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Regulation of Arabidopsis Flower Development

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INTRODUCTION

Plant development is governed by intrinsic and environmental factors that regulate the identity and activity of meristems, organized tissues of pluripotent "stem" cells, that together determine plant form and architecture. However, little is known about how these factors act at the molecular level to affect meristem identity and function. Genetic studies in Arabidopsis and other plant species such as snapdragon, petunia, and maize have revealed a hierarchy of regulatory genes that function together to promote the formation of the floral meristem and to regulate floral organogenesis. Mutations in these genes result in dramatic defects in flower development that can affect both meristem identity and organ development. Table 1 lists the Arabidopsis genes that are known to control meristem and organ identity and their snapdragon equivalents.

One class of regulatory genes, the homeotic organ identity genes, have provided important insights into the genetic and molecular mechanisms that govern floral organ identity and development (Bowman et al., 1991; Coen and Meyerowitz, 1991; Coen and Carpenter, 1993, this issue; van der Krol and Chua, 1993, this issue). In Arabidopsis, these genes include *APETALA1* (*AP1*), *APETALA2* (*AP2*), *APETALA3* (*AP3*), *PISTILLATA* (*PI*), and *AGAMOUS* (*AG*) (Table 1). By contrast, our understanding of the genetic and molecular processes that govern the establishment and maintenance of the floral meristem is still in its infancy. Genetic studies have identified six genes in Arabidopsis that play an important role in the regulation of floral meristem identity and the pattern of meristem development (Table 1). One of these genes, *TERMINAL FLOWER1* (*TFL1*), ensures that the inflorescence and floral meristems remain functionally distinct. The other five genes, *LEAFY* (*LFY*), *CAULIFLOWER* (*CAL*), *AP1*, *AP2*, and *AG* together control the pattern of flower development by regulating floral meristem identity. In this review, our discussion will focus on how the analysis of these six genes has provided new genetic, molecular, and physiological insights into the regulation of flower development in Arabidopsis.

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SEPARATION BETWEEN INFLORESCENCE AND FLORAL MERISTEMS IS MAINTAINED BY *TERMINAL FLOWER1*

Reproductive development in Arabidopsis is controlled by the activities of the inflorescence and the floral meristems. Each of these meristems can be distinguished by its pattern of apical growth and organogenesis. For example, as illustrated in Figure 1, the inflorescence meristem is characterized by a pattern of indeterminate growth that under long-day growth conditions (16-hr light/8-hr dark) results in the production of two to four cauline leaves and lateral or secondary inflorescences, followed by the production of flowers. Each of these structures is produced by a meristem or organ primordium that was produced by and emerged from the flanks of the inflorescence meristem. By contrast, the floral meristem displays a determinate pattern of cell division and organogenesis, resulting in the production of four concentric rings or whorls of floral organs (sepals, petals, stamens, and carpels) that comprise the Arabidopsis flower (Figure 1). Although the inflorescence and floral meristems are closely related both spatially and by cell lineage, each meristem must maintain a separate identity and follow a different developmental pathway to carry out its unique functions. They do so in part through the activity of the *TFL1* gene.

TFL1 is responsible for the maintenance of the inflorescence meristem and the regulation of floral meristem production. *tfl1* mutants are characterized by both early flowering and the conversion of the inflorescence meristem into a terminal flower (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). This conversion limits the development of the normally indeterminate inflorescence and results in a dramatic loss of both lateral branches and flowers, as illustrated in Figure 2. In addition, *tfl1* plants often produce one or two lateral branches that are terminated by a single wild-type flower.

Genetic studies have shown that several genes, including *LFY*, *AP1*, and *AP2*, are involved in the establishment of the floral meristem (Irish and Sussex, 1990; Huala and Sussex, 1992; Weigel et al., 1992; Coen and Carpenter, 1993, this issue). For example, mutations in *lfy* cause a partial conversion of the floral meristem to an inflorescence (Table 1). It has been suggested that *TFL1* and these floral meristem–promoting genes function antagonistically in the inflorescence and floral meristems to maintain the separation of meristem functions

Table 1. Genes Involved in the Regulation of Meristem and Floral Organ Identity in Arabidopsis

Genetic Function/ Gene Locus	Mutant Phenotype	Proposed Molecular Function(s)	Snapdragon Homolog	References[a]
Inflorescence Meristem Identity				
TERMINAL FLOWER (TFL1)	Early flowering; conversion of inflorescence to floral meristem	Negative regulator of *LFY*, *AP1*, and *AP2*	ND[b]	1, 2
Floral Meristem Identity				
AGAMOUS (AG)	Indeterminate and repetitive pattern of floral organogenesis resulting in a "flower-within-a-flower" [(sepal, petal, petal)$_n$]	Putative transcription factor	*PLENA (PLE)*	3, 4, 5, 6
APETALA1 (AP1)	Production of axillary flowers	Putative transcription factor	*SQUAMOSA (SQUA)*	7, 8, 9
APETALA2 (AP2)	Similar to *ap1* under short-day growth conditions	Negative regulator of *AG*	ND	10, 11, 12, 13
CAULIFLOWER (CAL)	Phenotypically wild type; however, *ap1 cal* double mutants display a conversion of the floral meristem to an inflorescence	ND	ND	14
LEAFY (LFY)	Partial conversion of floral meristems to inflorescence shoots	Putative transcription factor Positive regulator of *AP3* and *PI*	*FLORICAULA (FLO)*	15, 16, 17, 18
Floral Organ Identity				
AGAMOUS (AG)	Homeotic conversion of stamens to petals and of carpels to sepals	Putative transcription factor Negative regulator of *AP1*, *AP2*, and *AP3*	*PLENA (PLE)*	3, 4, 5, 6
APETALA1 (AP1)	Homeotic conversion of sepals to leaves; absence of petals	Putative transcription factor	*SQUAMOSA (SQUA)*	7, 8, 9
APETALA2 (AP2)	Homeotic conversion of sepals to leaves or carpels and of petals to stamens	Negative regulator of *AG*	ND	10, 11, 12, 13
APETALA3 (AP3)	Homeotic conversion of petals to sepals and of stamens to carpels	Putative transcription factor Positive regulator of *PI*	*DEFICIENS (DEF)*	19, 20
PISTILLATA (PI)	Similar to *ap3*	Putative transcription factor	*GLOBOSA (GLO)*	21, 22

