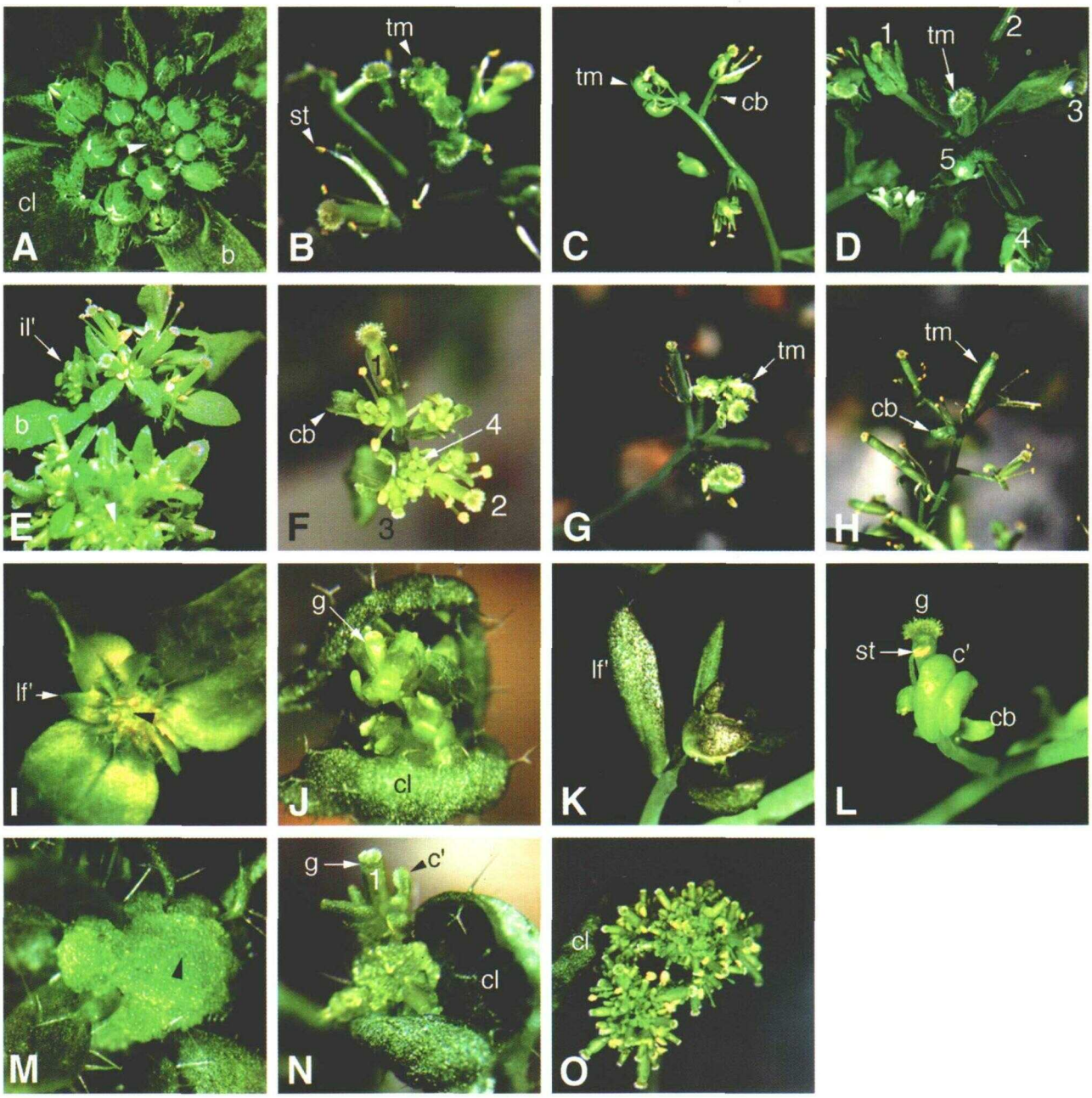

Figure 5. Phenotypes of Primary Inflorescences and Flowers.

(A) *lfy-5* primary inflorescence. The arrowhead indicates the indeterminate inflorescence center.
(B) 35S–*AG lfy-5* primary inflorescence.
(C) Terminal structure of the 35S–*AG lfy-5* primary inflorescence.
(D) Terminal structure of the *lfy-5 tfl1-2* primary inflorescence. Numbers indicate axillary flowers of the terminal structure.
(E) *ap1-1* primary inflorescence. The inflorescence apex is indicated with an arrowhead. The first-arising lateral meristem developed as an inflorescence shoot.
(F) 35S–*AG ap1-1* primary inflorescence. Numbers indicate the order of flowers.
(G) Terminal structure of the 35S–*AG ap1-1* primary inflorescence.
(H) Terminal structure of the *ap1-1 tfl1-2* primary inflorescence.
(I) *lfy-5 ap1-1* primary inflorescence. An arrowhead indicates the inflorescence center.
(J) 35S–*AG lfy-5 ap1-1* primary inflorescence.

Ectopic *AG* Function Promotes Determinate Floral Fate in Indeterminate Lateral Meristems of Floral Meristem Identity Mutants

To test whether the alteration of meristem identity by constitutive *AG* expression requires the functions of the floral meristem identity genes *LFY* and *AP1*, we generated 35S–*AG* plants with *lfy-5* and/or *ap1-1* mutant backgrounds and examined the effects of constitutive *AG* expression on apical and lateral meristem identity. In addition, to examine whether *AG* also requires the function of another floral meristem identity gene, *CAULIFLOWER* (*CAL*) (Bowman et al., 1993; Kempin et al., 1995), to promote determinate floral meristem identity, we examined the effect of ectopic *AG* expression on indeterminate lateral meristems of *ap1-1 cal-1* double mutants.

35S–AG lfy-5

As shown in Figures 5A and 6A, the *lfy-5* mutation causes partial conversion of floral meristems to shoot meristems. Consequently, *lfy-5* mutants have an increased number of branches in primary inflorescences (9.2 ± 1.2 from a total of six *lfy-5* plants examined versus 3.0 ± 0.6 from a total of 10 wild-type plants examined) and shootlike features in early-arising lateral floral buds. The *lfy-5* floral buds are subtended by a bract and consist of bract/sepal mosaic organs enclosing a number of secondary buds (Figures 6A and 6B). The effects of *lfy* mutations are attenuated acropetally such that late-arising lateral meristems develop into flowers without shootlike features (Schultz and Haughn, 1991; Weigel et al., 1992). However, because *LFY* positively controls the expression of *AP3* and *PISTILLATA* (*PI*) (Weigel and Meyerowitz, 1993), which are required for petal and stamen identity (Bowman et al., 1989, 1991), petal and stamen development is greatly affected even in the late-arising *lfy* flowers (Weigel et al., 1992).

