The Plant Cell, Vol. 7, 1485–1499, September 1995

UNUSUAL FLORAL ORGANS Controls Meristem Identity and Organ Primordia Fate in Arabidopsis

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A novel gene that is involved in regulating flower initiation and development has been identified in Arabidopsis. This gene has been designated *UNUSUAL FLORAL ORGANS* (*UFO*), with five corresponding nuclear recessive alleles designated *ufo-1* to *ufo-5*. Under short day–length conditions, *ufo* homozygotes generate more coflorescences than do the wild type, and coflorescences often appear apical to the first floral shoot, resulting in a period of inflorescence development in which regions of floral and coflorescence shoots are produced alternately. *ufo* enhances the phenotype of weak *leafy* alleles, and the double mutant Ufo-1 Apetala1-1 produces only coflorescence-like shoots, suggesting that these two genes control different aspects of floral initiation. Floral development was also altered in Ufo plants. Ufo flowers have an altered organ number in all whorls, and organs in the first, second, and third whorls exhibit variable homeotic transformations. Ufo single and double mutant phenotypes suggest that the floral changes result from reduction in class B floral homeotic gene expression and fluctuations in the expression boundaries of class C function and *FLO10*. Surprisingly, in situ hybridization analysis revealed no obvious differences in expression pattern or level in developing Ufo flowers compared with that of the wild type for any class B or C gene studied. We propose that *UFO* acts in concert with known floral initiation genes and regulates the domains of floral homeotic gene function.

INTRODUCTION

The mature Arabidopsis plant consists of a basal rosette and several nodes bearing lateral inflorescence shoots (coflorescences; Weiberling, 1965), which are followed by nodes bearing flowers in a racemous arrangement until senescence. Lateral shoots must therefore select one of two fates: vegetative (rosette and coflorescence) or floral (Shannon and Meeks-Wagner, 1991, 1993; Schultz and Haughn, 1993).

An understanding of the process of fate selection is crucial to an analysis of development in any organism. In Arabidopsis, such processes have been dissected by analysis of mutations that affect the ability of primordia to select fates appropriate to their spatial position (for reviews, see Shannon and Meeks-Wagner, 1993; Ma, 1994; Meyerowitz, 1994; Weigel and Meyerowitz, 1994). Of those that affect flowering, there are two broad categories: those that affect both floral initiation and floral organ type, and those that affect primarily floral organ type.

Studies of mutants impaired in the process of vegetative-to-reproductive transition in the primary shoot have led to the proposal of a model to explain fate choices in the inflorescence (Schultz and Haughn, 1993; Haughn et al., 1995). In this model, control of transitions from one type of node to the next is achieved by a signal (termed COPS, for control of phase switching) whose strength changes acropetally. As the signal strength passes through threshold levels, fate choices by lateral shoot and organ primordia, and thus the node type, also change.

The transition from coflorescence to floral node production has been studied in some detail, and loci involved in floral initiation have been termed FLIP (floral initiation process) or floral meristem identity genes. FLIP genes, namely, *LEAFY* (*LFY*), *APETALA1* (*AP1*), *CAULIFLOWER* (*CAL*), and *APETALA2* (*AP2*), have partially redundant roles in the FLIP. A mutation in any FLIP gene results in generation of flowers with coflorescence features; however, the severity of this phenotype depends on which FLIP gene is nonfunctional (Huala and Sussex, 1992; Bowman et al., 1993; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993; Weigel and Meyerowitz, 1993). *LFY* has the strongest effect, followed by *AP1*/*CAL* and *AP2*. It has been proposed that FLIP genes become active at a particular threshold level of COPS activity (Schultz and Haughn, 1993; Haughn et al., 1995) to direct lateral shoots to adopt a floral fate.

Determination of organ type within the flower occurs shortly after the initiation of floral organ primordia (Bowman et al., 1989) and is controlled at least partially by FLIP gene activity (Huala and Sussex, 1992; Bowman et al., 1993; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993; Weigel and Meyerowitz, 1993). Three major classes of floral organ determination genes have been identified. The FLIP genes *AP1* and *AP2* affect organ identity in the first three whorls but play a major role in determining perianth structures and have been designated class A genes (Komaki et al., 1988; Bowman et

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al., 1989, 1991, 1993; Kunst et al., 1989; Schultz and Haughn, 1993; Weigel and Meyerowitz, 1993; Gustafson-Brown et al., 1994; Jofuku et al., 1994). Class B genes, namely, *PISTILLATA* (*PI*) and *APETALA3* (*AP3*), are required for fate selection by primordia in whorls two and three (Bowman et al., 1989, 1991; Hill and Lord, 1989; Jack et al., 1992). The class C gene *AGAMOUS* (*AG*) is required for fate determination in reproductive whorls and floral meristem determinacy (Bowman et al., 1989, 1991; Yanofsky et al., 1990; Drews et al., 1991). A model has been proposed in which determination of floral organ type is governed through the combined action of class A, B, and C genes (Haughn and Somerville, 1988; Kunst et al., 1989; Bowman et al., 1991).

The mechanisms by which these genes might be spatially or temporally regulated have been elucidated by the *flo10* (floral mutant 10) mutation. Flo10 flowers generate additional stamen whorls, indicating that class B gene activity is present beyond the third whorl. Thus, it appears that the *FLO10* (also named *SUPERMAN*) gene product establishes a boundary of class B gene expression between the third and fourth whorls (Schultz et al., 1991; Bowman et al., 1992). Mechanisms for establishing other floral homeotic gene expression boundaries are less clear.

Here, we report the isolation and characterization of floral homeotic mutants designated unusual floral organs (Ufo) that are defective in a novel gene, *UFO*. Our results suggest that, like the known FLIP genes, the *UFO* gene has a role both in initiating flower development and in regulating floral organ identity genes.

RESULTS

Mutant Isolation and Genetic Analysis

The phenotypes of five allelic ethyl methanesulfonate–induced mutations in both the Landsberg *erecta* (Ler) and Columbia-2 (Col-2) ecotypes of Arabidopsis were examined. Each mutant exhibited highly variable homeotic alterations in the second and third floral whorls and changes in organ number in all whorls. This phenotype was named Unusual Floral Organs (Ufo) (Haughn et al., 1994; Wilkinson and Haughn, 1994; Levin and Meyerowitz, 1995). The different mutant lines were designated as Ufo-1 (Col-2) and Ufo-2, Ufo-3, Ufo-4, and Ufo-5 (*Ler*). Before analysis, Ufo-1 was backcrossed to Col-2 at least four times, and other mutants were backcrossed to *Ler* at least twice. All lines segregated the mutant phenotype in a 3:1 ratio, indicating that each resulted from a single recessive nuclear mutation in the gene designated *UFO*. All mutant phenotypes were similar; however, some differences were observed between the *Ler* and Col-2 alleles (described later). The *ufo-1* allele was selected for further analysis.

To ensure that the *UFO* gene does not represent a previously known locus, *UFO* was mapped genetically. Phenotypic segregation from the cross Ufo-1 × W100 (Koornneef et al., 1987) indicated that the *UFO* gene is linked to the marker *ANGUSTIFOLIA* (*AN*) on chromosome 1. Figure 1 summarizes recombination data from a cross of Ufo-1 to chromosome 1 marker line MSU7 (*eceriferum5* [*cer5*], *distorted trichomes2* [*dis2*], *gibberellic acid resistant4* [*ga4*]; *er*) (Koornneef and Hanhart, 1983). No mutations affecting floral morphology have been mapped to this region.

Ufo Rosette and Inflorescence Morphology

Figures 2 and 3 and Table 1 provide data concerning the rosette and inflorescence morphology of wild-type, Ufo-1, and Ufo-3 plants. When under continuous light (CL) conditions, Ufo mutants exhibit a slight but significant increase in coflorescence number compared with the wild type, whereas rosette development is not significantly affected (Figures 2A and 2B and Table 1). Unlike the wild type, the meristems of all Ufo primary and coflorescence shoots terminate in a structure resembling either a normal pistil or fused sepal–carpel organs (Figure 3M). The number of nodes bearing flowers produced before termination in Ufo-1 is approximately the same as the number of floral nodes generated before senescence in wild-type plants; however, alleles of *ufo* in the *Ler* background cause some mutants to terminate apical growth as early as the third floral node (Ufo-3: range of 3 to 13 floral nodes, mean of 7.9; compared with *Ler*: range of 24 to 36 floral nodes, mean of 30.3). Such observations suggest that part of the Ufo mutant phenotype includes premature termination of apical growth.

