The Plant Cell, Vol. 7, 1763–1771, November 1995

RESEARCH ARTICLE

The Arabidopsis *AGL8* MADS Box Gene Is Expressed in Inflorescence Meristems and Is Negatively Regulated by *APETALA1*

M. Alejandra Mandel[1] and Martin F. Yanofsky[2]

Department of Biology and Center for Molecular Genetics, University of California at San Diego, La Jolla, California 92093-0116

MADS box genes encode putative transcription factors that play important roles in plant and animal development. In plants, MADS box genes are involved in the early step of specifying floral meristem identity as well as the later step of determining the fate of floral organ primordia. Here, we describe the isolation and characterization of a new MADS box gene from Arabidopsis, designated *AGL8*. Although *AGL8* RNA does not accumulate during vegetative growth, it accumulates to high levels in the inflorescence apical meristem as well as in the inflorescence stem and cauline leaves. *AGL8* RNA is excluded from the young flower primordia that arise on the flanks of the inflorescence meristem but later accumulates in the walls of the developing carpels. The lack of *AGL8* RNA in floral meristems is due in part to the action of another MADS box gene, *APETALA1*, because *AGL8* RNA does accumulate in *apetala1* mutant flower primordia.

INTRODUCTION

Plant growth depends on meristems, which are groups of undifferentiated cells that give rise to the diverse parts of a plant. During the vegetative phase of Arabidopsis growth, the shoot apical meristem produces a small number of leaf primordia on its flanks that develop into a rosette. Responding to both environmental (e.g., temperature and light conditions) and internal (e.g., age) factors, flowering is induced. Upon floral induction, the vegetative apical meristem undergoes a transition into an inflorescence meristem that produces floral meristems on its flanks. In addition, secondary inflorescences begin to differentiate from the axils of preexisting leaf primordia (Hempel and Feldman, 1994). The increased elongation of internodes during the reproductive phase results in the separation of the basal rosette leaves from the later-arising cauline leaves. Floral meristems produce primordia in a whorled arrangement, in contrast to the inflorescence meristem, which produces primordia in a spiral. The different floral organs arise in precisely defined positions within four concentric whorls. These organs are, from the first (outermost) to the fourth (innermost) whorl, four sepals, four petals, six stamens, and two fused carpels.

Molecular and genetic studies have identified early-acting genes, such as *APETALA1* (*AP1*), *CAULIFLOWER* (*CAL*), and *LEAFY* (*LFY*) (Mandel et al., 1992; Weigel et al., 1992; Kempin et al., 1995), that specify the identity of floral meristems. These studies have also identified later-acting genes, such as *APETALA2* (*AP2*), *APETALA3* (*AP3*), *PISTILLATA* (*PI*), and *AGAMOUS* (*AG*) (Yanofsky et al., 1990; Jack et al., 1992; Goto and Meyerowitz, 1993; Jofuku et al., 1994), that determine the fate of floral organ primordia. Some of these genes have been shown to play multiple roles in flower development, such as *AP1*, which is involved in specifying both floral meristem and organ identity (for reviews, see Weigel and Meyerowitz, 1994; Yanofsky, 1995).

Five of these genes, *AP1*, *CAL*, *AP3*, *PI*, and *AG*, belong to the MADS box family of regulatory genes. MADS box genes encode DNA binding proteins that have a conserved N-terminal domain that shares similarity with transcription factors from yeast and mammals (Schwarz-Sommer et al., 1990). The name MADS derives from the initials of the first isolated members of this family: M for *MCM1*, a yeast gene that encodes PRTF, a transcription factor that regulates mating type–specific genes; A for *AG*; D for *DEFICIENS*, the *AP3* homolog first isolated in Antirrhinum; and S for SRF, serum response factor, a transcriptional regulator of c-*fos* (Norman et al., 1988; Passmore et al., 1988; Sommer et al., 1990; Yanofsky et al., 1990). In addition, the plant MADS box genes encode a second domain, the K domain, which has structural homology with a coiled-coil domain present in keratin, and it is thought to be involved in protein–protein interactions (Ma et al., 1991). The C terminus is rich in acidic amino acids, characteristic of transactivation

[1] Current address: Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.
[2] To whom correspondence should be addressed.

domains. MADS box genes in Arabidopsis have been referred to as *AGL* (*AG*-like) genes because their isolation followed the prior isolation of *AG*. A total of 17 MADS box genes have been isolated and characterized in Arabidopsis (Yanofsky et al., 1990; Ma et al., 1991; Jack et al., 1992; Mandel et al., 1992; Gotq and Meyerowitz, 1994; Kempin et al., 1995; Rounsley et al., 1995).

Here, we report the isolation and characterization of a new MADS box gene in Arabidopsis. It is designated *AGL8*. RNA blotting and in situ hybridization analyses suggest that *AGL8* may define a new class of gene that regulates inflorescence development. In addition, we characterize the regulatory interactions between *AGL8* and other genes involved in meristem and organ identity.

RESULTS

Sequence and Mapping of *AGL8*

A new Arabidopsis MADS box gene, designated *AGL8*, was isolated by reduced stringency hybridization with a probe specific for the *AGL3* MADS box (see Methods). The *AGL8* cDNAs contain a single long open reading frame as well as 5′ and 3′ untranslated regions and poly(A) tails of varying lengths (Figure 1). The predicted protein encoded by this cDNA has a calculated molecular mass of 27.5 kD, similar to previously characterized members of this family. This new AGL, like AG and the previously reported members of the family, has the highly conserved MADS domain. *AGL8* was placed on the Arabidopsis restriction fragment length polymorphism map (Hauge et al., 1993). *AGL8* maps on chromosome 5, ~1.5 centimorgans above *LFY* and 3 centimorgans below *cer3* (data not shown).