Ectopic *AG* function in *lfy-5* plants significantly reduced the size of inflorescences (Figures 5B, 6C, and 6N) and shootlike features of early-arising flowers (Figures 5B, 6C, and 6D). In general, the inflorescence structure of the 35S–*AG lfy-5* plants resembled those of the *lfy-5 tfl1-2* double mutant (Figures 5C and 5D) and other *lfy tfl1* double mutants (Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993). The primary inflorescence of 35S–*AG lfy-5* mutants produced two or three inflorescence branches and fewer than six lateral flowers (4.5 ± 1.3 lateral flowers before termination from a total of four 35S–*AG lfy-5* plants examined), each subtended by a bract (Figures 5B, 6C, and 6N), and was terminated by apical flowers (Figures 5C, 6N, and 6O). In addition to the homeotic conversion of sepals to carpelloid organs, all bracts of 35S–*AG lfy-5* plants were carpelloid (Figure 6N), whereas both rosette and cauline leaves maintained leaf identity, although they were abnormally curled up toward the adaxial surface (data not shown). Figure 6D shows a flower, derived from the first-arising lateral meristem, that consists of carpelloid sepals, staminoid petals, stamens, and a gynoecium, as found in flowers of 35S–*AG* plants with a wild-type *LFY* background (Figure 3). Although first-arising flowers occasionally produced one or two secondary lateral floral buds (data not shown), the shootlike indeterminate structures that are common in early-arising *lfy-5* buds were not observed in 35S-*AG lfy-5* plants.

35S–AG ap1-1

More than 20 early-arising flowers in the primary inflorescence of *ap1-1* mutants produced secondary lateral shoots and/or buds, each subtended by a bract and arranged in an incomplete spiral phyllotaxy (Figures 5E, 6E, and 6F; Bowman et al., 1993). The central region of these flowers was terminated eventually by the formation of a normal gynoecium (Figures 5E and 6F). As in *lfy* mutants, the inflorescence-like features of *ap1* flowers decreased acropetally during inflorescence development (Bowman et al., 1993). Because *AP1* also specifies the identity of sepal and petal (Irish and Sussex, 1990; Mandel et al., 1992), *ap1-1* flowers failed to produce these organs (Figures 5E and 6F). Ectopic *AG* function in *ap1-1* plants dramatically reduced inflorescence size as well as the inflorescence-like features of *ap1-1* flowers (Figures 5F, 6G, and 6H). The number of inflorescence branches in primary inflorescences was reduced (2.0 ± 0 per plant, $n = 4$ 35S–*AG ap1-1* plants; 4.1 ± 0.9 per plant, $n = 8$ *ap1-1* plants), and a small number of lateral flowers was made in the primary inflorescence (4.3 ± 1.3 from a total of four plants examined), which became terminated with flowers consisting of a central carpelloid organ surrounded by several radial flowers (Figure 5G). This inflorescence structure is similar

Figure 5. (continued).

(K) Late-arising *lfy-5 ap1-1* lateral shoot.

(L) Late-arising 35S–*AG lfy-5 ap1-1* lateral flower.

(M) *cal-1 ap1-1* primary inflorescence. An arrowhead indicates the inflorescence center.

(N) 35S–*AG cal-1 ap1-1* primary inflorescence. The gynoecium of the first-arising flower is indicated by 1.

(O) 35S–*AG cal-1 ap1-1* primary inflorescence at later development.

b, bract; c′, carpelloid organ; cb, carpelloid bract; cl, cauline leaf; g, gynoecium; il′; inflorescence-like structure; lf′, leaflike organ; st, stamen; tm, terminal structure.