Figures 2 and 3 and Table 1 show that vegetative growth of Ufo mutants in short days (SD) was clearly different from that of the wild type (Figures 2C and 2D). To ensure that these vegetative changes were not allele specific, two alleles, one in each of two genetic backgrounds, were examined. Mutants generate two to three times more coflorescence nodes than do wild-type plants, which bolt during the same interval. In addition, approximately one to three nodes generated prior to the first flower consist of a bract with no associated axial shoot (Figure 2D). Ufo plants in both ecotypes produce coflorescences apical to early floral nodes in both the primary and lateral inflorescence shoots (Figures 2D and 3A). Up to seven coflorescences can be produced consecutively in positions apical to the first flower before the ensuing flower is produced.

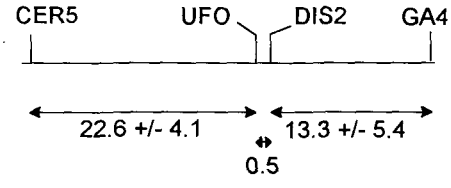

Figure 1. Map Position of the *UFO* Gene on Chromosome 1.

Results of linkage analysis from the cross *ufo-1/ufo-1* × *MSU7* (*Ler*; *ga4 dis2 cer5*; *er1*) are shown. Numbers below the arrows represent distances between markers in map units.

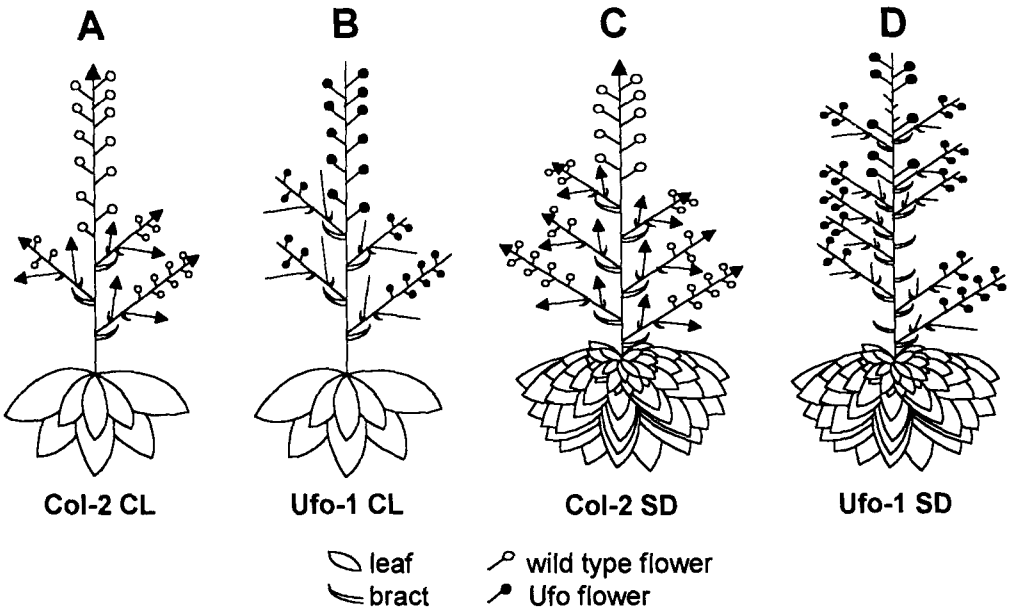

Figure 2. Structure of Wild-Type and Ufo Plants Grown under CL and SD Conditions.

Nodes with no lateral shoot are indicated as a bract with no associated inflorescence. Indeterminate inflorescences are indicated by arrows, and determinate inflorescences are indicated by lines without arrows.

(A) Morphology of a wild-type plant grown in CL.

(B) Morphology of Ufo-1 plants grown in CL.

(C) Morphology of wild-type plants grown in SD.

(D) Morphology of Ufo-1 plants grown in SD. Note the nodes from which no lateral shoot is produced.

These phases of coflorescence production can occur up to three times during the growth of an individual plant, with each coflorescence phase being separated by one to five Ufo-1 flowers. As in CL, all apical meristems terminate in carpel-like structures. Bracts or filamentous structures and stipules subtend most Ufo-1 flowers. Typically, morphologically normal bracts are limited to the most basal and most apical flowers (Figure 3B). In addition, SD-grown plants invariably generate numerous nodes in floral position from which no shoot of any kind was produced (Figure 3C). Ten or more such nodes appear consecutively after the first four to five flowers have been initiated and can be identified by the presence of reduced bract-like structures and stipules. Production of Ufo-1 flowers resumes thereafter; however, these unusual nodes continue to appear in scattered positions throughout the inflorescence.

Ufo Floral Morphology

Table 2 and Figure 3 illustrate the highly variable floral morphology of Ufo-1 plants. Homeotic changes are apparent in second and third whorl organs, whereas changes in organ number occur in all whorls. Floral defects are most severe in early flowers (Figure 3D). Unlike most other mutations affecting floral organ type, the fate chosen by an organ primordia can differ from that chosen by other primordia in the same whorl.

The first whorl of Ufo-1 flowers is generally unaffected. Additional sepals were rarely observed (two in 106 flowers); however, ontogenic analysis indicates that pairs of sepal primordia frequently appear in the position expected of a single primordium in the wild type (Figure 3E). All *ufo* alleles in the *Ler* background generate some flowers in which one or more first whorl organs are mosaics of sepal and carpel tissue (15 organs of 76 flowers scored). This becomes more pronounced in more apical flowers. Transformations of this type were never observed in Ufo-1 flowers.

Second whorl organ types in Ufo-1 flowers range from sepals and filamentous organs (similar to Figure 3J) in early flowers to normal petals or staminoid organs in later flowers. On the basis of cell surface features, most were a mosaic of two or more organ types. Typical second whorl organs are shown in Figures 3G and 3H. Relative numbers of obvious mosaic variations are listed in Table 2. Early flowers have fewer second whorl organs, and ontogenic analysis indicated that second whorl primordia are frequently absent when compared with the wild type. The absence of a second whorl organ primordium does not alter the placement of other second whorl