[a] (1) Shannon and Meeks-Wagner (1991); (2) Alvarez et al. (1992); (3) Bowman et al. (1989); (4) Yanofsky et al. (1990); (5) Carpenter and Coen (1990); (6) Bradley et al. (1993); (7) Irish and Sussex (1990); (8) Mandel et al. (1992); (9) Huijser et al. (1992); (10) Bowman et al. (1991); (11) Komaki et al. (1989); (12) Kunst et al. (1989); (13) Jofuku et al. (1993); (14) Bowman (1992); (15) Schultz and Haughn (1991); (16) Huala and Sussex (1992); (17) Weigel et al. (1992); (18) Weigel and Meyerowitz (1993); (19) Jack et al. (1992); (20) Sommer et al. (1990); (21) Hill and Lord (1989); (22) Tröbner et al. (1992).

[b] ND, not determined.

(Shannon and Meeks-Wagner, 1993). Double mutant studies showed that *TFL1* suppresses the activities of *LFY*, *AP1*, and *AP2* in the inflorescence meristem, and, conversely, that *LFY*, *AP1*, and *AP2* suppress *TFL1* activity in the floral meristem (Shannon and Meeks-Wagner, 1993). In addition, in situ hybridization analysis demonstrated at the molecular level that *TFL1* negatively regulates *LFY* gene expression in the inflorescence meristem (Weigel et al., 1992). Although it is not yet known how *TFL1* and *LFY*, *AP1*, and *AP2* carry out these functions, it is clear that the antagonistic relationship between these genes is essential for maintaining the separation between the inflorescence and floral meristems.

The effects of *tfl1* mutations on inflorescence development are determined in part by environmental conditions. Changes in both photoperiod and temperature have striking effects on mutant inflorescence development (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). For example, *tfl1* mutant plants grown under very long photoperiods (20-hr light/4-hr

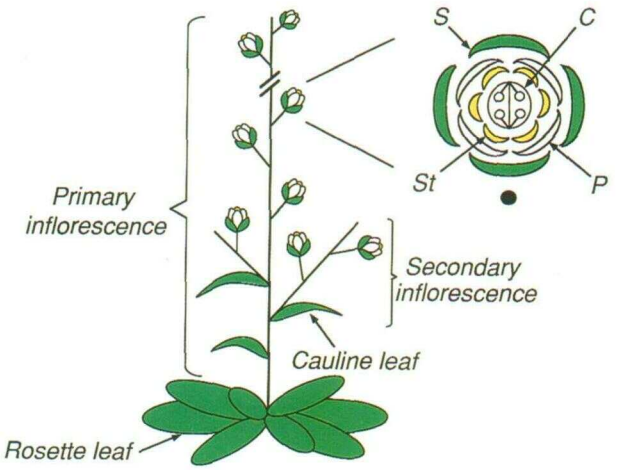

Figure 1. Schematic Representation of a Wild-Type Arabidopsis Inflorescence and Flower.

In Arabidopsis, the primary inflorescence consists of an inflorescence stem with a number of cauline leaves, lateral or secondary inflorescences, and flowers that are produced in a spiral pattern of phyllotaxis. The Arabidopsis flower consists of four concentric rings or whorls of floral organs: four sepals (S), four petals (P), six stamens (St), and two fused ovule-bearing carpels (C). The closed circle represents the position of the inflorescence meristem with respect to the flower.

dark) are phenotypically more extreme than *tfl1* plants grown under long-day conditions and may produce only a single flower (Shannon and Meeks-Wagner, 1991). By contrast, short day–grown *tfl1* plants (10-hr light/14-hr dark) are phenotypically much closer to wild type, producing both lateral inflorescences and flowers. Thus, long days enhance and short days suppress the *tfl1* mutant phenotype. *tfl1* mutants are also conditional with respect to temperature. Plants grown at 30°C display a more extreme phenotype than plants grown at the standard temperature of 22°C, whereas plants grown at 15°C are almost wild type (Alvarez et al., 1992). Although the molecular nature of the *tfl1* mutations are not yet known, these studies together suggest that *TFL1* may mediate the activity of a physiological inhibitor of floral meristem initiation (Alvarez et al., 1992).

LEAFY PROMOTES AND MAINTAINS FLORAL MERISTEM IDENTITY

LFY is a central player in the establishment of floral meristem identity and the regulation of flower homeotic gene expression. Mutations in *LFY* cause a partial block or delay in floral meristem production by the inflorescence meristem (Schultz