RNA Analyses

As a start toward determining the pattern of *AGL8* expression, RNA from roots, inflorescence stems, cauline leaves, and flowers was used in RNA blot hybridizations. A cDNA fragment containing only the 3′ end of the gene was used as a probe to avoid cross-hybridization due to homology with the MADS box region (Figure 2A). *AGL8* RNA was detected not only in floral tissues but also in stems and cauline leaves. No *AGL8* RNA was detected in roots (Figure 2B).

We also analyzed the levels of *AGL8* RNA in different mutant backgrounds to begin to determine possible interactions between this gene and the floral homeotic genes. RNAs from wild-type, *ap1-1*, *ap2-2*, *ap3-3*, *pi-2*, *ag-1*, and *ag-2* flowers were isolated and hybridized to the *AGL8*-specific probe. *AGL8* is expressed at similar levels in wild-type, *ap3-3*, and *pi-2* flowers. In contrast, *AGL8* RNA levels increase in *ap1-1* and *ap2-2* mutant flowers and decrease in *ag-1* and *ag-2* flower tissue (Figure 2C).

```
                                                    CCCAGAGAGACATAAGAAAGAAAGAGAGAGAGATACTT
                                                TGGTCATTTCAGGGTTGTCGTTTCTCTCTCTTGTTCTTGAGATTTTGAAGACAGAGAGAT
   1 ATGGGAAGAGGTAGGGTTCAGCTGAAGAGGATAGAGAACAAGATCAATAGGCAAGTTACT
   1  M  G  R  G  R  V  Q  L  K  R  I  E  N  K  I  N  R  Q  V  T

  61 TTCTCAAAGAGAAGGTCTGGTTTGCTCAAGAAAGCTCATGAGATCTCTGTTCTCTGCGAT
  21  F  S  K  R  R  S  G  L  L  K  K  A  H  E  I  S  V  L  C  D

 121 GCTGAGGTTGCTCTCATCGTCTTCTCTTCCAAAGGCAAACTCTTCGAATATTCCACCGAC
  41  A  E  V  A  L  I  V  F  S  S  K  G  K  L  F  E  Y  S  T  D

 181 TCTTGCATGGAGAGGATACTTGAACGCTATGATCGCTATTTATATTCAGACAAACAACTT
  61  S  C  M  E  R  I  L  E  R  Y  D  R  Y  L  Y  S  D  K  Q  L

 241 GTTGGCCGAGACGTTTCACAAAGTGAAAATTGGGTTCTAGAACATGCTAAGCTCAAGGCA
  81  V  G  R  D  V  S  Q  S  E  N  W  V  L  E  H  A  K  L  K  A

 301 AGAGTTGAGGTACTTGAGAAGAACAAAAGGAATTTTATGGGGGAAGATCTTGATTCGTTG
 101  R  V  E  V  L  E  K  N  K  R  N  F  M  G  E  D  L  D  S  L

 361 AGCTTGAAGGAGCTCCAAAGCTTGGAGCATCAGCTCGATGCAGCTATCAAGAGCATTAGG
 121  S  L  K  E  L  Q  S  L  E  H  Q  L  D  A  A  I  K  S  I  R

 421 TCAAGAAAGAACCAAGCTATGTTCGAATCCATATCTGCGCTCCAGAAGAAGGATAAAGCC
 141  S  R  K  N  Q  A  M  F  E  S  I  S  A  L  Q  K  K  D  K  A

 481 TTGCAAGATCACAACAATTCGCTTCTCAAAAAGATTAAGGAGAGGGAGAAGAAAACGGGT
 161  L  Q  D  H  N  N  S  L  L  K  K  I  K  E  R  E  K  K  T  G

 541 CAGCAAGAAGGACAATTAGTCCAATGCTCCAACTCTTCTTCAGTTCTTCTGCCTCAATAC
 181  Q  Q  E  G  Q  L  V  Q  C  S  N  S  S  S  V  L  L  P  Q  Y

 601 TGCGTAACCTCCTCCAGAGATGGCTTTGTGGAGAGAGTTGGGGGAGAGAACGGTGGTGCA
 201  C  V  T  S  S  R  D  G  F  V  E  R  V  G  G  E  N  G  G  A

 661 TCGTCGTTGACGGAACCAAACTCTCTGCTTCCGGCTTGGATGTTACGTCCTACCACTACG
 221  S  S  L  T  E  P  N  S  L  L  P  A  W  M  L  R  P  T  T  T

 721 AACGAGTAGAACTATCTCACTCTTTATAATATAATGATAATATAATTAATGTTTAATATT
 241  N  E  •

 781 TTCATAACATTCAGCATTTTTTTGGTGACTTATACTCATTATTAATACCGATATGTTTTA
 841 GCTAGTCATATTATATGTATGATGGAACTCCGTTGTCGAGACGTATGTACGTAAGCTATC
 901 ATTAGATTCACTGCGTCTTAAGAACAAAGATTCATATCTTGGTAATGATTTCTCATGAAA
 961 TA$_{n}$
```

Figure 1. Nucleotide and Deduced Amino Acid Sequences of the *AGL8* cDNA Clone.

The asterisk indicates a stop codon, and A_n represents a poly(A) tail. The MADS box region is underlined from amino acids 2 to 56, and the K box region is underlined from amino acids 92 to 158. The *AGL8* cDNA sequence has been submitted to GenBank as accession number U33473.