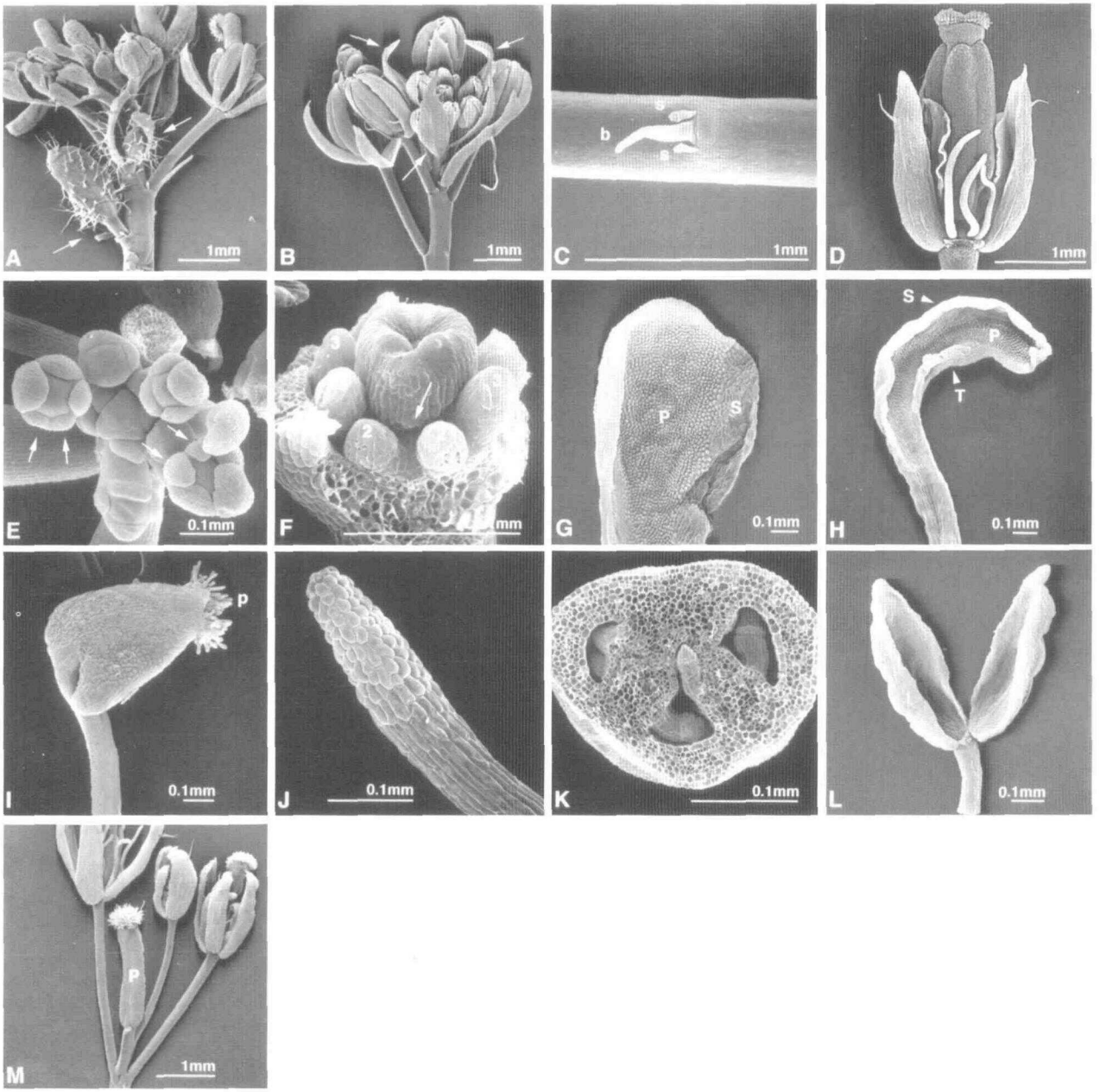

Figure 3. Morphology and Development of Ufo-1 Plants.

(A) Inflorescence of Ufo-1 plant grown in SD. Arrows indicate two coflorescence nodes that are separated by two flowers (one flower is hidden from view).
(B) Apical nodes of Ufo-1 plants grown in SD conditions. Arrows point to bracts subtending Ufo-1 flowers.
(C) Floral node from Ufo-1 plant grown in SD from which no lateral shoot was produced. Stipules (s) and bractlike structures (b) are visible.
(D) Typical mature Ufo-1 flower.
(E) Inflorescence of Ufo-1 plant grown in CL. Additional sepal primordia are visible on some developing flowers (arrows).
(F) Developing Ufo-1 flower. An organ primordium is missing from the third whorl position (arrow). Other primordia are numbered according to their position in the second (2) or third (3) whorl.
(G) Second whorl organ from Ufo-1. Regions of petal (P) and sepal (S) tissue are visible.
(H) Second whorl organ from Ufo-1. Regions of petal (P), sepal (S), and stamen (T) tissue are visible.
(I) Third whorl organ from Ufo-1. Stigmatic papillae (p) typical of carpels are visible at the tip of this organ.
(J) Filamentous organ from the third whorl of a Ufo-1 flower.
(K) Developing Ufo-1 gynoecium. Septa primordia have fused to generate three ovarial chambers.
(L) "Empty flower" from a Ufo-1 plant.
(M) The terminal apex of a Ufo-1 plant. A pistil-like (P) structure occupies the apex of the inflorescence.

Table 1. Comparison of Wild-Type and Ufo Plant Development in CL and SD Conditions

Conditions	Phenotype	Number of Rosette Leaves[a]	Number of Coflorescences[a]	Number of Plants Scored
CL	Ufo-1	9.1 ± 0.96	3.5 ± 0.63[b]	28
	Col-2	8.8 ± 0.51	2.5 ± 0.60	21
	Ufo-3	7.0 ± 0.53	2.8 ± 0.81[b]	22
	Ler	7.4 ± 0.83	1.6 ± 0.51	15
SD	Ufo-1	62.4 ± 8.95	15.9 ± 2.92[b]	25
	Col-2	62.3 ± 9.64	10.1 ± 1.80	25
	Ufo-3	37.8 ± 4.54	14.9 ± 1.27[b]	26
	Ler	33.1 ± 3.73	8.5 ± 1.22	26

[a] Values are given as mean ± standard deviation.
[b] Significant difference from appropriate wild-type strain at P = 0.05 level of significance by Student's *t* test.

primordia. No morphologically normal stamens ever appeared in the second whorl, although locules on petal–stamen mosaic organs could dehisce to produce pollen.

Typical third whorl organs are represented in Figures 3I and 3J. The relative numbers and types of transformations are listed in Table 2. The most frequent organs are filamentous. They are taller and thicker than those in the second whorl and are occasionally capped with stigmatic papillae (one of 106 flowers scored). Rare organ types included staminoid–carpels and, in later flowers, completely normal petals or stamens. Variations in organ number, both greater and fewer than six, were common in the third whorl. Most organs, however, developed in positions appropriate for the third whorl (Figure 3F).

Second and third whorl filamentous organs have no clear wild-type counterpart. The length of the organ is composed of cells with surface features typical of a stamen filament, whereas at the tip, cuticular thickenings similar to anther cell types are present (Figure 3J). Cross-sections revealed that, unlike stamen filaments, these organs are never vascularized.

The fourth whorl consists of two to four fused carpels (average of 2.98 with a modal number of three from 106 flowers scored). In many cases, ovaries from one or more carpels do not extend the full length of the gynoecium. The presence of extra carpels does not consistently correlate with organ loss from any other whorl. Ontogenic analysis indicated that up to four fourth whorl primordia are initiated (Figure 3K).

Flowers consisting solely of two to three sepals appear with a frequency of approximately one to two per plant (Figure 3L). Flowers produced immediately before and after these unusual "empty flowers" show no obvious differences from the typical Ufo-1 floral phenotype.

Ufo-1 flowerlike shoots that developed under SD conditions differ only slightly from those of plants in CL. Petal–stamen mosaic organs are rare, although sepal–stamen mosaic organs and sepal–carpel mosaic organs appear occasionally in second and third whorl positions. Fourth whorl carpels frequently are not fused. Other alleles of *ufo*, however, produce additional whorls of floral organs in SD. Up to four additional sepals were observed in Ufo-2, apparently forming a whorl interior and alternate to the second whorl sepaloid organs. An additional whorl of stamens, staminoid–carpels, and filamentous organs (similar to the third whorl of Ufo-1) was generated prior to the production of a gynoecium.

Changes in organ number have been associated with changes in meristem size (such as in Clavata mutant flowers; Leyser and Furner, 1992; Clark et al., 1993). Measurement of Ufo floral and apical meristems revealed that the diameter of Ufo-1 meristems was slightly but significantly larger than that of the wild type; however, in Ufo-3, the differences were not significant.

Double Mutant Analysis

The Ufo-1 phenotype suggests that the product of the *UFO* gene is required for at least two developmental processes: (1) selection of floral fate by lateral meristems, and (2) fate selection by floral organ primordia. To characterize further the role of *UFO* in these processes, double mutants were constructed between *ufo-1* and the floral meristem and organ identity mutations *pistillata* (*pi-1*), *apetala3* (*ap3-1*), *apetala2* (*ap2-6* and *ap2-1*), *agamous* (*ag-1*), *flo10* (*flo10-1*), *leafy* (*lfy-1* and *lfy-2*), and *apetala1* (*ap1-1*). Figure 4 shows the phenotypes of double mutant combinations. Because several double mutant constructs required crossing into the *Ler* ecotype, F_2 plants of a cross between *ufo-1* (Col-2) and the *Ler* wild type were examined to determine whether the *Ler* genetic background modifies the Ufo-1 phenotype. No significant modifications were detected in the F_2 generation of this cross.