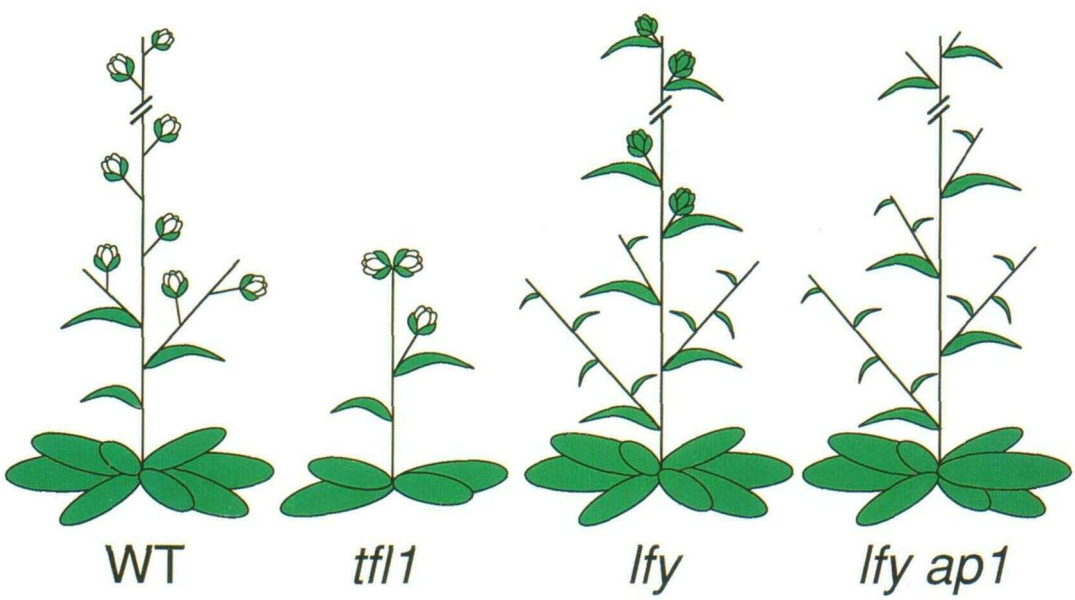

Figure 2. Genetic Control of Floral Meristem Production by *TERMINAL FLOWER1*, *LEAFY*, *APETALA1*, and *APETALA2*.

WT, *tfl1*, *lfy*, and *lfy ap1* refer to schematic representations of wild-type, *terminal flower1*, *leafy*, and *leafy apetala1* plants, respectively. *tfl1* mutants are characterized by early flowering and the conversion of the inflorescence meristem into a terminal flower (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). Under long-day conditions, mutant plants are characterized by the lack of lateral shoots and the production of only a few flowers. Severe *lfy* mutants are characterized by a partial block or delay in floral meristem production by the inflorescence meristem (Schultz and Haughn, 1991; Weigel et al., 1992). Flowers are replaced by shoots or flowerlike shoots (represented by green flowers). *lfy ap1* double mutants are characterized by a strong block in floral meristem production (Huala and Sussex, 1992; Weigel et al., 1992). Flowers are replaced by lateral shoots. *lfy ap2-1* mutants are similar in phenotype to *lfy ap1* double mutants (Huala and Sussex, 1992).

and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992). Thus, in strong *lfy* mutants, the first flowers produced by the primary inflorescence are replaced by shoots that are characterized by extended internodes, spiral phyllotaxis, and an indeterminate pattern of growth (Figure 2). As the inflorescence continues to grow, the lateral shoots become progressively more compact and flowerlike. However, these "flowers" are still phenotypically mutant, bearing sepals and carpel-like organs but no petals or stamens.

At the molecular level, *LFY* belongs to an evolutionarily conserved family of plant genes that includes the snapdragon gene *FLORICAULA* (*FLO*) (Weigel et al., 1992). Like *lfy* mutants, *flo* mutants are defective in the transition from inflorescence to floral meristem (Coen et al., 1990). Phenotypically, however, *flo* mutants are much stronger than *lfy* mutants, showing a complete conversion of flowers to shoots. It has been hypothesized that the proteins encoded by both *FLO* and *LFY* may function as transcription factors because they contain a proline-rich region as well as acidic and basic regions that are often found in eukaryotic transcription factors (Coen et al., 1990; Weigel et al., 1992). However, no other sequence similarity between LFY/FLO and other eukaryotic transcription factors is detectable, suggesting that these genes encode a novel class of plant regulatory protein.

One function of *LFY* may be to act as a genetic "trigger" for flower development by positively regulating homeotic gene expression. Consistent with this proposed function, *LFY* is the first known gene in the cascade of flower-specific meristem and organ identity genes to be activated during flower development (Weigel et al., 1992). As shown in Figure 3, *LFY* gene expression is detectable in the floral anlage even before the floral primordium becomes visible. As flower development progresses, *LFY* is expressed continuously and uniformly in young floral primordia. During organ development, *LFY* is highly expressed in the primordia of all four types of floral organs and appears to act as a positive regulator of *AP3*, *PI*, and *AG* gene expression (Weigel and Meyerowitz, 1993). *LFY* expression is transient, however, with transcript levels decreasing to undetectable levels in all four types of floral organs shortly before they reach maturity.

Recent studies have shown that the *LFY* gene is also required for the maintenance of floral meristem identity. Changes in photoperiod can dramatically alter floral meristem identity and development in both homozygous and heterozygous *lfy* mutant flowers (J.K. Okamuro, B.G.W. den Boer, C. Lotys, and K.D. Jofuku, manuscript submitted). For example, under long-day conditions, heterozygous flowers are phenotypically wild type. By contrast, Figure 4 shows that under short-day