AGL8 RNA Accumulates in Two Distinct Phases

The temporal and spatial pattern of *AGL8* expression was analyzed by RNA in situ hybridizations in wild-type inflorescences of Arabidopsis. *AGL8* RNA was first detected in the inflorescence meristem as soon as the plant switches from vegetative to reproductive development (Figures 3A and 3B). As the inflorescence stem elongates, *AGL8* RNA accumulates in the inflorescence meristem and in the stem (Figures 3C and 3D). Although *AGL8* RNA was detected in the apical inflorescence meristem, *AGL8* expression was not detected at the initial stages (1 and 2) of flower development (stages defined by Smyth et al., 1990), when the young floral primordia arise on the flanks of the inflorescence meristem (Figures 3C and 3D). At approximately stage 3, *AGL8* expression resumes in the center of the floral dome in the region that corresponds to the fourth whorl and is excluded from all other primordia and the pedicel

(Figures 3C and 3D). At later stages, *AGL8* expression becomes localized to the carpel walls, in the region that constitutes the valves of the ovary (Figures 3E to 3J). No *AGL8* RNA was detected in the ovules, style, stigmatic tissues, or the septum that divides the ovary.

AGL8 Is Negatively Regulated by *AP1*

AGL8 RNA accumulates in the inflorescence stem and meristem, and it is excluded from the floral meristem at the earliest stages of flower formation. The loss of *AGL8* expression coincides with the onset of expression of the meristem identity gene *AP1* (Mandel et al., 1992). Although *AP1* RNA is not expressed in the inflorescence meristem, *AP1* is expressed throughout the floral meristem during stages 1 and 2 of flower development, and at stage 3, *AP1* expression abates from the central dome due to the negative regulation by *AG* (Gustafson-Brown et al., 1994). At later stages, *AP1* expression becomes localized to sepals and petals. To determine whether *AP1* is responsible, at least in part, for the loss of expression of *AGL8* at the initial stages of flower development, we analyzed *AGL8* expression in an *ap1* mutant background. *ap1-1* mutant flowers, which have normal third and fourth whorl organs, have leaflike organs in the first whorl instead of sepals and lack petals in the second whorl. In the axils of first whorl organs, secondary flowers arise, which reiterate this phenotype, and tertiary flowers can arise in the axils of leaflike organs of secondary flowers (Irish and Sussex, 1990).

In *ap1-1* mutant inflorescences, *AGL8* RNA accumulates in the inflorescence meristem and in the carpel walls of developing flowers, as in the wild type. However, in contrast to the wild type, *AGL8* RNA accumulates in stage 1 and 2 flower primordia in *ap1* mutants. Furthermore, *AGL8* RNA accumulates in the first whorl leaflike organs and throughout the floral pedicel (Figures 4A and 4B). Secondary flowers arising in the axils of first whorl organs show a similar pattern of *AGL8* expression. The expanded pattern of *AGL8* expression in *ap1* mutant flowers suggests that AP1 negatively regulates *AGL8* in young flower primordia and in sepals (and perhaps in petals) later in flower development.

Although these studies provide insight into the regulation of *AGL8*, they fail to shed light on the factor(s) that prevents *AGL8* from being expressed in third whorl organs. One candidate for repressing *AGL8* expression in stamens is AP3, because AP3 regulates organ identity in the second and third whorls (Jack et al., 1992). Flowers of *ap3-3* mutants have sepals in the first and second whorls and carpels in the third and fourth whorls. In the *ap3-3* mutant, *AGL8* RNA is not expressed during stages 1 and 2 of flower development. However, when its expression is reestablished during stage 3, the domain in the center of the floral meristem expands to what appears to correspond to the third and fourth whorls (Figures 4C and 4D). At later stages, *AGL8* RNA accumulates in the carpel walls of the third and fourth whorl organs (Figures 4E and 4F), and it is not expressed in first and second whorl sepals. These results suggest that AP3 can formally be considered a negative regulator of *AGL8* in the third whorl.

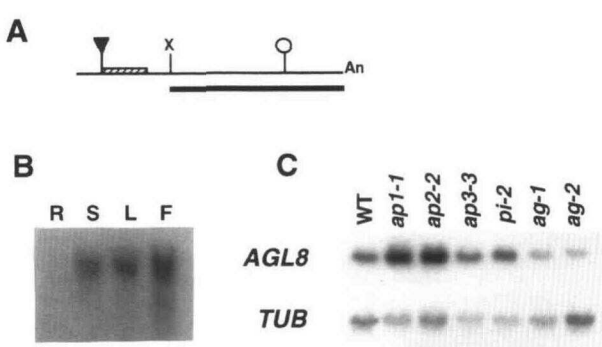

Figure 2. RNA Gel Blot Analyses of *AGL8*.

(A) Diagram of the *AGL8*-specific probe. ▼, start codon; ○, stop codon; hatched bar, MADS box; solid bar, probe; An, poly(A) tail; X, XbaI.
(B) RNA blot hybridization of wild-type tissues with the *AGL8*-specific probe shown in **(A)**. F, flowers; L, cauline leaves; R, roots; S, stems.
(C) RNA gel blot hybridization of the wild type (WT) and different mutant floral tissues with *AGL8*. A tubulin (*TUB*) probe was used as a loading control (Marks et al., 1987).