Table 2. Numbers of Different Organ Types in Second and Third Whorls of Ufo-1 Flowers

Whorl	Petals	Filamentous Organs	Petal–Sepals	Petal–Stamens	Stamens	Carpels	Sepals	Absent[a]	Additional[b]
Second	109	102	92	55	0	0	22	28	0
Third	5	424	0	0	22	13	0	164	9

Organs in unambiguous positions were scored for organ type and whorl position in 106 flowers grown at 22°C. Organs in ambiguous positions were not scored.
[a] The total number of missing organs in the indicated whorl for all flowers scored.
[b] The frequency of additional organs appearing within a whorl.

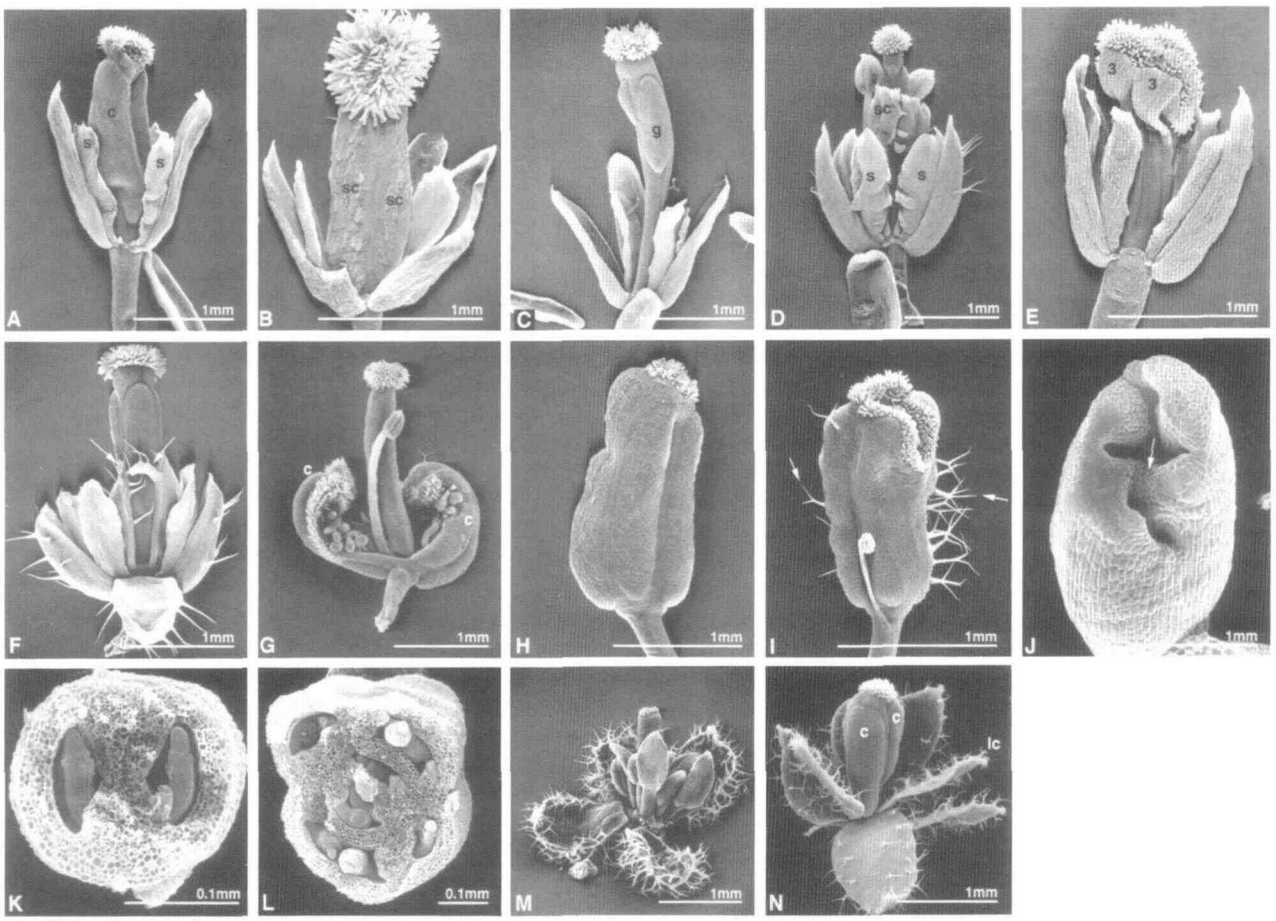

Figure 4. Single and Double Mutant Phenotypes of *ufo-1* and *pi-1*, *ap3-1*, *ap2-6*, or *ap2-1*.

(A) Pi-1 flower. Second whorl sepals (s) and third whorl carpels (c) are visible.

(B) and **(C)** Ufo-1 Pi-1 double mutant flowers. In **(B)**, sepal/carpels (sc) are fused in a gynoecial-like structure. In **(C)**, a slender, solid gynoecial structure (g) is visible with ovary tissue in the upper regions. Some perianth tissue has been removed for clarity.

(D) Flower from an Ap3-1 plant grown at 16°C. Second whorl sepals (s) and third whorl stamen–carpel organs (sc) are visible. The front sepal has been removed for clarity.

(E) and **(F)** Flowers from Ufo-1 Ap3-1 double mutant plants grown at 16°C. In **(E)**, carpel-like organs (3), apparently arising from the third whorl, are seen fused in the center of the flower. The front sepal has been removed for clarity. In **(F)**, two sepal-like organs (arrows) are visible in typical third whorl positions.

(G) Ap2-6 flower. Carpels (c) develop in first and second whorl positions.

(H) Ap2-6 flower showing fusion of all perianth whorl organs.

(I) Ufo-1 Ap2-6 flower. Trichomes (arrows) typical of leaf tissue are visible on the fused outer carpelloid organs.

(J) Developing Ufo-1 Ap2-6 flower. Additional carpels (arrow) appear late in flower development.

(K) Developing Ufo-1 Ap2-6 flower in cross-section. Two ovary chambers are visible at this stage of development.

(L) Developing Ufo-1 Ap2-6 flower in cross-section. This flower is older than the one shown in **(K)**. Four outer ovary chambers are visible surrounding two inner ovary chambers.

(M) Mature Ap2-1 flower.

(N) Mature Ufo-1 Ap2-1 flower. Second whorl leaf/carpel (lc) and third whorl fused carpels (c) are visible.

Ufo-1 Ap3-1 and Ufo-1 Pi-1

Ufo flowers generate petaloid and petal–stamen mosaic organs in both the second and third whorls. Variation in the expression domain of class C organ identity genes between whorls 2 and 3 could explain such homeotic changes. To test this hypothesis, double mutants were constructed between *ufo-1* and *ap3-1* or *pi-1*.

Figures 4A and 4D show the phenotypes of mutations in the genes *PI* and *AP3*. Pi-1 and Ap3-1 phenotypes involve the transformation of petal to sepal and stamen to carpel (Bowman et al., 1989, 1991; Hill and Lord, 1989; Jack et al., 1992).

Double mutants and mutants homozygous at one locus and heterozygous at the other from both the *ufo-1* × *pi-1* and *ufo-1* × *ap3-1* F_2 populations have almost indistinguishable phenotypes but are distinct from the single mutant parents. We refer to this as the double mutant phenotype, which suggests that the *ufo-1* allele exhibits genetic dominance in a class B mutant background. The double mutant phenotype could be considered additive with respect to Ufo, Pi, and Ap3 single mutant phenotypes.

Organs were assigned to whorls based on their position within the flower, and ambiguous cases were not used in organ-type analysis. Double mutant flowers consist of sepal, carpel, sepal–carpel mosaic, and filamentous organs. Unlike Pi and Ap3 single mutant phenotypes, second and third whorl organs exhibit both sepal and carpel features, which is consistent with the appearance of reproductive tissue in the perianth whorls and of perianth tissue in the reproductive whorls of Ufo-1 single mutant flowers. Gynoecial structures from double mutants often produce little or no ovary tissue. Typical Ufo-1 Pi-1 flowers are shown in Figures 4B and 4C. Table 3 lists the frequencies of second and third whorl homeotic transformations from 142 Ufo-1 Ap3-1 double mutants grown at 16°C. Flowers from this population (Figures 4E and 4F) have slightly weaker transformations than those in Ufo-1 Pi-1.