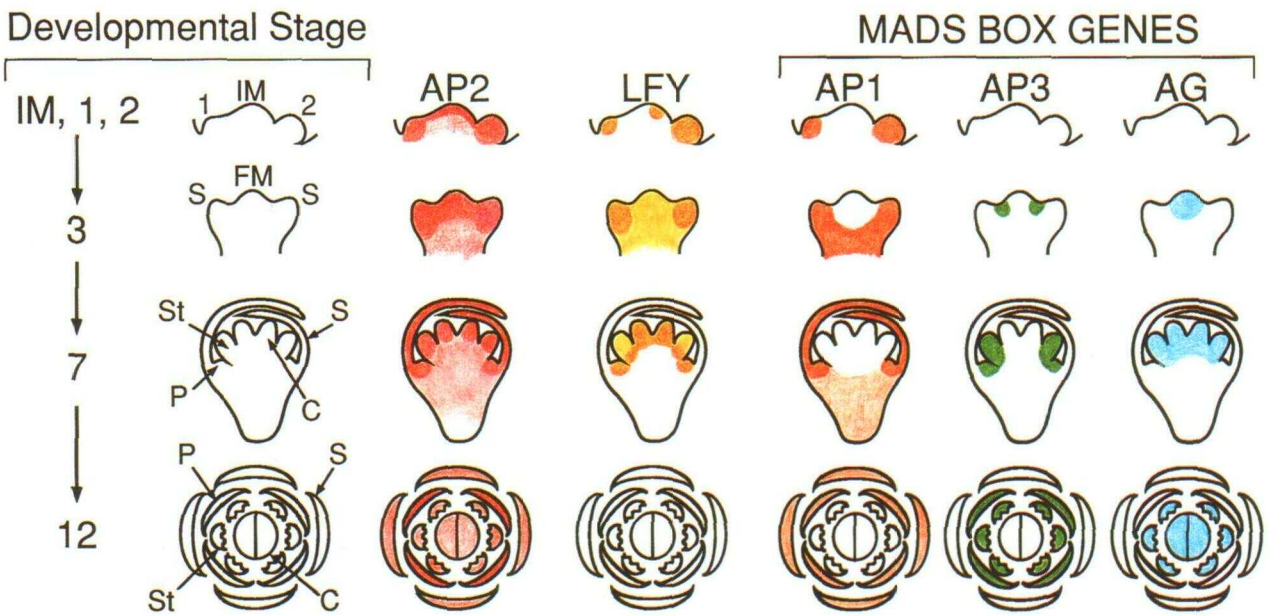

Figure 3. Temporal and Spatial Regulation of Arabidopsis Meristem and Organ Identity Gene Expression during Flower Development.

This diagram illustrates both the temporal and spatial patterns of gene expression during flower development for the meristem and organ identity genes *APETALA2* (*AP2*) (K.D. Jofuku, B.G.W. den Boer, M. Van Montagu, and J.K. Okamuro, manuscript submitted), *LEAFY* (*LFY*) (Weigel et al., 1992), *APETALA1* (*AP1*) (Mandel et al., 1992), *APETALA3* (*AP3*) (Jack et al., 1992), and *AGAMOUS* (*AG*) (Drews et al., 1991). Successive stages of flower development are represented by longitudinal sections through the inflorescence meristem (IM), the floral meristem (FM), and stage 1, 2, 3, and 7 flowers (as described by Smyth et al., 1990). Mature stage 12 flowers are represented by transverse sections showing all four types of floral organs: sepals (S), petals (P), stamens (St), and carpels (C). Regions of darker and lighter shading within a flower represent quantitative differences in transcript levels for *AP2*, *LFY* and *AP1*.

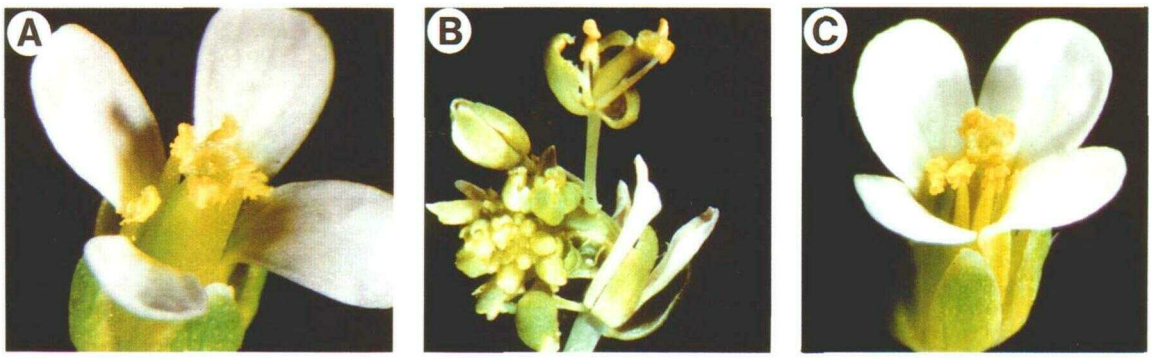

Figure 4. Physiological Control of Floral Meristem Identity in Heterozygous *leafy* Flowers.

(A) A heterozygous *leafy-6* (*lfy-6*) flower under long-day growth conditions. Heterozygous *lfy* flowers are morphologically and functionally normal. **(B)** Short-day–induced floral reversion in a heterozygous *lfy-6* flower. The mutant flowers are initially phenotypically wild type, characterized by a normal complement of sepals, petals, stamens, and carpels. However, instead of forming a normal fruit, the ovary becomes swollen and is eventually forced open by the growth of an ectopically formed inflorescence (J.K. Okamuro, B.G.W. den Boer, C. Lotys, and K.D. Jofuku, manuscript submitted). The ectopic inflorescence is characterized by elongated internodes, spiral phyllotaxis, and the production of many lateral flowers. **(C)** Exogenously applied gibberellin suppresses floral meristem reversion in heterozygous *lfy* flowers. Gibberellin-treated heterozygous *lfy* flowers produce a normal complement of floral organs under short-day conditions (J.K. Okamuro, B.G.W. den Boer, C. Lotys, and K.D. Jofuku, manuscript submitted).

conditions (9-hr light/15-hr dark), these flowers undergo a reversion to inflorescence development after producing a normal complement of floral organs. Thus, a heterozygous "mutant" flower consists of sepals, petals, stamens, carpels, and an ectopic inflorescence. This photoperiod-dependent transformation or reversion to inflorescence development clearly demonstrates that floral meristem identity and determinacy are not irreversibly fixed in the floral meristem. Moreover, it shows that the floral meristem is capable of sustained growth and development even after forming a normal complement of floral organs. Although the phenomenon of floral reversion is not unique to Arabidopsis and has been demonstrated for several plant species, including *Triticum aestivum* (wheat), *Impatiens balsamina*, and *Anagallis* (reviewed by Battey and Lyndon, 1990), *LFY* is the first gene to be implicated in the control of this process. Thus, the characterization of *LFY* function may provide clues into the molecular mechanism that regulates the maintenance of floral meristem identity under changing growth conditions.