AGL8 Expression in *ag*, *ap2*, and *clavata1* (*clv1*) Mutant Backgrounds

To provide further insights into the regulation and function of *AGL8*, we analyzed the pattern of *AGL8* expression in other mutant tissues.

ag

ag-1 mutant flowers have petals replacing stamens in the third whorl, and the fourth whorl carpels are replaced by a new flower that reiterates the pattern (sepal, petal, petal)$_n$ (Bowman et al., 1991). *AGL8* RNA expression in *ag-1* mutants, as in the wild type, accumulates in the inflorescence meristem but not in the young flower primordia (Figures 4G and 4H). Even though carpels are not present in *ag* mutant flowers, *AGL8* RNA accumulates in the fourth whorl region of stage 3 flowers and continues to accumulate in the central meristematic region later in flower development (Figures 4I and 4J).

ap2

ap2-2 mutant flowers have carpels in the first whorl instead of sepals, lack petals in the second whorl, and have fewer stamens in the third whorl, and the fourth whorl has normal or unfused carpels (Bowman et al., 1991). In this mutant, *AGL8* RNA is expressed in a similar way as in wild-type

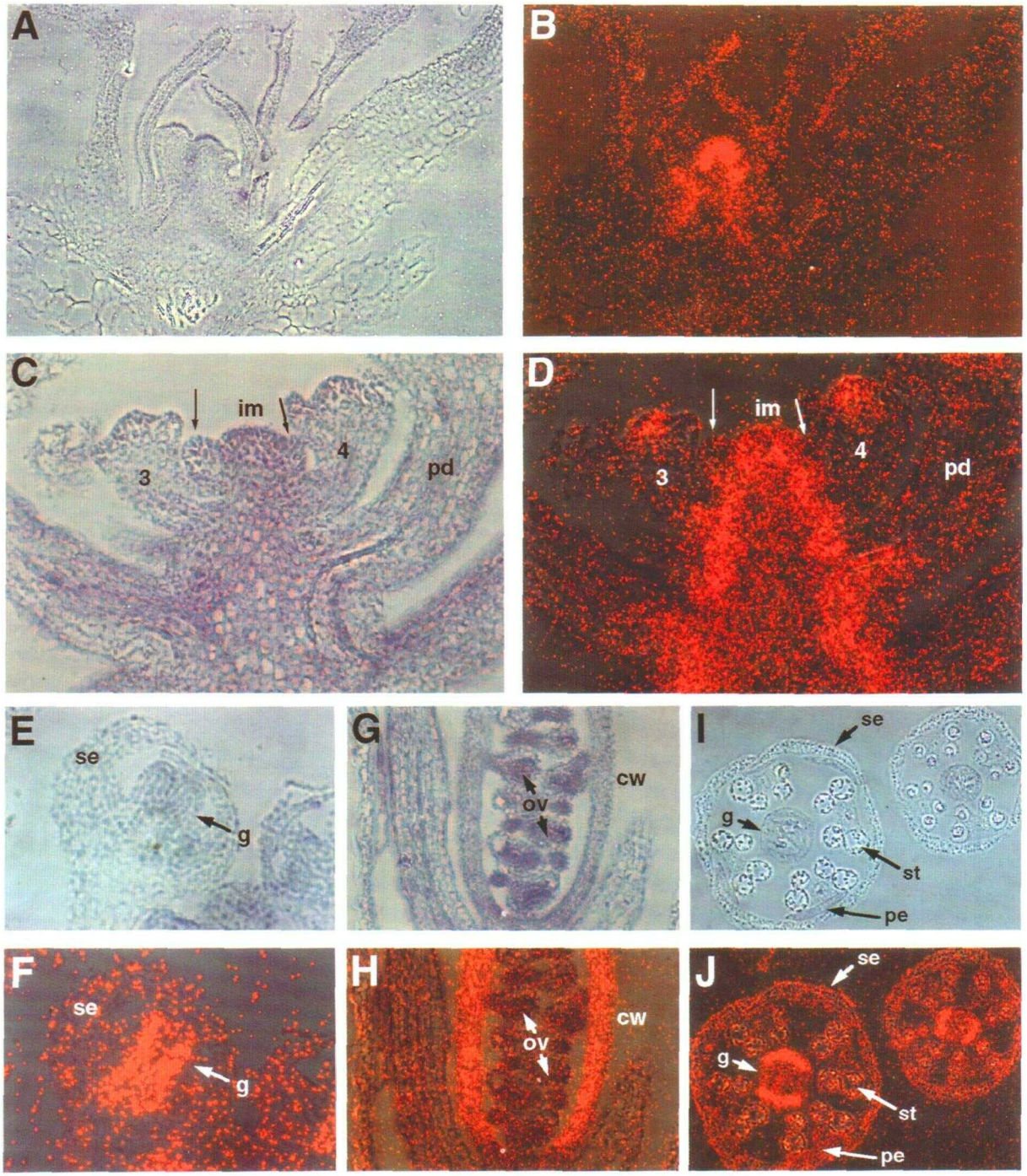

Figure 3. *AGL8* Expression in Arabidopsis Wild-Type Tissues.

Longitudinal sections (**[A]** to **[H]**) and transverse sections (**[I]** and **[J]**) of wild-type plant tissues hybridized with the *AGL8*-specific antisense mRNA probe are shown. **(A)**, **(C)**, **(E)**, **(G)**, and **(I)** are bright-field micrographs of tissue sections. **(B)**, **(D)**, **(F)**, **(H)**, and **(J)** are bright/dark-field (red filter) double exposures.

(A) and **(B)** *AGL8* RNA accumulates in the inflorescence meristem at the apex of a wild-type plant immediately after the transition to flowering, 14 days post-germination under continuous light.

(C) and **(D)** A wild-type inflorescence. The arrows indicate floral meristems at stage 1 (right) and 2 (left). The other numbers indicate floral primordia at stages 3 and 4.

(E) and **(F)** A stage 7 flower.

(G) and **(H)** Part of a carpel of a stage 12 flower.