The appearance of reproductive tissue in the second whorl and perianth tissue in the third whorl of Ufo-1 Ap3-1 and Ufo-1 Pi-1 flowers suggests that the *ufo-1* mutation causes aberrant expression of class C organ identity function.

Ufo-1 Ap2-6, Ufo-1 Ap2-1, and Ufo-1 Ag-1

Many aspects of the Ufo-1 floral phenotype are similar to those observed in Pi and Ap3 mutant flowers, and double mutant phenotypes with *ap3* or *pi* and alleles of class A and C genes have been well characterized (Bowman et al., 1989, 1991). To determine whether the *ufo-1* allele behaves in a similar manner, double mutant lines were constructed between *ufo-1* and alleles of *ap2* and *ag*. As shown in Figures 4G, 4H, and 4M, mutations in the *AP2* gene affect all whorls but have strong effects on first and second whorl organ type (Bowman et al., 1989; Kunst et al., 1989). The *ap2-6* allele reduces organ number and causes perianth organs to develop as carpel or leaf–carpel mosaics (Figures 4G and 4H; Kunst et al., 1989). The *ap2-1* allele causes first whorl organs to develop as leaves and second whorl organs to develop as petal–stamen mosaics (Figure 4M; Bowman et al., 1989). Fewer third whorl organs are produced in Ap2-6, and these develop as stamens or carpelloid stamens.

Flowers of Ufo-1 Ap2-6 and Ufo-1 Ap2-1 double mutants exhibit an additive phenotypic interaction similar to that described for Pi-1 Ap2-2 and Pi-1 Ap2-1 double mutants (Bowman et al., 1989, 1991). Outer whorl organs of Ufo-1 Ap2-6 flowers are similar to those of Ap2-6 flowers but invariably fuse to enclose the remaining floral organs (Figure 4I). In the earliest stages, flowers are composed of two fused carpels (Figure 4K). As these flowers mature, additional carpels appear. The additional organs most likely arose from the third and fourth whorls, although we were unable to determine their exact origin (Figure 4J). Mature flowers typically consist of four fused outer and two fused inner carpels (Figure 4L).

Ufo-1 Ap2-1 flowers (Figure 4N) generate four first whorl primordia and two to four large second whorl primordia that develop as leaf or leaf–carpel mosaic organs. Third and fourth whorls generate small numbers of primordia that fuse soon after initiation, making individual whorl designation difficult. These primordia develop to produce a fused or open multicarpellate structure in the position of the gynoecium.

Ag-1 flowers exhibit third whorl transformations of stamen to petal, and in place of the fourth whorl, three whorls of organs—sepal, petal, petal—are generated. This pattern repeats several times to generate a nested flower phenotype (Figure 5A; Yanofsky et al., 1990; Bowman et al., 1991).

Ufo-1 Ag-1 flowers have an additive phenotype similar to that of Pi-1 Ag-1 (Bowman et al., 1989, 1991). Double mutant flowers consist entirely of sepal, sepal–petal, and filamentous organs (Figure 5B). Organ primordia are initiated in the wild-type position for up to three whorls before becoming disrupted by floral meristem enlargement. Mature flowers are "fan shaped" and generate large numbers of organ primordia with no apparent whorled pattern (Figure 5C). A similar enlargement was observed in Pi-1 Ag-1 floral meristems (J. Bowman, personal communication). Unlike Ufo-1 single mutant plants and all other double mutant combinations examined, apical growth in Ufo-1 Ag-1 plants does not terminate with the production of a pistil-like structure. Rather, a flowerlike structure consisting of numerous whorls of sepal and sepal–carpel mosaic organs is produced at the apex of all inflorescences. Unlike the Ag-1 phenotype, where internode elongation occurs between nested flowers (Yanofsky et al., 1990; Schultz et al., 1991), no internode elongation is apparent within Ufo-1 Ag-1 flowers, even in the absence of the *erecta* mutation.

Table 3. Frequency of Organ Types Appearing in Second and Third Whorls of Ufo-1 Ap3-1 Double Mutant Flowers

Whorl	Sepals	Sepal–Carpels	Filamentous Organs with Trichomes	Filamentous Organs without Trichomes	Filamentous Organs Fused to Gynoecium
Second	49	14	3	4	0
Third	60	93	22	175	30

Numbers of each organ type appearing in 142 flowers grown at 16°C; organs in ambiguous positions were not scored.

Ufo-1 Flo10-1

To investigate further the Pi and Ap3-like features of Ufo-1, double mutants were generated that carried *ufo-1* and an allele of the *FLO10* gene, a negative regulator of *AP3/PI* activity.

In Flo10-1 flowers, carpels were replaced by stamens and stamen–carpel mosaic organs (Figure 5D). Whorls 1, 2, and

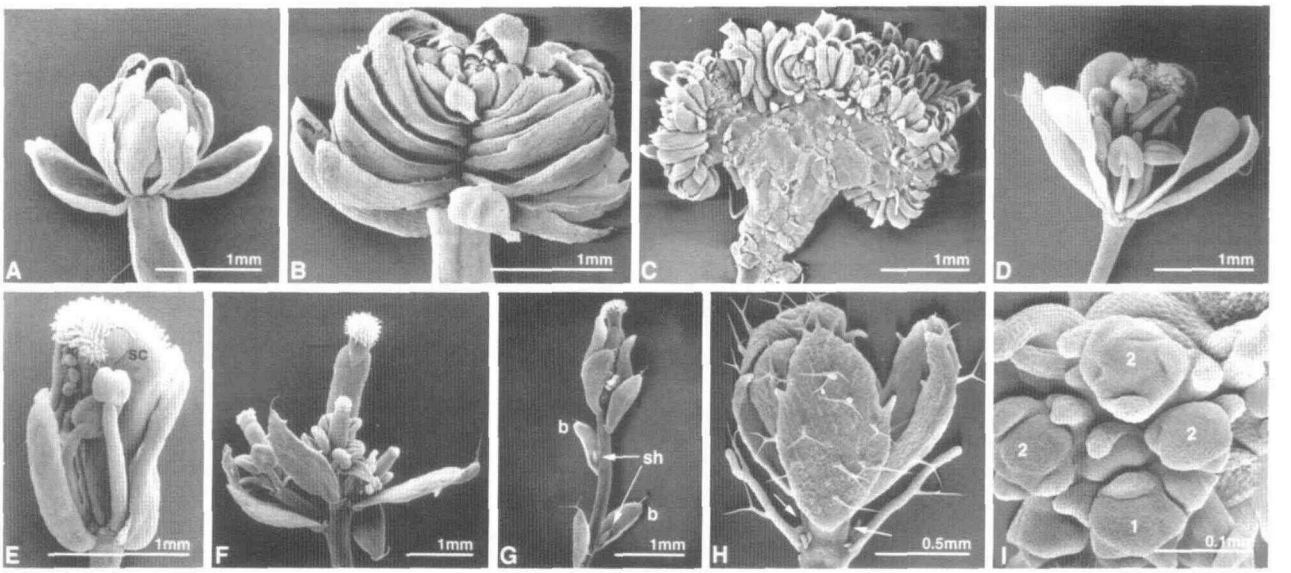

Figure 5. Single and Double Mutant Phenotypes of *ufo-1* and *ag-1*, *flo10-1*, or *ap1-1*.

(A) Mature Ag-1 flower.
(B) Mature Ufo-1 Ag-1 flower.
(C) Mature Ufo-1 Ag-1 flower. Mature organs have been removed to reveal the "fan-shaped" floral structure.
(D) Mature Flo10-1 flower.
(E) Ufo-1 Flo10-1 flower. The first whorl sepal has been removed. Third whorl stamen–carpel (sc) organs are visible.
(F) Mature Ap1-1 flower.
(G) Mature Ufo-1 Ap1-1 lateral shoot from a position normally occupied by a flower in wild-type plants. Bracts (b) subtending tertiary shoots (sh) are visible. A pistil-like structure can be seen at the apex of the shoot.
(H) Shoot from apical node of a Ufo-1 Ap1-1 plant. Internodes (arrows) show less elongation than in earlier shoots (compare with **[G]**), giving the shoot a more flowerlike appearance.
(I) Apex of Ufo-1 Ap1-1 inflorescence. The primary inflorescence (1) and lateral inflorescences (2) all generate lateral shoots in a spiral phyllotaxy.