APETALA1 CONTROLS FLORAL MERISTEM AND ORGAN IDENTITY AT THE TRANSCRIPTIONAL LEVEL

AP1 plays a dual role in the regulation of floral meristem identity and the control of floral organ identity and development. *ap1* mutants are characterized by the partial conversion of the floral meristem into an inflorescence meristem, resulting in the production of secondary flowers within the axils of the first whorl floral organs (Irish and Sussex, 1990; Bowman, 1992). The flowers produced by *ap1* mutants also show a homeotic conversion of sepals into bractlike leaves, the loss of petals, and a normal complement of stamens and carpels. In addition, recent experiments have shown that the inflorescence-like character of the *ap1* mutant is enhanced under short-day growth conditions, suggesting that *AP1*, like the meristem identity gene *LFY*, is involved in the physiological regulation of floral meristem identity and development (J.K. Okamuro, B.G.W. den Boer, C. Lotys, and K.D. Jofuku, unpublished results).

The role of *AP1* in the establishment of floral meristem identity is clearly revealed in double mutant studies by combining *ap1* with other meristem mutations. For example, *cauliflower* (*cal*) is a genetic enhancer of the *ap1* floral phenotype (Bowman, 1992). *cal* mutants have little if any discernible phenotype of their own. However, *cal* can transform the floral meristem into an inflorescence when in double mutant combination with *ap1*. Thus, *AP1* and *CAL* function together to promote the transition from inflorescence to floral meristem. The molecular nature of the *AP1/CAL* interaction is not yet known.

The establishment of the floral meristem is also due in part to a synergistic interaction between *AP1* and *LFY*. For example, by combining the *lfy* and *ap1* mutations together in a *lfy ap1* double mutant, flower production is blocked and floral meristems are converted into shoots, as shown in Figure 2 (Huala and Sussex, 1992; Weigel et al., 1992). This synergism is also reflected at the molecular level. In situ hybridization experiments have shown that *ap1* and *lfy* single mutants do not strongly suppress the flower-specific expression of the homeotic organ identity genes *AP3* and *PI*. By contrast, *AP3* and *PI* gene expression is completely suppressed in most "flowers" of the *lfy ap1* double mutant (Weigel and Meyerowitz,

1993). Although the molecular basis of the interaction between *AP1* and *LFY* and its effects on homeotic gene expression have not yet been determined, it is clear that *AP1* and *LFY* function cooperatively to promote floral meristem identity.

Molecular studies have shown that there is a strong correlation between the temporal and spatial regulation of *AP1* gene expression and its genetic functions in the control of floral meristem and organ identity and development (Mandel et al., 1992). *AP1* transcripts are first detectable in very young floral buds, before the emergence of the first organ primordia and after the onset of *LFY* gene expression (Figure 3). As flower development progresses, *AP1* gene expression expands to include both sepal and petal organ primordia but is excluded from developing stamens and carpels by another homeotic gene, *AG* (Mandel et al., 1992). This antagonistic relationship between *AP1* and *AG* establishes an important boundary of homeotic gene activity between the petal and stamen primordia in the developing flower. Similarly, the temporal and spatial boundaries of *AG* gene expression are controlled in part by an antagonistic interaction with *AP2* (Drews et al., 1991). Together, these interactions illustrate an important regulatory mechanism in pattern formation that is used by both plants and animals (Coen and Meyerowitz, 1991; Nüsslein-Volhard, 1991).

How does *AP1* regulate flower meristem identity and organ development at the molecular level? *AP1* belongs to an evolutionarily conserved family of transcription factors that includes the Arabidopsis homeotic genes *AG*, *AP3*, and *PI* as well as their snapdragon homologs *SQUAMOSA* (*SQUA*), *PLENA* (*PLE*), *DEFICIENS* (*DEF*), and *GLOBOSA* (*GLO*) (Table 1). Each of these regulatory factors is characterized by a highly conserved 58–amino acid DNA binding domain called the MADS box (derived from yeast MCM1 [*minichromosome maintenance-1*; Ammerer, 1990], Arabidopsis AG [Yanofsky et al., 1990], snapdragon DEF [Sommer et al., 1990], and human SRF [serum response factor; Norman et al., 1988]). Much of what is known about the functions of these proteins comes from in vitro studies of MCM1 and SRF (reviewed by Treisman and Ammerer, 1992). These studies showed that MCM1 and SRF function as dimers to regulate gene expression in response to extracellular signals and that their activity is regulated in part by their interactions with cell-specific accessory transcription factors. MCM1 and SRF share a high degree of sequence similarity within a 91–amino acid "core domain" that includes the MADS box. This core domain contains all sequences necessary for DNA recognition and binding, protein dimerization, and interaction with accessory transcription factors (Norman et al., 1988; Ammerer, 1990; Christ and Tye, 1991; Mueller and Nordheim, 1991; Primig et al., 1991).