(I) and **(J)** Carpels of a stage 12 (*left*) and a stage 9 (*right*) flower.

cw, carpel wall; g, gynoecium; im, inflorescence meristem; ov, ovule; pd, pedicel; pe, petal; se, sepal; st, stamen.

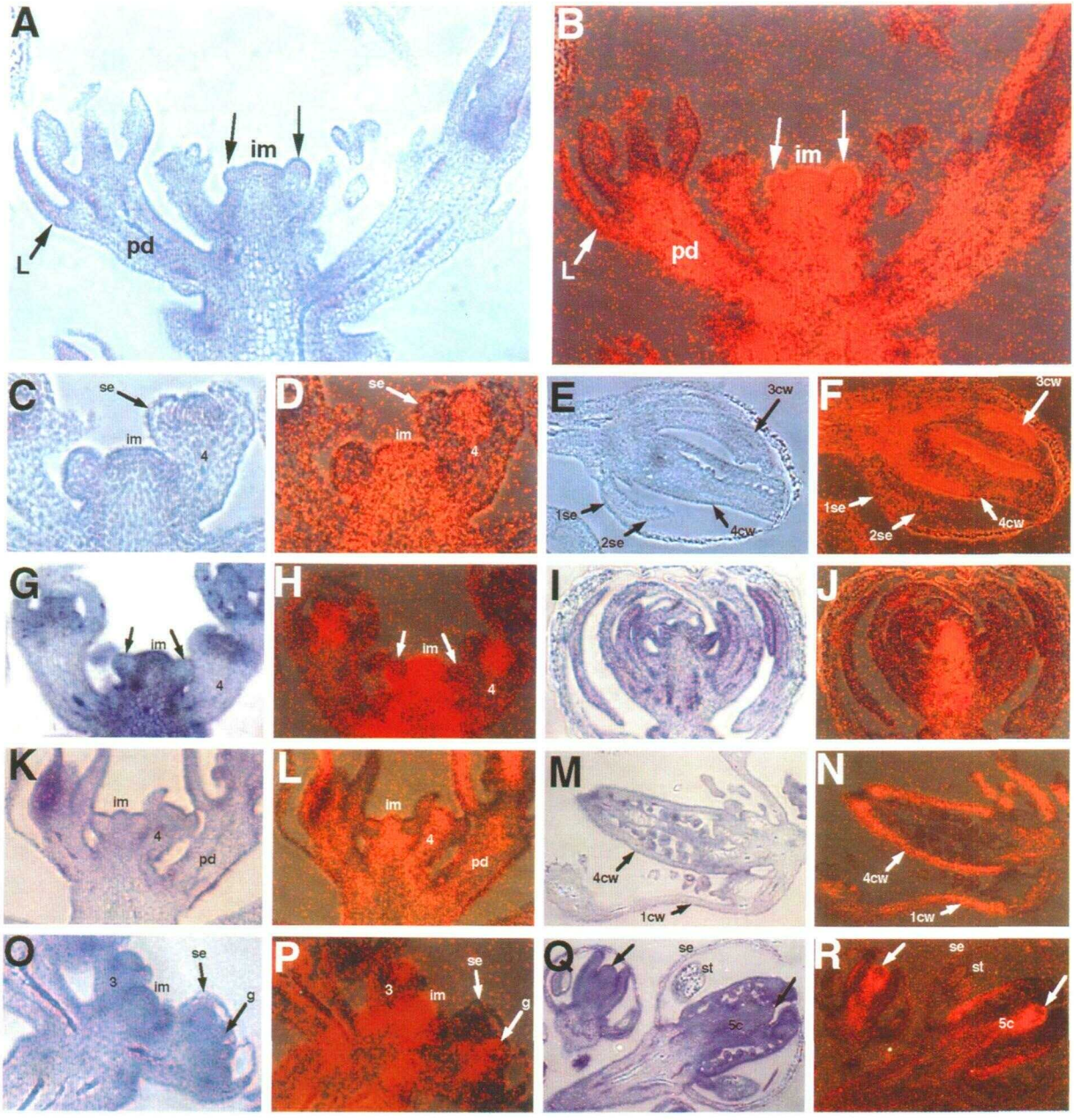

Figure 4. *AGL8* Expression in the Floral Tissues of Mutants.

Longitudinal sections of floral tissues of mutants hybridized with the *AGL8*-specific antisense mRNA probe. **(A)**, **(C)**, **(E)**, **(G)**, **(I)**, **(K)**, **(M)**, **(O)**, and **(Q)** are bright-field micrographs. **(B)**, **(D)**, **(F)**, **(H)**, **(J)**, **(L)**, **(N)**, **(P)**, and **(R)** are bright/dark-field (red filter) double exposures.

(A) and **(B)** An *ap1-1* inflorescence. Arrows indicate two young floral primordia (stage 1 at left and stage 2 at right). Compare the expression of *AGL8* at similar stages of wild-type floral primordia.

(C) and **(D)** An *ap3-3* inflorescence with a stage 4 flower.

(E) and **(F)** An *ap3-3* flower at a later stage.

(G) and **(H)** An *ag-1* inflorescence. Arrows indicate two young floral primordia at stage 2 of flower development. A stage 4 flower is indicated.

(I) and **(J)** An older *ag-1* flower.

(K) and **(L)** An *ap2-2* inflorescence with a stage 4 flower.

(M) and **(N)** An older *ap2-2* flower.

(O) and **(P)** A *clv1-7* inflorescence with an approximately stage 3 floral primordium.