3 were initiated normally in organ position, type, and number (Schultz et al., 1991; Bowman et al., 1992). This phenotype is believed to result from *AP3* and *PI* expression in the fourth whorl.

Ufo-1 Flo10-1 double mutant flowers have characteristics of both single mutants (Figure 5E). First whorl organs are sepals. Few second whorl organs appear (eight organs in 39 flowers), but all show the range of transformations seen in second whorl organs of Ufo-1. Lateral third whorl organs usually mature to freestanding stamens, whereas medial third whorl organs are stamens or mosaics of stamen and carpel tissue that fuse with other carpelloid organs, forming an open gynoecial structure. Thus, as expected, fewer fourth whorl stamens are produced in double mutant flowers than in Flo10-1 alone, suggesting that the *ufo-1* mutation suppresses the Flo10 phenotype in the fourth whorl, possibly by reducing class B activity. However, the appearance of third whorl stamens and staminoid organs in all double mutant flowers was surprising because Ufo-1 does not generate staminoid structures until the latest flowers. Thus, the *flo10-1* mutation appears to suppress the Ufo-1 phenotype in the third whorl.

Ufo-1 Ap1-1, Ufo-1 Lfy-1, and Ufo-1 Lfy-2

Several aspects of the Ufo-1 phenotype suggest that mutant plants are defective in initiating the floral program. To study this effect further, double mutants were constructed using the *ufo-1* allele and mutant alleles of two FLIP genes, *AP1* and *LFY*.

Strong mutant alleles of *AP1* (*ap1-1*) generate flowers that exhibit a lack of perianth structures and the replacement of these with bracts from which axillary "floral" shoots arise (Figure 5F; Irish and Sussex, 1990; Bowman et al., 1993; Schultz and Haughn, 1993).

Strong *LFY* mutant alleles (e.g., *lfy-1*) cause all lateral shoots to develop as coflorescences. All primary and lateral shoot apical meristems terminate development with the production of carpel-like structures. All other floral organs are absent. Weaker alleles (e.g., *lfy-2*) generate more coflorescence-like structures than do the wild type; however, some "flowers" are generated from which new lateral shoots may arise. Lfy-2 floral organs show a wide range of transformations and are often mosaics of two or more organ types (Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992).

Ufo-1 Ap1-1 double mutants in CL produce a phenotype similar to that of a strong Lfy mutant. A rosette of seven to 10 leaves is formed, followed by up to four coflorescence nodes subtended by normal bracts. All other lateral meristems develop as coflorescence-like shoots, occasionally subtended by filamentous structures (Figure 5G). Each lateral shoot consists of up to 11 nodes (mean of 6.46 nodes per coflorescence), with a leaf or leaf–carpel mosaic organ and axillary meristem at each node. The amount of carpel tissue within these mosaic organs increases acropetally, and terminal organs frequently fuse. As in both Ufo-1 and Lfy plants, all apical and lateral shoots terminate in a pistil-like structure. Elongation does not occur in some of the more apical lateral shoots, giving them a flowerlike appearance, although neither sepal, petal, nor stamen tissues can be seen (Figure 5H). Ontogenic analysis revealed that primordia on lateral shoots have a spiral phyllotaxy similar to that of an inflorescence (Figure 5I). No lateral shoots produce organs in an obviously whorled pattern. Thus, the inflorescence structure of the double mutant is similar to that seen in strong Lfy mutants; however, unlike Lfy, only the most basal lateral shoots are subtended by normal bracts.

Because the *ufo-1* mutation exhibits primarily a floral phenotype in CL, it was anticipated that mutations at the *LFY* locus should be partially or completely epistatic to *ufo-1*. Indeed, the F_2 populations of both *ufo-1* × *lfy-1* and *ufo-1* × *lfy-2* crosses exhibit wild type, Ufo-1, and a range of Lfy-like phenotypic classes in a ratio indicating that the double mutant phenotypes are Lfy-like.

To assist in identifying double mutant individuals, Lfy-2 plants were crossed with the linked marker line *ufo-1 dis2* that was generated during the mapping of *UFO*. Lfy-2 Ufo-1 Dis2 plants from the F_2 progeny of this cross consistently generate fewer floral-like nodes and terminate apical growth earlier than Lfy-2 alone, suggesting that, as expected, *ufo-1* enhances the weak Lfy-2 phenotype.

Ufo-1 Tfl1-14

The *TERMINAL FLOWER1* (*TFL1*) gene product is believed to be a regulator of FLIP gene expression (Shannon and Meeks-Wagner, 1991; Bowman et al., 1993; Schultz and Haughn, 1993). To examine further the role of *UFO* in the FLIP process, Ufo-1 Tfl1-14 double mutant plants were constructed.

Mutations at the *TFL1* locus cause the replacement of coflorescences with flowers and early termination of growth when the shoot apex itself becomes a floral meristem (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992).

Ufo-1 Tfl1-14 plants have an inflorescence structure similar to that of Tfl1-14 alone; however, all flowers, including the terminal flower, exhibit transformations similar to those seen in Ufo-1 single mutant individuals. Notably, organ types in Ufo-1 Tfl1-14 double mutant flowers are typical of those observed in the latest flowers of Ufo-1 single mutant plants.

In Situ Hybridization

In situ hybridization analysis was performed on sections of inflorescences from Ufo-1, Ufo-3, Ufo-4, and Ufo-5 plants using FLIP and organ identity genes as probes. Antisense RNA was hybridized to the sections, and flowers up to stages 6 or 7 of development were examined for the presence of the transcript. The results are shown in Figure 6.

Although the expectation was that class C gene expression would be variable, in situ hybridization with the *AG* antisense probe revealed no clear case in which *AG* expression level or pattern was altered compared with that of the wild-type floral primordia (Figures 6A and 6B). However, ectopic *AG* transcript was detected in sections of apical meristems late in inflorescence development (Figure 6C). In these instances, the transcript appeared in a ring of cells surrounding the apical meristem. In inflorescences that had terminated growth, the *AG* transcript was detected in the terminal carpel structures.

It was expected from single and double mutant analyses that class B gene expression should be reduced in Ufo flowers. However, in situ hybridization analyses using the *PI* antisense RNA probe revealed no clear differences in expression pattern or intensity compared with wild type at early stages (Figures 6D and 6E). In slightly older flowers, the *PI* transcript remained detectable at high levels in the presumptive second and third whorl organs but in some cases appeared to be more restricted to the outer cell layers of these structures when compared with the wild type (Figure 6F). Similar results are observed using *AP3* antisense probes.

In situ hybridization analysis of *AP1* (Figures 6G to 6I) and *LFY* transcripts in Ufo-1 flowers and inflorescences revealed no differences in pattern or intensity compared with that of the wild-type plants.