AP1, like other plant MADS box–containing proteins, shows no significant homology to MCM1 or SRF outside the MADS box (Mandel et al., 1992). To date, the only flower homeotic proteins studied in detail with respect to their structure and function are AG, DEF, and GLO (Mueller and Nordheim, 1991; Schwarz-Sommer et al., 1992; Tröbner et al., 1992; Shiraishi et al., 1993). In vitro studies have shown that the MADS box domain from AG and DEF, and by inference AP1, is sufficient for DNA recognition and binding (Mueller and Nordheim, 1991; Shiraishi et al., 1993). However, these experiments do not address the question of what sequences in these proteins are responsible for their ability to interact with other proteins to activate or repress gene transcription. It has been shown that the conserved carboxyl region of the SRF/MCM1 core domain mediates the interactions of SRF and MCM1 with cell-specific accessory transcription factors, and it is possible that this region may serve a similar function for other MADS box–containing proteins, such as AP1. Alternatively, sequences found elsewhere in these proteins could serve this function.

The plant MADS box–containing genes share a conserved 65–amino acid region that is located near the carboxyl end of the MADS box domain and is not found in either MCM1 or SRF. This second domain of homology, called the K-box because it bears structural similarity to the interacting region of keratins, is capable of forming a coiled-coil structure due to the propensity of regions within this domain to form amphipathic α-helices (Ma et al., 1991). The formation of coiled-coils mediates protein–protein interactions in both structural and regulatory proteins such as yeast GCN4 (O'Shea et al., 1989) and the BZLF1 gene product of the Epstein–Barr virus (Flemington and Speck, 1990) and could function similarly for AP1 and other plant MADS box–containing proteins to mediate dimerization or possible interactions with accessory transcription factors.

AGAMOUS IS INVOLVED IN THE TERMINATION OF FLOWER DEVELOPMENT

The floral homeotic gene *AG* plays a critical role in regulating the second half of flower ontogeny—the development of stamens and carpels and the termination of flower development. *ag* flowers are characterized by the absence of carpels and the homeotic conversion of stamens to petals. In addition, the floral meristem displays an indeterminate or repetitive pattern of organogenesis that results in a (sepal, petal, petal)$_n$ structure referred to as a flower-within-a-flower (Bowman et al., 1989). Together, these mutant characteristics indicate that *AG* is necessary for the genetic control of both floral meristem determinacy and floral organ identity.

The effects of *ag* mutations on floral organ identity may be due in part to the role of *AG* as a negative regulator of *AP1* gene expression. In wild-type flowers, *AP1* activity is restricted to developing sepals and petals (Mandel et al., 1992). In *ag* flowers, however, the *AP1* gene is ectopically expressed in third whorl organ primordia and thus may be responsible for the homeotic conversion of stamens to petals (Irish and Sussex, 1990; Mandel et al., 1992). By contrast, the indeterminate or repetitive pattern of *ag* flower organogenesis does not result

from the ectopic activity of either *AP1* or *LFY*, as shown by double mutant studies (Irish and Sussex, 1990; Weigel et al., 1993).

Under short-day conditions, *ag* flowers may also undergo a heterochronic reversion of the floral meristem to an inflorescence meristem similar to that seen in flowers from plants heterozygous for the *lfy* mutation (J.K. Okamuro, B.G.W. den Boer, C. Lotys, and K.D. Jofuku, manuscript submitted). That is, after producing several whorls of sepals and petals, the floral meristem reverts to the production of axillary floral buds, extended internodes, and spiral phyllotaxis, as shown in Figure 5. These mutant flowers are usually found along the primary inflorescence at the normal point of transition from shoot to flower production. This short-day–induced reversion from flower to inflorescence meristem in *ag* flowers again demonstrates that floral meristem identity is not fixed but is maintained physiologically by *AG* as well as by *LFY*.

AG, like *AP1* and *LFY*, shows a temporal and spatial pattern of gene expression that reflects its genetic functions. *AG* RNA transcripts appear later in flower development than either *LFY* or *AP1* transcripts and are first detectable in the central region of the flower primordium after the sepal primordia have begun to emerge from the flanks of the floral meristem (Figure 3). As organogenesis progresses, *AG* gene expression is restricted to developing stamens and carpels and is undetectable in both sepals and petals. Both the temporal and spatial pattern of *AG* gene expression is determined in part by the homeotic gene *AP2*, which negatively regulates *AG* gene expression in early floral buds, in sepals, and in petals (Drews et al., 1991). The molecular nature of the *AP2–AG* interaction is not yet known.

APETALA2 FUNCTIONS THROUGHOUT FLOWER DEVELOPMENT

AP2, like *AG*, is involved in the genetic control of both meristem identity and floral organ identity. *ap2* mutant flowers are characterized by a broad spectrum of related phenotypes, and mutations in *AP2* affect the identity or development of all four floral organs (Komaki et al., 1988; Bowman et al., 1989, 1991; Kunst et al., 1989; K.D. Jofuku, B.G.W. den Boer, M. Van Montagu, and J.K. Okamuro, manuscript submitted). Severe *ap2* mutant flowers are characterized by the homeotic transformation of sepals into ovule-bearing carpels, the absence of petals, a reduction in stamen number, and defects in carpel fusion. By contrast, weak *ap2* mutant flowers are characterized by the homeotic conversions of sepals into leaves and of petals into pollen-producing stamens, and they contain a normal complement of stamens and carpels.