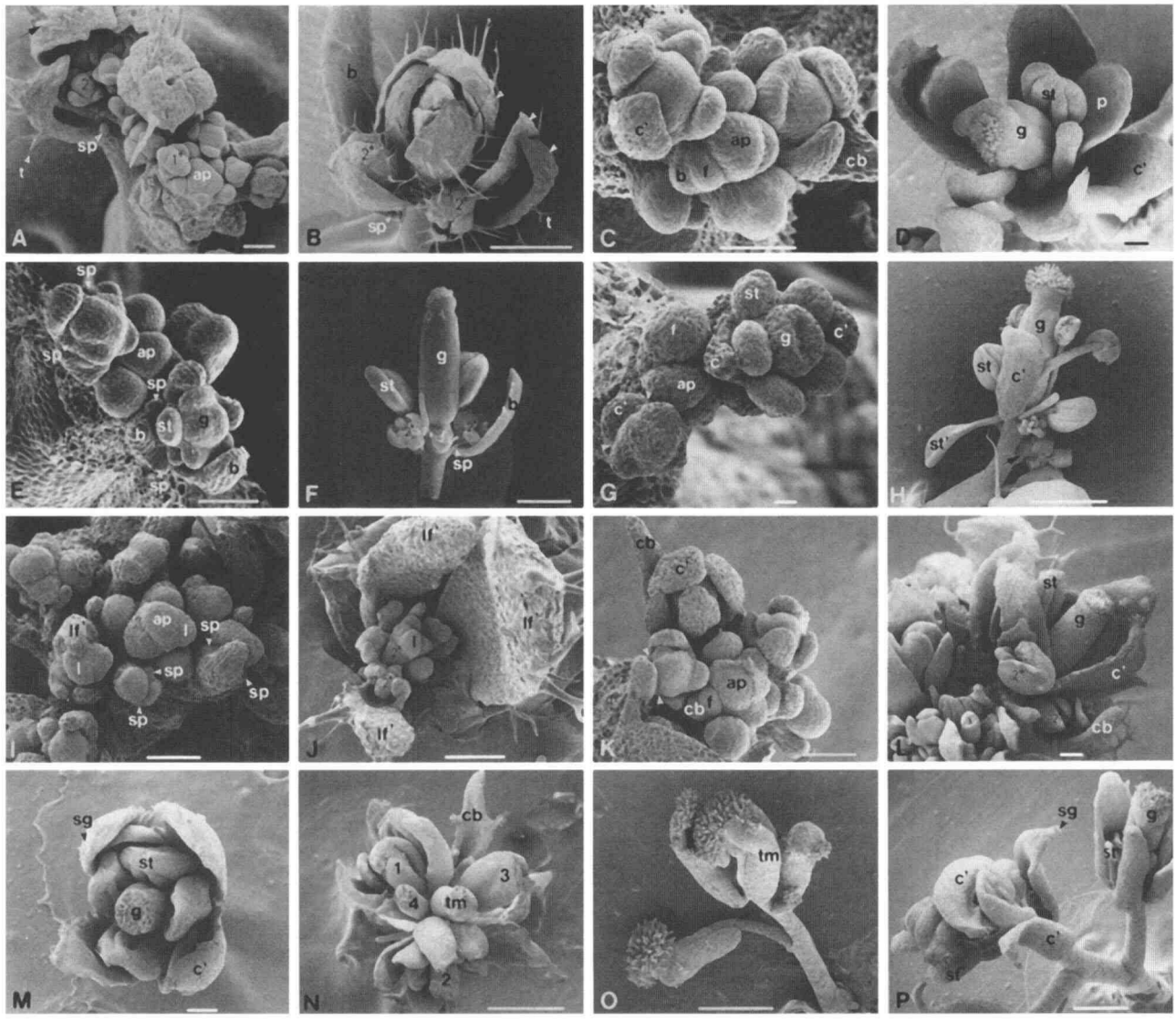

Figure 6. Scanning Electron Microscopy of Inflorescence Apices and Flowers.

(A) Apex of the young *lfy-5* primary inflorescence. An arrow indicates a long cell in a sepal/leaf organ.
(B) Third-arising *lfy-5* flower. Arrowheads indicate layers of sepal/leaf organs.
(C) Apex of the young 35S–*AG lfy-5* primary inflorescence.
(D) Early-arising 35S–*AG lfy-5* flower.
(E) Apex of the *ap1-1* primary inflorescence.
(F) Intermediate-arising *ap1-1* flower.
(G) Apex of the young 35S–*AG ap1-1* primary inflorescence. There are no stipules at the base of carpelloid organs (arrowheads).
(H) First-arising flower in the 35S–*AG ap1-1* primary inflorescence. An arrow indicates the primary inflorescence.
(I) Apex of the young *lfy-5 ap1-1* primary inflorescence.
(J) Early-arising *lfy-5 ap1-1* lateral structure.
(K) Apex of the young 35S–*AG lfy-5 ap1-1* primary inflorescence. There are stipules at the base of carpelloid organs (arrowheads).
(L) First-arising flower in the 35S–*AG lfy-5 ap1-1* primary inflorescence. Subsequent flowers are also seen at lower left.
(M) Late-arising 35S–*AG lfy-5 ap1-1* flower.
(N) Entire 35S–*AG lfy-5* primary inflorescence. Numbers indicate the order of lateral flowers.
(O) Terminal structure of the 35S–*AG lfy-5* primary inflorescence.
(P) Terminal structure of the 35S–*AG lfy-5 ap1-1* primary inflorescence.
ap, apical inflorescence meristem; b, bract; c′, carpelloid organ; cb, carpelloid bract; f, floral meristem; g, gynoecium; l, lateral shoot meristem; lf′, leaflike organ; p, petal; sg, stigma; sp, stipule; st, stamen; st′, staminoid organ; t, branched trichome; tm, terminal structure; 1°, primary lateral bud; 2°, secondary lateral bud. In **(A)**, **(E)**, **(G)**, **(I)**, and **(K)**, bars = 50 μm; in **(B)**, **(D)**, **(F)**, and **(N)** to **(P)**, bars = 500 μm; in **(C)**, bar = 10 μm; in **(H)**, **(J)**, **(L)**, and **(M)**, bar = 100 μm.

to those of *ap1-1 tfl1-2* (Figure 5H) and *ap1-10 tfl1-10* plants (Schultz and Haughn, 1993). Even the first-arising flowers were no longer highly branched (Figures 5F and 6H), having at most two or three lateral secondary flowers like the late-arising *ap1-1* flowers (Figure 6F). As in the *ap1-1 tfl1-2* plant (Figure 5H), each bract subtending a secondary flower was transformed into a carpelloid organ (Figure 5F).

35S–AG lfy-5 ap1-1

Lateral meristems in *lfy-5 ap1-1* plants form shoots instead of flowers (Figures 5I and 5K; Weigel and Meyerowitz, 1993); that is, early-arising lateral shoots and inflorescence apical shoots are indistinguishable (Figures 6I and 6J), indicating that inflorescence shoots are converted to sterile shoots with leaves. Much later in inflorescence development, lateral meristems eventually give rise to organs exhibiting some floral traits (Weigel and Meyerowitz, 1993). However, no staminoid/petaloid organs were observed even in late-arising *lfy-5 ap1-1* flowers. Previous observations that transcripts of *AP3* and *PI* genes are rarely detected in *lfy-5 ap1-1* mutants, as in *lfy-6 ap1-1* mutants (Weigel and Meyerowitz, 1993), explain the absence of petals and stamens.

Ectopic *AG* function in *lfy-5 ap1-1* plants substantially restored floral fate to lateral meristems (Figures 5J and 5L). Early-arising lateral meristems produced carpelloid organs with or without axillary floral buds in the outermost whorl and then produced staminoid organs and a central gynoecium to the center (Figures 6K and 6L). Surprisingly, late-arising flowers (approximately seventh- and later-arising flowers) exhibited the phyllotaxy and determinacy of normal flowers; that is, these flowers had carpelloid organs, stamens, and a central fused gynoecium with a whorled phyllotaxy (Figures 5L, 6M, and 6P). However, as occurred in 35S–*AG lfy-5* plants, most flowers were subtended by a carpelloid bract (Figures 5L, 6K, and 6L). Finally, after producing a small number of lateral flowers (<10; Figure 5J), the 35S–*AG lfy-5 ap1-1* inflorescence apex was terminated by abnormal flowers consisting of carpelloid organs, staminoid organs, and unfused or fused carpels, but not petals or petaloid organs (Figure 6P).