DISCUSSION

UFO Is Involved in the Floral Initiation Process

Most data from *ufo-1* single and double mutant analyses support the interpretation that *UFO* has a role in initiating floral development in lateral shoots (FLIP). The wide array of homeotic transformations observed in Ufo-1 flowers has also been observed in flowerlike structures from weak Lfy and Ap1 mutants (Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992; Bowman et al., 1993; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993). Like FLIP mutant individuals, Ufo-1 plants exhibit an enhanced number of coflorescence-like lateral shoots in SD beyond that seen in the wild type. Bracts or bractlike structures subtend many "floral" shoots in both Leafy and Ufo-1 and are particularly pronounced in the most apical nodes. Ufo shows an acropetal decrease in phenotypic severity, enhances flower-to-coflorescence

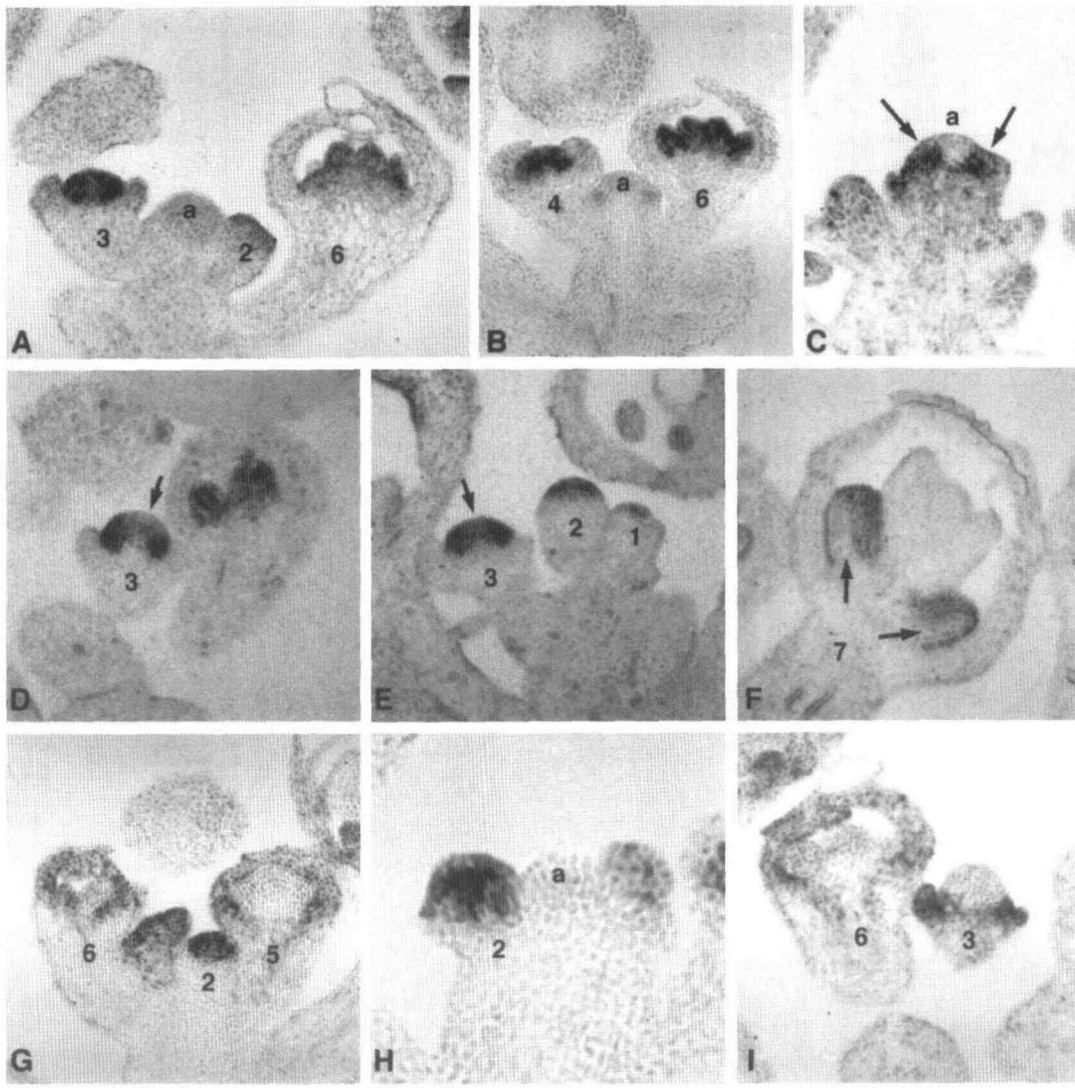

Figure 6. In Situ Hybridization of FLIP and Organ Identity Genes to Wild-Type and Ufo Inflorescences.

Numbers on floral primordia indicate the stage of flower development according to Smyth et al. (1990). (a), inflorescence apex.

(A) In situ hybridization of the *AG* antisense RNA probe to a wild-type inflorescence (×160).

(B) In situ hybridization of the *AG* antisense RNA probe to a *ufo-1* inflorescence (×130).

(C) In situ hybridization of the *AG* antisense RNA probe to a *ufo-4* shoot apex. Hybridization is seen in a ring around the apical meristem (arrows) but is absent from the apex itself. This most likely represents an apex in the process of initiating terminal carpel structures (×170).

(D) In situ hybridization of the *PI* antisense RNA probe to wild-type flowers. Strong hybridization can be detected in the stage 3 flower; however, the transcript is lower in the central region of the dome (arrow) (×140).

(E) In situ hybridization of the *PI* antisense RNA probe to *ufo-5* floral primordia. Direct comparisons can be made between the stage 3 primordium shown here and the wild-type primordium in **(D)**. Strong signal can be detected in the stage 2 and 3 primordia; however, the transcript is lower in the central region of the dome (arrow) (×140).

(F) In situ hybridization of the *PI* antisense RNA probe to *ufo-5* floral primordia. Strong hybridization is apparent in the third whorl organs; however, cells on the interior of these organ primordia exhibit lower amounts of *PI* transcript (arrows) (×140).

(G) In situ hybridization of the *AP1* antisense probe to wild-type floral primordia. Strong hybridization is apparent throughout the stage 2 floral primordium and is present in first and second whorl organs in older flowers (×130).

(H) In situ hybridization of the *AP1* antisense probe to *ufo-1* floral primordia. Strong hybridization can be detected throughout the stage 2 primordium (×250).

(I) In situ hybridization of the *AP1* antisense probe to *ufo-1* floral primordia. Hybridization can be detected in the first and second whorl positions of the stage 3 and stage 6 floral primordia shown (×170).

transformations in combination with Ap1 or Lfy alleles, exhibits numerous transformations consistent with reduction of class B function, and exhibits termination of apical meristem growth accompanied by production of carpelloid structures. Each of these features is common among FLIP genes (Irish and Sussex, 1990; Huala and Sussex, 1992; Weigel et al., 1992; Schultz and Haughn, 1993). These similarities are compelling evidence that *UFO* plays a role in the FLIP process. A diagram in Figure 7 indicates the relative role of *UFO* in the FLIP compared with other genes involved in floral initiation.

Each FLIP gene examined has had a unique phenotype, having some features shared with other FLIP genes and other features specific to that locus. Ufo also has a unique phenotype and unique features. Unlike the Lfy and Ap1 phenotypes, the transition from coflorescence to flower production is pronounced such that any given shoot is conspicuously flowerlike or coflorescence-like. Moreover, in SD conditions, Ufo plants produce coflorescences apical to flowers and floral nodes from which no shoot is produced. This phenotype implies that the fate of lateral shoots in Arabidopsis is determined independently of adjacent lateral shoots. The fact that only flowerlike structures are generated in the most apical nodes of Ufo-1 indicates that, in these nodes, the fate selection mechanism is operating correctly. Thus, it appears that *ufo-1* interferes with the ability of the FLIP to interpret COPS signal correctly at near-threshold levels. The appearance of empty flowers suggests that, even if a floral fate is chosen, the commitment to this fate is weak or unstable in Ufo-1. Other aspects of floral morphology are altered in Ufo-1, and these are discussed in the next section.

Because *AP2* might be considered a FLIP gene (Irish and Sussex, 1990; Bowman et al., 1993; Schultz and Haughn, 1993), it is interesting that the Ufo-1 Ap2-1 and Ufo-1 Ap2-6 phenotypes are not synergistic with respect to their floral initiation phenotypes, as was observed in the Ufo-1 Ap1-1 double mutant phenotype. There are three possible explanations: (1) *AP2* plays a sufficiently minor role in the FLIP, and therefore no enhancement of FLIP mutant features was apparent in the double mutant; (2) the alleles chosen were weak enough, and therefore synergistic effects were not apparent; or (3) *UFO* and *AP2* have few redundant activities in the FLIP.

Molecular and genetic analyses have shown that individual FLIP genes are largely independent of each other for their transcription (Weigel et al., 1992; Bowman et al., 1993; Gustafson-Brown et al., 1994; Jofuku et al., 1994; Weigel and Meyerowitz, 1994). Thus, the wild-type transcript patterns of *LFY* and *AP1* in Ufo plants are not inconsistent with the designation of *UFO* as a FLIP gene.