(Q) and **(R)** Two *clv1-4* flowers at different stages of development. The arrows indicate the tip of the fifth whorl carpel that shows no *AGL8* RNA accumulation.

g, gynoecium; im, inflorescence meristem; L, leaflike organ; pd, pedicel; se, sepal; st, stamen; 1cw, first whorl carpel; 1se, first whorl sepal; 2se, second whorl sepal; 3cw, third whorl carpel; 4cw, fourth whorl carpel; 5c, fifth whorl carpel.

inflorescences, and it is absent from stage 1 and 2 flower primordia (Figures 4K and 4L). Between stages 3 and 4, *AGL8* transcripts are detected in the center of the floral primordium and in pedicels (Figures 4K and 4L). At later stages, *AGL8* becomes localized to the carpel walls of both the first and fourth whorl carpel tissues (Figures 4M and 4N).

clv1

clv1 mutant plants have enlarged apical and floral meristems, displaying an array of different phenotypes that includes fasciation, disrupted phyllotaxis, and extra floral organs and whorls. *clv* mutant flowers have a fifth whorl that usually consists of extra carpelloid tissue (Clark et al., 1993). *AGL8* RNA is strongly expressed in the inflorescence stem and meristem of *clv* mutants, and it is turned off during stages 1 and 2 (Figures 4O and 4P). *AGL8* expression resumes in the center of the flower primordia of stage 3 flowers, as in the wild type (Figures 4O and 4P). At later stages, *AGL8* RNA accumulates in the carpel walls in the fourth whorl and throughout the carpelloid tissues that proliferate in the fifth whorl, except in an area located at the tip of these tissues (Figures 4Q and 4R).

DISCUSSION

MADS box genes have been shown to play key roles in flower development, including the specification of floral meristem and floral organ identity. Here, we report the isolation and characterization of a new MADS box gene from Arabidopsis. We have designated it *AGL8.* Like other plant members of this family of genes, *AGL8* encodes a putative transcription factor that shares the highly conserved DNA binding MADS domain as well as the K domain, which is thought to be involved in protein–protein interactions (Ma et al., 1991). With this new member, the Arabidopsis MADS box family of genes includes at least 18 members.

In contrast to the majority of MADS box genes, which are expressed only in flowers, *AGL8* appears to be a novel MADS box gene whose RNA accumulates in two distinct phases of plant development. *AGL8* RNA was first detected immediately after the transition from vegetative to reproductive growth in the shoot apex. These data are supported by preliminary results using a 2.3-kb promoter fragment of *AGL8* fused to the β-glucuronidase (*GUS*) gene (Jefferson et al., 1987). Transgenic Arabidopsis plants harboring this construct showed no GUS activity during the vegetative phase of growth, but immediately after the transition to flowering, GUS activity was observed in the apical meristem and newly formed cauline leaves (M.A. Mandel and M.F. Yanofsky, unpublished observations). The high levels of *AGL8* RNA in the inflorescence meristem suggest a possible role for *AGL8* in maintaining inflorescence meristem identity or, alternatively, in promoting the initiation of flowers. Genetic studies have identified a gene, *TERMINAL FLOWER* (*TFL*) (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992), that is proposed to play a role in maintaining the identity of the inflorescence meristem. This conclusion is inferred, in part, from the fact that the apical and lateral inflorescence meristems of *tfl* mutants develop as flowers. *AGL8* may interact with *TFL* to promote inflorescence development. Alternatively, *AGL8* could mediate the induction of the floral meristem identity genes, such as *AP1*, *CAL*, and *LFY.* We previously proposed a regulatory hierarchy of MADS box gene expression, beginning with the onset of the meristem identity genes *AP1* and *CAL* (Rounsley et al., 1995). The onset of *AGL8* expression in the inflorescence apical meristem immediately after the transition to flowering suggests that *AGL8* could act at the top of this hierarchy either by inducing or repressing the meristem identity genes.

In contrast to the high levels of *AGL8* RNA present in the inflorescence apical meristem, no *AGL8* RNA was detected in the flower primordia when they first arise on the flanks of the inflorescence meristem. This suggests that some factor(s) prevents *AGL8* expression early in flower development. One candidate for such a factor is the meristem identity gene *AP1*, whose expression pattern is largely complementary to that of *AGL8*, because *AP1* RNA is not detected in the inflorescence meristem but is detected in young flower primordia (Mandel et al., 1992). Consistent with this idea is the fact that *AGL8* RNA does accumulate in young flower primordia of the *ap1* mutant. Although the timing of the onset of *AP1* expression coincides with the corresponding loss of *AGL8* RNA accumulation, additional experiments are necessary to establish whether AP1 represses *AGL8* through a direct or an indirect interaction. It is interesting to note that of 18 MADS box genes characterized in Arabidopsis, *AGL8* most closely resembles the floral meristem identity genes *AP1* and *CAL*, suggesting that *AGL8* has diverged relatively recently from these genes (Purugganan et al., 1995). This observation is particularly intriguing because these two classes of genes are expressed in inflorescence and floral meristems, respectively, and their expression patterns are complementary.

Although *AGL8* RNA is not expressed in young flower primordia, it does begin to accumulate later in flower development at about the time that sepal primordia begin to arise. This is also roughly the same time at which the organ identity genes *AP3*, *PI*, and *AG* begin to be expressed (Yanofsky et al., 1990; Drews et al., 1991; Jack et al., 1992; Goto and Meyerowitz, 1994). These data illustrate the two distinct phases of *AGL8* RNA accumulation, the first occurring during inflorescence development in the stem and cauline leaves and the second in the later stages of flower development. The expression of *AGL8* in the developing flowers is confined to the region from which the fourth whorl organ primordia later emerges. This is particularly evident as the flower begins to develop, because *AGL8* RNA accumulates throughout carpel primordia as they begin to form. Later in development, it is clear that *AGL8* RNA is specifically localized to the carpel walls and is absent from all other

cell types of the carpel. This feature distinguishes it from other characterized MADS box genes that are also expressed in carpels but whose RNAs accumulate in ovules (e.g., *AG*, *AGL2*, *AGL4*, *AGL5*, *AGL9*, *AGL11*, and *AGL13*) (Bowman et al., 1991b; Ma et al., 1991; Flannagan and Ma, 1994; Rounsley et al., 1995; Savidge et al., 1995; M.A. Mandel and M.F. Yanofsky, unpublished data).