35S–AG ap1-1 cal-1

CAL plays a redundant role in specification of floral meristem identity by interacting with *AP1*, and the effect of a *cal-1* mutation is apparent only when it is combined with an *ap1* mutation. As shown in Figure 5M, lateral meristems of *ap1-1 cal-1* double mutants are indeterminate. The primary meristems continuously produce indeterminate lateral meristems and never differentiate into any floral organs in early inflorescence development (Figure 5M), although very late arising lateral meristems eventually form functional carpels and stamens (data not shown). Ectopic *AG* function resulted in formation of flowers with central carpels surrounded by stamens upon the transition to flowering (Figure 5N). Although differentiation of floral organs was slower than that in wild-type plants (Figure 5N), all lateral meristems did differentiate into flowers (Figure 5O). Eventually, the apical meristems of 35S–*AG ap1-1 cal-1* plants were terminated by the production of multiple carpelloid organs (data not shown). 35S–*AG ap1-1 cal-1* inflorescences had more lateral flowers than did 35S–*AG ap1-1* inflorescences (Figures 5F and 5O). In contrast to 35S–*AG ap1-1* plants, early-arising flowers in 35S–*AG ap1-1 cal-1* plants were more determinate, that is, they often formed no secondary flowers (Figure 5N), whereas most subsequent flowers had some secondary flowers (data not shown). These observations demonstrate that *AP1* and *CAL* functions are not absolutely required for ectopic *AG* action to promote determinate floral fate in the central region of indeterminate apical and lateral meristems.

Overall, ectopic *AG* function essentially restored determinate floral fate to the central region of defective lateral meristems and suppressed indeterminate shoot features in both *lfy-5* and *ap1-1* single and double mutants as well as in *ap1-1 cal-1* double mutants. Furthermore, in all cases, primary shoot apices were terminated precociously with ectopic flowers. These results strongly suggest that ectopic *AG* function can promote determinate floral fate, at least in central regions of apical and lateral shoot meristems, in the absence of the normal functions of floral meristem identity genes.

Ectopic *AG* Function Accelerates the Transition to Flowering

In addition to promoting floral meristem identity in inflorescence shoot meristems, ectopic *AG* function also has an effect on the transition of shoot apical meristems from vegetative to reproductive growth. As shown in Figure 7A, 35S–*AG* plants grown under continuous lighting (CL) conditions flowered much earlier than did the wild-type and other control plants by all three criteria we had used (see Methods). Two additional independent 35S–*AG* lines (see Methods) also flowered precociously (shown only are the results for ATG-1w1 and ATG-1w2 in Figure 7B), whereas two control lines, one carrying the vector pMON530 only and the other carrying a 35S–*1MI* construct that encodes a truncated, nonfunctional AG protein (Mizukami et al., 1996), flowered as late as wild-type plants. This observation demonstrates that ectopic *AG* function accelerates the transition to flowering.

To examine whether *AG* is necessary in regulation of the transition to flowering normally, we measured flowering time of *ag-1* plants as well as plants carrying mutations in other floral meristem identity genes. Whereas flowering in *lfy-5* mutants was delayed slightly, plants homozygous for *ap1-1* and *ap2-1* mutations, the latter of which has a minor effect on floral meristem identity (Shannon and Meeks-Wagner, 1993), flowered almost at the same time as the wild type (Figure

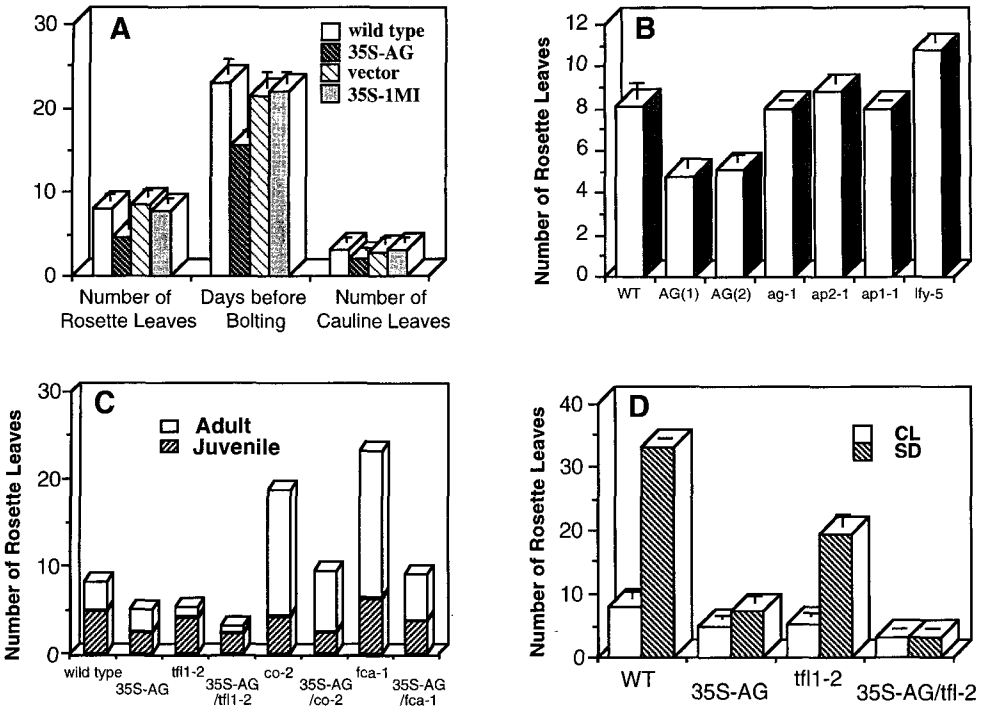

Figure 7. Effects of Ectopic *AG* Function on the Transition to Flowering.

(A) Comparison of flowering time between 35S–*AG* plants and the control plants. Three different criteria were used for measuring flowering time (see Methods). Wild type, wild-type Landsberg *erecta* plants; 35S–AG, 35S–*AG* plants (line ATG-1w1); vector, transgenic plants carrying the pMON350 vector (Mizukami and Ma, 1992); 35S–1MI, transgenic plants carrying a 35S–*1MI* construct encoding a nonfunctional, truncated AG protein (Mizukami et al., 1996).

(B) Flowering time in floral meristem identity mutants and 35S–*AG* plants. WT, wild type; AG(1), line ATG-1w1; AG(2), line ATG-1w2.

(C) Effect of the 35S–*AG* transgene on flowering time and vegetative phase transition in early- (*tfl1-2*) and late- (*co-2* and *fca-1*) flowering mutants. Numbers of juvenile and adult rosette leaves that were developed before flowering are shown.