The effects of *ap2* mutations on flower organ identity are due in part to the role of *AP2* in the temporal and spatial regulation of *AG* gene expression. In wild-type flowers, *AG* gene activity is confined to developing stamens and carpels. In strong *ap2* mutants, however, the *AG* gene is activated at an earlier stage of flower development and is ectopically expressed in both first and second whorl organ primordia, resulting in the homeotic conversion of sepals to carpels and an absence of petals (Drews et al., 1991). The loss of second and third whorl organs in strong *ap2* mutants is due to ectopic *AG* gene activity, because these organs are restored in *ap2 ag* double mutants (Bowman et al., 1991). This negative effect of *AG* on

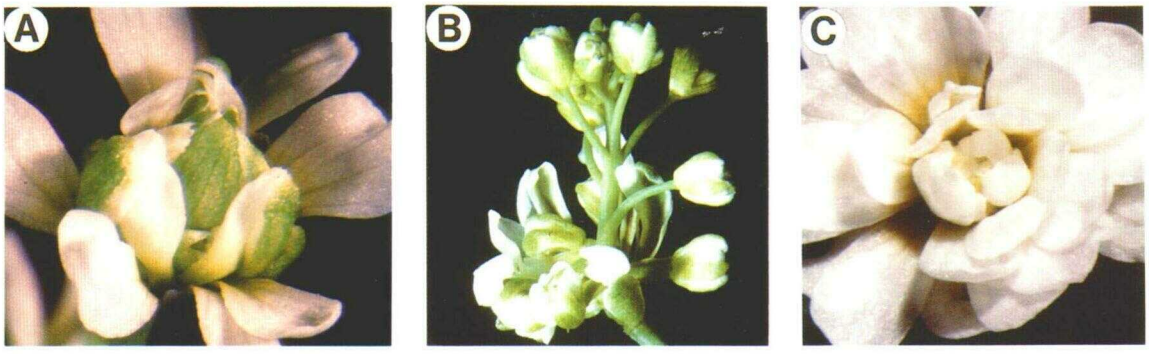

Figure 5. Physiological Control of Floral Meristem Identity in *agamous* Flowers.

(A) An *agamous-1* (*ag-1*) mutant flower grown under standard long-day conditions (16-hr light/8-hr dark). The mutant flower phenotype consists of the repeating flower-within-a-flower pattern of organogenesis (sepal, petal, petal)$_n$.

(B) Short-day–induced floral meristem reversion of an *ag* flower. After producing several whorls of sepals and petals, the floral meristem reverts to an inflorescence meristem under short-day conditions (J.K. Okamuro, B.G.W. den Boer, C. Lotys, and K.D. Jofuku, manuscript submitted). The ectopic inflorescence displays both elongated internodes, spiral phyllotaxis, and the production of lateral flowers.

(C) Exogenously applied gibberellin suppresses floral meristem reversion in short-day–induced *ag* flowers.

petal development was clearly demonstrated by Mizukami and Ma (1992), who induced "*ap2*-like" mutants in transgenic wild-type plants by the constitutive ectopic expression of a chimeric *AG* gene in the flower.

In addition to its role in the control of floral organ identity, *AP2* also contributes to the establishment of floral meristem identity. In response to short-day photoperiod, several weak *ap2* mutants will produce secondary flowers in the axils of the first whorl organs of the flower and will fail to make petals (Komaki et al., 1988; Shannon and Meeks-Wagner, 1993; J.K. Okamuro, B.G.W. den Boer, and K.D. Jofuku, unpublished observations). This short-day phenotype resembles that of *ap1* flowers grown under long-day conditions and suggests that *AP2* and *AP1* affect similar processes in flower development. In addition, genetic studies have shown that *AP2* promotes floral meristem development through synergistic interactions with both *AP1* and *LFY*. For example, mutations in *lfy* do not block flower development completely. However, weak mutations in *ap2*, such as *ap2-1*, when in combination with *lfy*, can completely block the formation of flowers, resulting in the conversion of whorled flowers into shoots (Huala and Sussex, 1992). Moreover, flowers are converted into indeterminate inflorescence branches in *ap2-1 ap1-1* double mutants (Irish and Sussex, 1990). The mechanism by which these three genes together promote floral meristem development has not yet been determined.

The molecular analysis of *AP2* gene expression has shown that *AP2* is expressed continuously in both the inflorescence and floral meristems and in all four types of floral organs (Figure 3; K.D. Jofuku, B.G.W. den Boer, M. Van Montagu, and J.K. Okamuro, manuscript submitted). The sustained expression of *AP2* during inflorescence and early flower development provides a striking contrast to the sequential and spatially overlapping expression domains of the MADS box–containing genes *AP1*, *AP3*, and *AG* and to the transient expression of *LFY* in all four types of floral organs. Thus, one important conclusion from the molecular analysis of *AP2* gene expression is that its expression during flower development overlaps with the expression of all three MADS box–containing genes, including *AG*. This indicates that *AG* does not suppress *AP2* gene transcription in stamens and carpels as was proposed by Meyerowitz et al. (1991) and that *AP2* expression in these organs does not suppress *AG* gene expression as it does in sepals and petals. Thus, it appears that *AP2* alone is not capable of suppressing *AG* gene activity but may function cooperatively with an unidentified gene whose activity is restricted to sepals and petals.

Finally, mRNA gel blot studies have shown that *AP2* gene expression is not restricted to flowers and the inflorescence meristem. *AP2* is also expressed at low levels in leaf, stem, and root (K.D. Jofuku, B.G.W. den Boer, M. Van Montagu, and J.K. Okamuro, manuscript submitted; B.G.W. den Boer, unpublished results). Although *ap2* mutants have no visible defects in vegetative development under long-day growth conditions (Bowman et al., 1989), short-day studies suggest that *AP2* does have a cryptic function in stem development (J.K. Okamuro, unpublished results).