The negative regulation of *AGL8* by AP1 occurs not only in young flower primordia but also later in the developing organs and floral pedicel. This conclusion is based on the observation that *AGL8* RNA accumulates in the pedicel and first whorl leaflike organs in *ap1* mutant flowers and that *AGL8* is not expressed in the pedicel and first whorl organs in the wild type. AP1 may also prevent *AGL8* expression in second whorl organs, although this is difficult to confirm because these organs fail to develop in *ap1* mutant plants. Although *AGL8* may be negatively regulated by AP1 in sepals and petals, and *AGL8* is normally expressed in carpels, these data do not explain the lack of *AGL8* RNA in stamens. One candidate for a factor that prevents *AGL8* expression in third whorl organs is AP3, which is involved in specifying stamen identity (Jack et al., 1992). *AGL8* is expressed in the third whorl carpels that develop in *ap3* mutant flowers, consistent with the idea that AP3 could act directly or indirectly to prevent *AGL8* expression in third whorl organs.

An interesting aspect of *AGL8* regulation arises from the analyses of *AGL8* RNA accumulation in *ag* mutants. RNA blot hybridizations showed decreased levels of *AGL8* RNA in *ag* mutant flowers. *ag* mutant flowers have only sepals and petals, and *AGL8* RNA does not accumulate in these organs in wild-type flowers. RNA in situ analyses of *ag* mutant flowers show that there is no expression of *AGL8* in sepals and petals but that *AGL8* is expressed in the meristematic region at the center of the flower. Because the center of the *ag* mutant flower continues to give rise to organ primordia, it is indeterminate, analogous in this sense to the inflorescence apical meristem, which is also indeterminate and continuously gives rise to flower primordia. *AGL8* may play a role in maintaining this indeterminacy.

Mutations in the *clv1* locus show a variety of phenotypes. They affect apical meristem size, cause fasciation, and in the strongest alleles, can alter the number of floral organs in all whorls and create additional whorls inside the carpels. Because the fifth whorl carpelloid organs that develop in *clv* mutants develop as an inflorescence in the *clv1 ap1* double mutant, it was suggested that AP1 may by necessary to maintain the floral meristem identity of the inner region of *clv* mutant flowers (Clark et al., 1993). In *clv1* mutant flowers, *AGL8* RNA accumulates in the fourth whorl carpel walls and throughout the carpelloid tissue in the fifth whorl, except in the tip, where *AP1* is expressed. Perhaps the inflorescence character of the fifth whorl in *clv1 ap1* double mutant flowers is due in part to the activity of *AGL8*.

The isolation and characterization of *AGL8* reported here define a novel Arabidopsis MADS box gene that is one of the earliest acting regulatory genes after the transition to flowering. These studies suggest that *AGL8* functions in the inflorescence apical meristem, perhaps to maintain inflorescence meristem identity. It will be interesting to determine whether *AGL8* interacts directly with other genes involved in early events in flower development and to identify and characterize loss-of-function alleles for this gene.

METHODS

Isolation of *AGL8*

A cosmid library of *Arabidopsis thaliana* Landsberg *erecta* genomic DNA (Yanofsky et al., 1990) was screened under reduced stringency conditions with a 550-bp EcoRI fragment of the cDNA clone of *AGL3* (for *AGAMOUS* [*AG*]-like; pCIT2280) that contains the MADS box (Ma et al., 1991). In addition to several clones that corresponded to *AGL3*, a new gene was identified and designated *AGL8*. An internal 264-bp XbaI-PvuII fragment of the *AGL8* gene containing the first exon and a portion of the first intron was used to screen, under stringent conditions, an ecotype Columbia cDNA library constructed with poly(A)$^+$ RNA from vegetative and floral tissues (Elledge et al., 1991), and two clones corresponding to *AGL8* were isolated.

The *AGL8* cDNA was excised from the λ YES vector using the reported procedure (Elledge et al., 1991) and subsequently subcloned into pGEM7Zf(+) (Promega). The cDNA clones were double-strand sequenced using the Sequenase Version 2.0 DNA sequencing kit (U.S. Biochemical Corp.) according to the manufacturer's protocol. Sequence analyses were performed using the Mac Vector program (International Biotechnologies, New Haven, CT).

RNA Isolation and Blot Analyses

Total RNA from roots, stems, cauline leaves, and flowers (through stage 9) was isolated from wild-type Landsberg *erecta* plants and from flowers of different mutants according to Crawford et al. (1986). The RNA was size fractionated by gel electrophoresis under denaturing conditions in agarose gels with formaldehyde, transferred to Hybond-N membranes (Amersham Corp.), and hybridized according to standard procedures with ^{32}P-labeled specific probes. The *AGL8* probe used was an ~650 bp XbaI-EcoRI fragment of the 3′ end of the cDNA clone (shown in Figure 2A). This probe did not contain any MADS box sequence.