(D) Effect of photoperiods on the early flowering phenotype caused by the *tfl1* mutation and/or ectopic *AG* function. Numbers of rosette leaves formed before flowering under CL and SD photoperiods are shown.

Bars in 7A, 7B, and 7D indicate the standard deviation.

7B); this result essentially agreed with previous observations (Shannon and Meeks-Wagner, 1993). *ag-1* plants exhibited no abnormality in the timing of their transition to flowering (Figure 7B). This result demonstrates that the absence of *AG* function does not alter flowering time, indicating that *AG* is not required for controlling the normal transition to flowering.

Ectopic *AG* Function Accelerates the Vegetative Phase Change of the Apical Shoot Meristem

In general, shoot apical meristems require the acquisition of meristematic competence to respond to floral-inducing stimuli (McDaniel et al., 1992). The successive alteration of vegetative shoot apical meristems from the noncompetent (juvenile) to the competent (adult) phase is associated with morphological changes of leaves, in particular, the appearance of the trichomes on the adaxial and abaxial surface of leaves (Poethig, 1990). To investigate whether ectopic *AG* function accelerates the physiological transition of vegetative shoot apical meristems, we measured the duration of the juvenile phase, namely, the number of rosette leaves lacking abaxial trichomes, in plants grown under CL conditions. As shown in Figure 7C, the duration of both juvenile and adult vegetative phases was shortened in 35S–*AG* plants, although the relative proportion of the two phases was generally not altered by ectopic *AG* function (data not shown). This clearly demonstrates that ectopic *AG* function also accelerates the vegetative phase transition.

Ectopic *AG* Function Can Substitute for Internal and External Floral Inductive Stimuli

Physiological changes associated with competence of shoot apical meristems to undergo the flowering transition are reg-

ulated by external and internal floral inductive stimuli mediated by photoperiods and/or genetic background. To investigate whether *AG* function in the promotion of the transition to flowering depends on such floral inductive stimuli, we examined the effects of noninductive SD photoperiods on the flowering time of 35S–*AG* plants. Whereas flowering in *tfl1-2* plants as well as in wild-type plants was severely delayed under SD conditions compared with that under CL conditions (Figure 7D), 35S–*AG* plants exhibited only a slight delay of flowering under SD conditions (Figure 7D), although the inflorescences had more flowers before terminating than under CL conditions (data not shown). Strikingly, the effect of SD photoperiods on the flowering transition was completely eliminated in 35S–*AG tfl1-2* plants; these plants flowered as early under SD conditions as they did under CL conditions (Figure 7D). Furthermore, SD photoperiods did not alter the vegetative phase change in 35S–*AG tfl1-2* plants (Figure 7C). These results demonstrate that floral promotion activity by ectopic *AG* function is affected less by photoperiods and can substitute for floral promotion stimuli supplied by inductive photoperiods.

Recent genetic studies on the transition to flowering have revealed that there are at least two groups of genes that promote flowering (Koornneef et al., 1991; Martinez-Zapater et al., 1994): one group (which includes *FCA*) is involved in the constitutive flowering promotion pathway, whereas the other group (which involves *CO*) participates in the promotion of flowering under inductive photoperiods. Mutation of these genes results in a late-flowering phenotype. To test whether ectopic *AG* function is regulated by these floral promotion genes, we examined the effects of mutations in these genes on flowering time of 35S–*AG* plants. As shown in Figure 7C, the duration of vegetative development in both *co-2* and *fca-1* plants is greatly extended relative to that of wild-type plants. Ectopic *AG* function in these late-flowering mutant plants substantially reduced the duration of vegetative growth, restoring flowering time to that of nontransgenic, wild-type plants (Figure 7C). In addition, the vegetative phase transition was also accelerated by ectopic *AG* function (Figure 7C). These results demonstrate that the function of these floral promotion genes can be substituted at least partly by ectopic *AG* function, suggesting that *AG* may act through an independent pathway.

Table 1. Specification of Apical and Lateral Meristem Fate by Floral Meristem Identity Genes

Genotype	Apical Meristem (AM) Identity[a]	Lateral Meristem (LM) Identity[a]	*AG* Function[b] (AM/LM)	References[c]
Wild type	I	F	−/+	(1)
lfy	I	I–F	−/±[d]	(2, 3, 4)
ap1	I	I–F	−/±[d]	(5, 6)
ag	I	F (F–I)	−/−	(7, 8)
tfl1	F	F	+/+	(9)
lfy ap1	I	I	−/−[d]	(4, 6, 10, 11)
ap1 cal	I	I	±/±[d]	(6, 12)
lfy tfl1	F	F	±/±[d]	(10, 11)
ap1 tfl1	F	F	±/±[d]	(10, 11)
ag tfl1	F (F–I)[e]	F (F–I)[e]	−/−	(9)
lfy ap1 tfl1	I	I	−/−[d]	(10, 11)
35S–*AG*	F	F	+/+[f]	(8)
35S–*AG lfy*	F	F	+/+[f]	(8)
35S–*AG ap1*	F	F	+/+[f]	(8)
35S–*AG tfl1*	F	F	+/+[f]	(8)
35S–*AG lfy ap1*	F	F	+/+[f]	(8)
35S–*AG ap1 cal*	F	F	+/+[f]	(8)

[a] I and F indicate indeterminate inflorescence shoots and determinate flowers, respectively.
[b] *AG* function/expression is normally restricted to the center of floral meristems. (+) and (−) indicate the presence and the absence, respectively, of *AG* function. (±) indicates that *AG* function is present, but presumably not as normal as that in wild-type floral meristems.
[c] (1) Smyth et al. (1990); (2) Schultz and Haughn (1991); (3) Huala and Sussex (1992); (4) Weigel et al. (1992); (5) Irish and Sussex (1990); (6) Bowman et al. (1993); (7) Bowman et al. (1989, 1991); Yanofsky et al. (1990); (8) this study; (9) Shannon and Meeks-Wagner (1991); Alvarez et al. (1992); (10) Schultz and Haughn (1993); (11) Shannon and Meeks-Wagner (1993); (12) Kempin et al. (1995).
[d] Spatial and temporal expression pattern of *AG* mRNA is affected.
[e] Determinate floral state of both apical and lateral floral meristems are unstable (Y. Mizukami and H. Ma, unpublished result).
[f] *AG* is ectopically expressed throughout the plants.