How does *AP2* carry out its genetic functions at the molecular level? DNA sequence analysis has shown that *AP2* encodes a protein that is distinct from all known plant, fungal, and animal regulatory proteins (K.D. Jofuku, B.G.W. den Boer, M. Van Montagu, and J.K. Okamuro, manuscript submitted). However, the ability of *AP2* to repress *AG* gene expression in the flower and the presence of a putative nuclear localization signal sequence in the AP2 protein suggests that it may function as a transcription factor. In addition, the AP2 protein contains a highly acidic serine-rich domain that is structurally similar to regions found in several nucleic acid binding proteins. Another feature of the AP2 protein is a 69–amino acid repeated motif, the *AP2*-domain, that is evolutionarily conserved in AP2-like proteins from plants as divergent as petunia, snapdragon, and rice (B.G.W. den Boer, A. Gerats, M. Van Montagu, E.S. Coen, J.K. Okamuro, and K.D. Jofuku, unpublished results). Sequences within the *AP2*-domain are theoretically capable of forming amphipathic helical structures that may mediate protein–protein interactions. Thus, one possible function of this domain may be to mediate interactions between AP2 and the products of other homeotic genes such as *AP1* or *LFY*.

HORMONAL CONTROL OF FLORAL MERISTEM DEVELOPMENT

Arabidopsis is a quantitative long-day plant. Changes in photoperiod and temperature can dramatically affect the timing and duration of vegetative and inflorescence growth (Langridge, 1957; Bernier et al., 1993, this issue) but do not normally alter the wild-type pattern of floral organogenesis or inflorescence development (Shannon and Meeks-Wagner, 1991). This developmental homeostasis or resistance to environmental perturbations occurs in both plants and animals and is referred to as canalization (Waddington, 1942). Recent experiments have shown that an important function of the network of meristem identity genes is to maintain the normal pattern of flower development under changing environmental conditions. Each of the five mutants we have just described—*lfy*, *ag*, *ap1*, *ap2*, and *tfl1*—has a classic long-day "signature" phenotype with respect to the identity or pattern of flower or inflorescence development. Each phenotype, however, can be dramatically altered by changes in photoperiod or temperature. Thus, under short-day conditions, the floral meristems of *lfy*, *ag*, *ap1*, and *ap2* mutants display new traits that are normally characteristics of an inflorescence meristem, and the inflorescence meristem of the *tfl1* mutant is more wild type under short-day than long-day conditions. The effects of short-day photoperiod on flower development in these mutants suggest that these genes must normally function together to maintain the normal

pattern of inflorescence and floral meristem development despite varying physiological conditions.

How does wild-type Arabidopsis maintain a normal pattern of flower and inflorescence development under changing environmental conditions? A wealth of studies in numerous plant species have implicated the hormone gibberellin as an important regulator of flowering and flower development in higher plants (reviewed by Zeevaart, 1983; Kinet et al., 1985; Chailakhyan and Khrianin, 1987; Bernier et al., 1993, this issue). Moreover, gibberellin levels can be affected by changes in both photoperiod and temperature due to changes in the activities of specific enzymes in the gibberellin biosynthetic pathways (Metzger and Zeevaart, 1980; 1982; Gilmour et al., 1986; Hazebroek and Metzger, 1990; Hazebroek et al., 1993). One clue that gibberellin might be involved in the physiological control of floral induction and inflorescence development in Arabidopsis comes from a recent study by Wilson et al. (1992), who showed that flowering can be completely suppressed in the gibberellin biosynthesis mutant *ga1* under short-day conditions. Moreover, short-day–grown *ga1* mutants can be induced to flower if treated with exogenous gibberellin, thus demonstrating a direct correlation between flowering and gibberellin under short-day conditions in Arabidopsis.

If the effects of short days on flower development in *ag*, *ap1*, *ap2*, and *lfy* plants are due to reduced gibberellin levels, then the exogenous application of gibberellin should reverse the effects of short-day conditions on floral meristem identity. Recent experiments have shown that this is indeed the case (J.K. Okamuro, B.G.W. den Boer, C. Lotys, and K.D. Jofuku, manuscript submitted). The short-day–induced reversion of the floral meristem to an inflorescence in both *lfy* heterozygous and *ag* homozygous mutants is fully suppressed by the application of exogenous gibberellin (Figures 4 and 5). Moreover, the effects of short-day photoperiod on *ap1* and *ap2* floral meristem identity are also suppressed by gibberellin (J.K. Okamuro, B.G.W. den Boer, C. Lotys, and K.D. Jofuku, unpublished data). Together, these experiments suggest that the effects of short-day photoperiod on floral meristem identity and development in these mutants are mediated by a decrease in gibberellin levels (J.K. Okamuro, B.G.W. den Boer, C. Lotys, and K.D. Jofuku, manuscript submitted).

What insight do these physiological studies provide regarding the regulation of inflorescence and flower development in Arabidopsis? One possible explanation for the effects of short-day photoperiod and gibberellin on inflorescence and floral meristem identity in these mutants is that the expression of one or more of the meristem identity genes *AP1*, *AP2*, *AG*, or *LFY* is positively regulated, either directly or indirectly, by gibberellin. Thus, when gibberellin levels are reduced under short-day conditions, the inflorescence character of the floral meristem in *lfy*, *ap1*, or *ag* mutants is conditionally enhanced, perhaps due to increased expression of *TFL1*, the antagonist of the floral meristem–promoting genes. Conversely, under long-day conditions, when gibberellin levels are high, *tfl1* mutants are phenotypically enhanced; that is, the inflorescence meristem is transformed into a floral meristem due to the ectopic expression of the floral meristem–promoting genes.

CONCLUSION

Plant reproduction is governed by both intrinsic and environmental factors that coordinate the activities of the inflorescence and floral meristems. Many of the genes that regulate floral meristem identity and development have been identified and characterized in Arabidopsis. These studies have provided much of our current knowledge on the genetic and molecular regulation of flower development. In addition, recent physiological studies utilizing floral meristem mutants have implicated the plant hormone gibberellin as an important regulator of Arabidopsis flower development. Together, these studies suggest that an integrated molecular and physiological understanding of how flower development is regulated may soon be possible.

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