In Situ Hybridizations

Preparation of tissues and hybridization conditions were the same as previously described (Drews et al., 1991) with minor modifications. The *AGL8* ^{35}S-labeled antisense mRNA probe was synthesized with T7 RNA polymerase from a SalI digest of pAM497, which is a 580-bp subclone of the 3′ end of the *AGL8* cDNA generated by polymerase chain reaction in the pCRII vector (Invitrogen, San Diego, CA). The probe was used at a final concentration of 2.5 × 10^7 cpm/mL. Slides were exposed for 4 to 5 weeks.

Image Processing

Photographic slides were scanned and digitized using a Coolscan (Nikon Inc., Melville, NY). Additional processing was performed using Adobe Photoshop 2.5 (Adobe Systems Inc., Mountain View, CA). Composite figures were printed using a Tektronix Phaser IIsdx (Tektronix Inc., Wilsonville, OR) dye sublimation printer.

ACKNOWLEDGMENTS

We thank Beth Savidge for assistance with RNA gel blots and Judith Bender and Gerald R. Fink for additional *AGL8* map information. We also thank Steve Rounsley, Sarah Liljegren, and Beth Savidge for comments on the manuscript. This work was supported by grants from the National Science Foundation (No. IBN 94-18436 and No. DCB 90-18749).

Received June 1, 1995; accepted August 28, 1995.

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.

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

Bowman, J.L., Drews, G.N., and Meyerowitz, E.M. (1991b). Expression of the Arabidopsis floral homeotic gene *AGAMOUS* is restricted to specific cell types late in flower development. Plant Cell **3,** 749–758.

Clark, S.E., Running, M.P., and Meyerowitz, E.M. (1993). *CLAVATA1*, a regulator of meristem and flower development in *Arabidopsis*. Development **119,** 397–418.

Crawford, N.M., Campbell, W.H., and Davis, R.H. (1986). Nitrate reductase from squash: cDNA cloning and nitrate regulation. Proc. Natl. Acad. Sci. USA **83,** 8073–8076.

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.

Elledge, S.J., Mulligan, J.T., Ramer, S.W., Spottswood, M., and Davis, R.W. (1991). λ YES: A multifunctional DNA expression vector for the isolation of genes by complementation of yeast and *Escherichia coli* mutations. Proc. Natl. Acad. Sci. USA **88,** 1731–1735.

Flannagan, C.A., and Ma, H. (1994). Spatially and temporally regulated expression of the MADS-box gene *AGL2* in wild type and mutant *Arabidopsis* flowers. Plant Mol. Biol. **26,** 581–595.

Goto, K., and Meyerowitz, E.M. (1994). Function and regulation of the *Arabidopsis* floral homeotic gene *PISTILLATA*. Genes Dev. **8,** 1548–1560.

Gustafson-Brown, C., Savidge, B., and Yanofsky, M.F. (1994). Regulation of the Arabidopsis floral homeotic gene *APETALA1*. Cell **76,** 131–143.

Hauge, B.M., Hanley, S.M., Cartinhour, S., Cherry, J.M., Goodman, H.M., Koorneef, M., Stam, P., Chang, C., Kempin, S.A., Medrano, L., and Meyerowitz, E.M. (1993). An integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. Plant J. **3,** 745–754.

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

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

Jack, T., Brockman, L.L., and Meyerowitz, E.M. (1992). The homeotic gene *APETALA3* of Arabidopsis thaliana encodes a MADS box and is expressed in petals and stamens. Cell **68,** 683–697.

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, D.K., 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.

Kempin, S.A., Savidge, B., and Yanofsky, M.F. (1995). Molecular basis of the *cauliflower* phenotype in *Arabidopsis*. Science **267,** 522–525.

Ma, H., Yanofsky, M.F., and Meyerowitz, E.M. (1991). *AGL1-AGL6*, an *Arabidopsis* gene family with similarity to floral homeotic and transcription factor genes. Genes Dev. **5,** 484–495.

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.

Marks, M.D., West, J., and Weeks, D.P. (1987). The relatively large beta-tubulin gene family of *Arabidopsis thaliana* contains a member with an unusual transcribed 5′ noncoding sequence. Plant Mol. Biol. **10,** 91–104.

Norman, C., Runswick, M., and Pollock, R. (1988). Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-*fos* serum response element. Cell **55,** 989–1003.

Passmore, S., Maine, G.T., Elble, R., Christ, C., and Tye, B.K. (1988). A *Saccharomyces cerevisiae* protein involved in plasmid maintenance is necessary for mating of *MATα* cells. J. Mol. Biol. **204,** 593–606.

Purugganan, M.D., Rounsley, S.D., Schmidt, R.J., and Yanofsky, M.F. (1995). Molecular evolution of flower development: Diversification of the plant MADS-box regulatory gene family. Genetics **140,** 345–356.

Rounsley, S.D., Ditta, G.S, and Yanofsky, M.F. (1995). Diverse roles for MADS box genes in Arabidopsis development. Plant Cell **7,** 1259–1269.

Savidge, B., Rounsley, S.D., and Yanofsky, M.F. (1995). Temporal relationship between the transcription of two Arabidopsis MADS box genes and the floral organ identity genes. Plant Cell **7,** 721–733.

Schwarz-Sommer, Z., Huijser, P., Nacken, W., Saedler, H., and Sommer, H. (1990). Genetic control of flower development by homeotic genes in *Antirrhinum majus*. Science **250,** 931–936.

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.

Sommer, H., Beltrán, J.P., Huijser, P., Pape, H., Lönnig, W.-E., Saedler, H., and Schwarz-Sommer, Z. (1990). *Deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum*

majus: The protein shows homology to transcription factors. EMBO J. **9**, 605–613.

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

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.

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., and Meyerowitz, E.M. (1990). The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. Nature **346**, 35–39.