DISCUSSION

The key difference in the property of floral meristems from that of shoot meristems is that the former is determinate and produces a defined number of floral organ primordia, whereas the latter is indeterminate and continuously gives rise to lateral meristems and organ primordia. On the basis of our results presented here, in addition to previous results summarized in Table 1, *AG* plays an indispensable role in regulation of floral meristem determinacy, which provides the final definition of floral meristem identity. First, our loss-of-function analysis clearly demonstrated that *AG* is required for preventing the conversion of floral meristems to inflorescence shoot meristems even in the *LFY* and *AP1* background. Furthermore, our gain-of-function analysis revealed that constitutive *AG* function can promote the determinate floral pattern at the center of reproductive meristems in the *lfy* and/or *ap1* mutant background. Finally, we showed that ectopic *AG* function can generate precocious meristematic competence to flowering and floral promotion activity in the vegetative shoot apical meristem. These results strongly suggest that *AG* is a principal developmental switch that functions in the final definition of determinate floral meristem identity.

AG: A Developmental Switch That Defines Floral Meristem Identity

Previous phenotypic analysis of loss-of-function *ag* mutants suggested that *AG* is not involved in the formation of initial floral pattern (Bowman et al., 1989, 1991). Similarly, our observation has revealed that lateral meristems of the *ag-1 co-2* inflorescence as well as the shifted *ag-1* inflorescence initially exhibited a pattern characteristic of floral meristems, which give rise to floral organ primordia in concentric whorls. As they developed, however, these indeterminate floral meristems were transformed into inflorescence shoot meristems that produced lateral flowers in a spiral phyllotaxy. This provides evidence that floral meristems that have been initiated by *LFY* and *AP1* functions are neither determinate nor completely specified as floral meristems in the absence of *AG* function, such that those floral meristems can be transformed into inflorescence shoot meristems. Therefore, *AG* is indeed required for the final definition of floral meristem identity. In Antirrhinum, the ortholog of *AG*, *PLENA*, has also been shown to be required for floral meristem determinacy (Bradley et al., 1993), suggesting that the function of *AG* orthologs may also be required in other flowering plants to define the final floral meristem identity.

It has been reported that *LFY*, *AP1*, and *AG* mRNAs were not detected in the indeterminate inflorescence shoot apex (Drews et al., 1991; Mandel et al., 1992; Weigel et al., 1992). Our results with gain-of-function 35S–*AG* plants have demonstrated that constitutive *AG* function can promote determinate floral fate in the inflorescence shoot apical meristem, as illustrated in Figure 8A, suggesting that *AG* function is sufficient to promote determinate floral meristems. Furthermore, the determinate phenotype of 35S–*AG* inflorescences was enhanced by the *tfl1-2* mutation, which causes ectopic expression of *LFY* and *AP1* in the apical meristem and production of terminal flowers (Weigel et al., 1992; Gustafson-Brown et al., 1994). This indicates that the effect of constitutive *AG* function and those of ectopic *LFY* and *AP1* functions are additive in promoting determinate floral fate in the indeterminate inflorescence apex, suggesting that the phenotype of 35S–*AG* inflorescences was not directly due to ectopic expression of *LFY* and *AP1* in the inflorescence apex. Finally, ectopic *AG* function could promote determinacy in the indeterminate central region of apical and lateral meristems of the *lfy ap1* inflorescence and restore the production of determinate flowers. Together, these results indicate that even though *AG* expression in the floral meristem center normally is controlled by *LFY* and *AP1* functions, *AG* function in promoting determinate floral fate at the indeterminate center of reproductive meristems is independent of *LFY* and *AP1* functions.

Because *lfy-5* is not a null allele (Weigel et al., 1992), it is possible that promotion of the determinate floral meristem by ectopic *AG* function in *lfy-5 ap1-1* plants may rely on residual *LFY* function. However, this is not likely to be the case for the following reasons. It is known that the *ap1-1 lfy-5* double mutant has phenotypes as severe as those in the double mutant of *ap1-1* with *lfy-6*, a presumed null allele (Weigel et al., 1992). Similarly, dramatic enhancement of phenotypes is also observed when the intermediate *ap1-4* allele is combined with *lfy-5* (Bowman et al., 1993). Thus, *LFY* and *AP1* mutually enhance each other's function (Bowman et al., 1993), and the *LFY* activity in *lfy-5 ap1-1* plants is as low as that in *lfy-6 ap1-1* plants. Therefore, it is unlikely that residual *LFY* function contributes to the definition of floral meristem identity by *AG* in the *lfy-5 ap1-1* apical and lateral meristems.

As in 35S–*LFY* plants, however, the determinate growth of 35S–*AG* reproductive meristems was attenuated by mutations in other floral meristem identity genes such that 35S–*AG* inflorescences produced more lateral flowers before termination and the early-arising flowers had a few axially secondary flow-

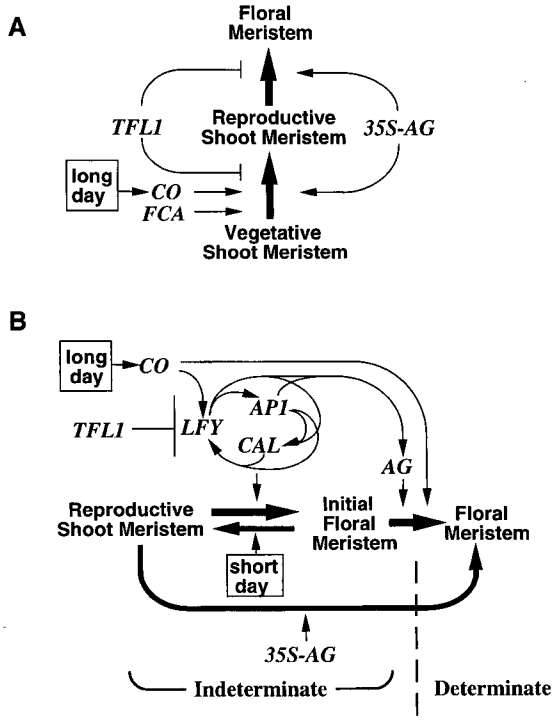

Figure 8. Models for Gene Actions Controlling Reproductive Meristem Identities.

Only the genes that are discussed in this report are shown here. These models have incorporated elements from previous models (Bowman et al., 1993; Schultz and Haughn, 1993; Martinez-Zapater et al., 1994).

(A) Control of apical meristem identity.

(B) Control of lateral meristem identity.

The thick arrows indicate developmental processes. The thin arrows represent positive interactions or effects, whereas the thin lines with a thin bar represent negative interactions or effects.

ers in the first whorl. Thus, ectopic *AG* function may not be sufficient for the complete suppression of lateral meristem formation in the peripheral region of the reproductive meristems in *lfy* and/or *ap1* mutant background, although it is sufficient to promote determinacy at the meristem center. It is possible that during early inflorescence development, determination of meristem growth by *AG* requires the function(s) of some other gene(s) that is spatially and/or temporally expressed only in the central region of floral meristems (see below).

Ectopic *AG* Function Promotes Meristematic Competence and Floral Promotion Activity

The existence of vegetative growth in 35S–*AG* plants before the transition to flowering demonstrates that ectopic *AG* function cannot alter the activity of the shoot apical meristem immediately from the vegetative to the floral state. Similar results were reported previously with 35S–*LFY* and 35S–*AP1* plants (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995). These observations together indicate that a certain physiological and molecular state(s) of meristematic cells is required for responding to meristem identity gene actions. It has been shown that the shoot apical meristem must first become competent to respond to floral stimuli (McDaniel et al., 1992). Although the molecular basis of meristematic competence remains unknown, the competence is likely necessary for the establishment of determinate floral meristem fate by floral meristem identity genes.

Despite the existence of vegetative growth in 35S–*AG* plants, ectopic *AG* function dramatically reduced the duration of the vegetative phase in these plants, resulting in early flowering. An important aspect of this phenotype is that ectopic *AG* accelerates the vegetative phase change from noncompetent juvenile phase to competent adult phase. Thus, ectopic *AG* most likely functions in accumulating meristematic competence in the vegetative shoot apical meristem. Similarly, the adult phase was also shortened in 35S–*AG* plants, suggesting that ectopic *AG* can function in increasing a floral promotion factor(s) or meristematic sensitivity to perceive it (Figure 8A). In fact, ectopic *AG* function substantially suppresses the delay of the flowering transition caused by reduction of flowering inductive stimuli in late-flowering mutants and in plants grown under noninductive conditions. Therefore, *AG* may generate floral promotion activity.

An early flowering phenotype was also reported with 35S–*LFY* plants that were grown under SD conditions (Weigel and Nilsson, 1995). However, ectopic *LFY* function truncates only the adult phase but not the juvenile phase of vegetative development. This 35S–*LFY* phenotype indicates that *LFY* neither functions in promoting meristematic competence nor activates floral genes such as *AG* during the juvenile phase of vegetative development. Thus, LFY action is likely restricted to the meristems that are competent to flower. On the contrary, early flowering under both SD and long-day conditions was observed in 35S–*AP1* plants (Mandel and Yanofsky, 1995), as in 35S–*AG* transgenic plants, suggesting that constitutive *AP1* function also acts as a floral promoter. However, the early-flowering phenotype of 35S–*AP1* plants could be mediated by the function(s) of a downstream gene(s) controlled by AP1 (Mandel and Yanofsky, 1995), such as *AG*.

How can floral promotion activity generated by *AG* contribute to Arabidopsis reproductive development? *AG* is unlikely to be involved in the transition of the shoot apical meristem to flowering because the loss-of-function *ag* mutation has no effect on flowering time, and *AG* expression could not be detected during vegetative development. It is known that the floral promotion activity is not only necessary for the transition to flowering but also for the maintenance of reproductive development (Battey and Lyndon, 1990; Martinez-Zapater et al., 1994). Therefore, it is most likely that *AG* may normally contribute to maintaining flower development by producing floral promotion activity within flowers.

It was recognized previously that floral promotion activity at the apical meristem continues to increase as the primary inflorescence develops (Bowman et al., 1993; Schultz and Haughn, 1993; Martinez-Zapater et al., 1994). Weigel and Nilsson (1995) have suggested that such accumulation of floral promotion activity can later partially substitute for the functions of the floral meristem identity genes such as *LFY* and *AP1* during inflorescence development. Because ectopic *AG* function increases floral promotion activity, restoration of floral meristem identity in the 35S–*AG lfy ap1* lateral meristems can be explained by the result of precocious accumulation of floral promotion activity due to ectopic *AG* function.

Gene Action That Controls Floral Meristem Identity

Our results with 35S–*AG* plants clearly demonstrate that *AG* plays an indispensable role in the determination of floral meristem identity. These results, in addition to previous observations (Bowman et al., 1993; Okamuro et al., 1993; Schultz and Haughn, 1993; Haughn et al., 1995), suggest that the determinate floral fate of lateral meristems is progressively acquired because of functions of at least two sets of genes that regulate distinct aspects of floral meristem identity, as shown in Figure 8B. The early floral meristem, designated as the initial floral meristem, in which secondary lateral meristem formation is inhibited and perianth organs are produced in a whorled phyllotaxy, is first established by functions of the floral meristem identity genes, such as *LFY* and *AP1*, in the peripheral region of the inflorescence shoot meristem (Bowman et al., 1993; Kempin et al., 1995). The determinate floral fate is then specified by *AG* such that the

cells in the central region of floral meristems become committed to the final determinate and reproductive state. In the absence of *AG* function, the cells in the indeterminate central region repeat the pattern of the early state indefinitely. It is likely that floral promotion activity supplied by inductive photoperiods is required to maintain the early floral pattern, probably through positively regulating a floral meristem identity gene(s) (Putterill et al., 1995). Therefore, when *AG* function is absent, mutations or growth conditions that reduce such floral promotion activity cause conversion of the initial indeterminate floral meristem to the indeterminate inflorescence shoot meristem. On the contrary, when *AG* function is present, initial floral meristems are specified rapidly to become determinate floral meristems, and thus conversion to inflorescence shoot meristems is prevented.

Our results also revealed that ectopic *AG* function only restored normal flowering time in late-flowering mutants, *co* and *fca*, suggesting that *AG* functions in a pathway independent of these genes. The early-flowering *tfl1* mutation, on the other hand, enhanced the effects of ectopic *AG* function on both vegetative and reproductive meristems. A recent study on the spatial and temporal expression pattern of *centroradialis* (*cen*), the presumptive *TFL1* ortholog of Antirrhinum, has suggested that CEN and therefore TFL1 may mediate an inhibitory signal of flowering or prevent a flowering signal from reaching the apical meristem (Bradley et al., 1996). Thus, the lack of such an inhibitory factor in the *tfl1* mutant may help ectopic *AG* to alter the activity of apical meristems to acquire the determinate floral fate.

As mentioned above, entire AG action in determination of reproductive meristems may rely on another gene function(s) that is present only in the meristem center or after the establishment of the initial floral meristem. One intriguing candidate for such genes is the *SHOOT MERISTEMLESS* (*STM*) gene, encoding a KNOTTED class homeodomain protein, which functions in the formation and/or maintenance of the shoot apical meristem (Long et al., 1996). In adult plants, although *STM* mRNA is not accumulated in floral meristems at early stages, it is expressed in the central region of floral meristems before the onset of *AG* expression (Long et al., 1996), suggesting that *STM* may function in the floral meristem center, as does *AG*. Therefore, *STM* may help to define the domain of AG action for specifying determinate floral fate. This possibility can be tested by ectopically expressing both *AG* and *STM* in floral meristems at early stages.

In conclusion, we have shown that *AG* promotes determinacy and transition to flowering independent of the functions of early-acting genes, contributing to final determination of lateral meristem identity. AG, as a transcription factor, most likely controls genes that are involved in cell proliferation and differentiation in the floral meristem center. Further study designed to identify the AG target genes will help to explain how AG switches the developmental state from indeterminate to determinate growth and how AG can induce floral promotion activity during flower development.

METHODS

Plants

The wild-type *Arabidopsis thaliana* plants and all mutants used in this study were in the Landsberg *erecta* background. The mutant alleles have all been described previously: *ag-1*, Bowman et al. (1989); *ap1-1*, Irish and Sussex (1990); *lfy-5*, Weigel et al. (1992); *cal-1*, Bowman et al. (1993); *tfl1-2*, Alvarez et al. (1992); and *co-2* and *fca-1*, Koornneef et al. (1991). The T_0 generation of 35S–*AG* transformants carrying the 35S–*ATG-1* or 35S–*ATG-34* transgene was described previously (Mizukami and Ma, 1992). The transgenic line ATG-1w1 was used for all of the experiments involving 35S–*AG* plants, and ATG-1w2 and ATG-34W1 were used for flower induction analysis.

Growth Conditions

For analyses of floral phenotype and flowering time, we grew plants in growth rooms or chambers at 25°C under continuous illumination with fluorescent lights (inductive continuous lighting [CL] conditions). A photoperiodic cycle of 8 hr of light/16 hr of dark was used for short-day (SD) conditions in 25°C growth chambers. Floral reversion experiments were performed by transiently shifting plants that had grown under CL conditions and had produced >10 mature flowers in the primary inflorescence into complete darkness for 24 or 48 hr, either by turning off the lights in the growth room or by placing plants in a sealed box in a darkroom.

Genetic Analysis

Segregation of double mutants was close to the expected ratio of 1:15 in the F_2 generation. Because *ag-1* plants are sterile, *ag-1*/+ heterozygotes were identified by a mild phenotype of increased number of carpels (Mizukami and Ma, 1995) and crossed with the *co-2* plants; seeds from *ag-1*/+, *co-2*/+ F_1 plants were harvested. Double mutants segregated in the F_2 generation, as expected. To generate 35S–*AG tfl1-2*, 35S–*AG lfy-5*, and 35S–*AG ap1-1* plants, we performed crosses between each single mutant and transgenic plant homozygous for 35S–*AG*, and all plants in the F_1 generation showed the curly leaf phenotype observed in paternal 35S–*AG* plants (Mizukami and Ma, 1992). The 35S–*AG* transgenic genotype of these plants was further confirmed by polymerase chain reaction.

For each cross, ~20% of the plants with curly leaves in the F_2 generation had a novel phenotype. Because the phenotypic difference between homozygous and heterozygous 35S–*AG* was not significant, *lfy-5* or *ap1-1* plants homozygous for 35S–*AG* were identified by the absence of segregating plants showing a normal leaf phenotype in the F_3 generation. The *tfl1-2*/+, 35S–*AG*/− F_1 plants displayed phenotypes of inflorescence termination and slightly earlier flowering than did the paternal 35S–*AG* plants.

The introduction of the 35S–*AG* transgene into the *lfy-5 ap1-1* background was performed as follows. 35S–*AG*/−, *lfy-5*, or 35S–*AG*/−, *ap1-1* pistils were pollinated with pollen grains from putative *lfy-5*/+, *ap1-1*/+ plants, and progeny from self-pollination of each parental plant were examined to confirm the genotype of the parents. Seeds were collected from individual F_1 plants that had curly leaves (35S–*AG*/−) and *lfy-5* or *ap1-1* flowers. In F_2 generations, ~28% (close to the expected 25%) of the plants carrying the 35S–*AG* transgene dis-

played a novel phenotype, which was attributed to 35S–*AG lfy-5 ap1-1*.

To obtain 35S–*AG ap1-1 cal-1* plants, 35S–*AG* homozygous plants were crossed with *ap1-1 cal-1* plants; F_2 plants carrying the 35S–*AG* transgene were selected for kanamycin resistance, and two of 37 plants were 35S–*AG*/+, *ap1-1 cal-1* plants, as confirmed by the appearance of *ap1-1 cal-1* plants in the F_3 generation. For 35S–*AG co-2* and 35S–*AG fca-1* plants, seeds were harvested from individual 35S–*AG* F_2 plants exhibiting later flowering than did paternal 35S–*AG* plants, and the 35S–*AG*/−, *co-2* and 35S–*AG*/−, *fca-1* plants were identified by the reappearance of the single phenotype in the F_3 generation.

Phenotypic Analysis

Floral phenotypes were first analyzed using a dissecting light microscope (model STEMI SV8; Carl Zeiss, Thornwood, NY) and then by using a scanning electron microscope (model 5300; JEOL USA, Peabody, MA). Specimens for scanning electron microscopy were prepared as described previously (Mizukami and Ma, 1992). Flowering time was examined by using three different criteria: the numbers of (1) rosette leaves at the time of bolting, (2) days before the appearance of floral buds, and (3) cauline leaves in the primary inflorescence (Martinez-Zapater et al., 1994). Analysis of vegetative phase change was performed according to the previous observations in which the adult phase is associated with the appearance of a rosette leaf developing abaxial trichomes (Martinez-Zapater et al., 1994). Therefore, we considered rosette leaves lacking abaxial trichome as juvenile leaves, and by counting the number of juvenile leaves and total rosette leaves, we extrapolated the duration of juvenile or adult phase.

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