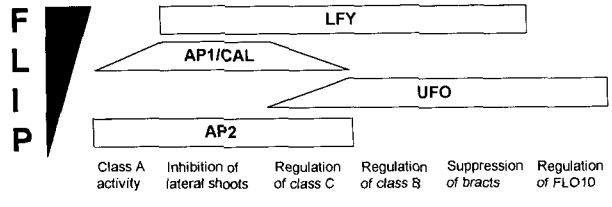

Figure 7. Role of *UFO* and Other FLIP Genes during Floral Initiation and Development.

The vertical axis represents the degree of participation of each gene in the FLIP, as indicated by the vertical gradient. The horizontal axis indicates several functions required for proper floral development. The genes *LFY*, *AP1*, *CAL*, *UFO*, and *AP2* are in horizontal boxes spanning the functions that appear to be regulated by each gene. Graded lines indicate that the given gene plays a lesser role in the associated floral function compared with other genes.

UFO Regulates Developmental Processes within the Flower

Figure 7 indicates the relative roles of UFO and other FLIP genes in the regulation of developmental processes within the flower. There appears to be significant overlap between the roles of *AP1* and *AP2* compared with that between the roles of *UFO* and *AP1/AP2*, suggesting that these two groups of genes control subsets of the functions governed by *LFY*. This may account, in part, for the stronger phenotype of Ufo Ap1 double mutants when compared with Ap2 Ap1 double mutants (Irish and Sussex, 1990; Schultz and Haughn, 1993).

Several lines of evidence indicate that *UFO* is needed to establish domains of function of the floral organ identity genes. The appearance of reproductive tissues in perianth whorls and perianth tissue in reproductive whorls of Ufo single mutant and Ufo Pi or Ufo Ap3 double mutants suggests that class C organ identity activity is variable from organ to organ in the first, second, and third whorls. Thus, *UFO* appears to have a role in regulating class C activity. The FLIP genes *AP2*, *AP1*, and *LFY* are also involved in regulating class C function, and many organ-type transformations observed in Ufo are similar to those observed in Ap2 and Ap1 individuals. For example, carpelloid first whorl organs are common in Ap2-6, and petal–stamen mosaic organs are observed in Ap1 (Bowman et al., 1993; Schultz and Haughn, 1993). However, unlike *AP1* and *AP2*, where mutations affect primarily first and second whorl organ type, *UFO* has its strongest effect on organ type in the second and third whorls.

The whorls with class B gene function (whorls 2 and 3) are affected the most by *ufo-1*, and many of the organ-type transformations in Ufo and double mutant combinations can be accounted for by the reduction in class B gene function. However, there is significant evidence that *UFO* does not represent a novel class B gene. No known mutant alleles of class B genes affect the expression of class A or C gene function, and no known class B genes affect floral initiation. In addition, no *ufo* allele affects the class B domain to the same extent as do mutations in *PI* or *AP3*.

Consistent with the apparent reduction in class B activity in Ufo flowers, *ufo* mutations are able to suppress the production

of stamens in the fourth whorl of Flo10 flowers. It is surprising, however, that *flo10* is able to suppress partially the effects of *ufo* mutations in the third whorl. The latter result could be explained if ectopic expression of *FLO10* were partially responsible for reducing class B activity in the third whorl. Nevertheless, ectopic *FLO10* expression cannot, by itself, account for all of the reduction of class B activity in Ufo flowers, because the second whorl organ number in Ufo Flo10 double mutant flowers is reduced and generates organ types similar to those found in Ufo alone. Thus, *UFO* must also have a role in regulating class B activity independent of *FLO10*.

Despite the phenotypic evidence supporting the fact that class B gene function is reduced and class C gene function varies spatially, in situ hybridization results revealed little difference in the amount or pattern of *PI*, *AP3*, or *AG* transcripts in Ufo flowers when compared with the wild type. Several possibilities might account for the apparent discrepancy between the Ufo phenotype and results from in situ hybridization. First, the regulation of the organ identity genes by *UFO* could be post-transcriptional. Second, the variability of the Ufo phenotype indicates that class B and C gene function is affected only in some flowers and only in particular regions within a given flower. In addition, organ-type transformations are seldom complete in Ufo flowers; thus, the effect of *UFO* on organ identity gene transcription may be subtle. Because it is difficult to quantitate steady state transcript levels with in situ hybridization results, such changes might not be easily detectable. Third, the Ufo phenotype might not depend on the specific class B and C genes used as probes for the in situ hybridization analyses. Because there are undoubtedly additional organ identity genes, it is possible that *UFO* exerts its effects through other, as yet unidentified, class B and C genes.

The production of carpels in terminating inflorescence shoot apices is a feature shared by many FLIP mutant phenotypes (Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992; Schultz and Haughn, 1993). Ufo plants are unique, however, in that primary meristem termination occurs much earlier than it does in other FLIP mutations. In situ hybridization analysis of terminating Ufo inflorescences showed that this event is correlated with the appearance of *AG* transcript in the apex of Ufo inflorescences, although it appears to be excluded from the meristematic region. Because no known FLIP gene has been shown to be transcribed in the apex of inflorescence shoots, it is unclear how FLIP genes regulate *AG* transcription in this region. It has been suggested from studies with male-sterile mutants and plants from which flowers have been surgically removed that normal flowers are involved in signaling the shoot apex to remain indeterminate (Hensel et al., 1994). However, *UFO* must have an additional role in this process, because the absence of the *UFO* gene product causes apical termination earlier than expected. Some sterile plants also generate carpelloid first whorl organs and bracts subtending the most apical floral nodes (Hensel et al., 1994), suggesting that some features commonly observed in FLIP mutant phenotypes might be due to sterility.

UFO Influences Other Aspects of Floral Development

Changes in organ number are common in mutations affecting floral initiation (Schultz and Haughn, 1993). The primary difference between *ufo* and other FLIP mutations is that such FLIP mutations as *ap2* and *ap1* exhibit only reductions in organ number, whereas Ufo flowers may also generate additional organs in the first, third, and fourth whorls as well as additional whorls of organs under SD conditions. Little is known about the regulation of organ and whorl number. Investigation of the Clavata mutant phenotypes (Leyser and Furner, 1992; Clarke et al., 1993) has revealed that increases in meristem size are associated with increased organ number. Measurements of floral meristems showed that Ufo-1 floral meristems were significantly larger than those of the wild type, but the differences were not significant in Ufo-3 plants. Because additional organs are rare and a maximum of one additional organ has been observed in a single flower, it is possible that the changes in meristem size are too small to be measured accurately. The unusual growth of Ufo Ag floral meristems further supports the suggestion that *UFO* has a role in regulating meristem size or identity.

Role of *UFO* in Flower Development

We have shown that the *UFO* gene is involved in at least two processes. First, it is required for floral initiation, in combination with the other FLIP gene members. Second, it has a role in regulating all classes of floral organ identity gene activity. In addition, in situ hybridization analysis of *UFO* transcript patterns late in flower development (Ingram et al., 1995) has shown that the *UFO* transcript is restricted to regions surrounding the petal primordia, suggesting that *UFO* may have an additional role in petal development. *UFO* is not unique in having multiple roles throughout flower development. It is becoming apparent that each FLIP gene differs in the degree to which it participates in the FLIP decision and in the particular subset of downstream developmental processes regulated by that gene (Bowman et al., 1993; Okamuro et al., 1993; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993). In Figure 7, the relative participation of UFO in the FLIP and subsequent developmental processes is compared with that of other FLIP genes. Ufo plants provide a clear example of the distinct roles that a FLIP gene plays in both floral initiation and later floral development.

METHODS

Plant Material and Growth Conditions

The *UNUSUAL FLORAL ORGANS* mutant line *ufo-1* was isolated from an ethyl methanesulfonate–mutagenized population of *Arabidopsis*

thaliana ecotype Columbia-2 (Col-2). Ufo-1 was backcrossed to the wild type a minimum of four times before phenotypic analysis of mutants segregating from backcross populations. The line segregating *ufo-2* (formerly *Polyhomeotic*) was a gift of E.M. Meyerowitz (California Institute of Technology, Pasadena, CA), and lines carrying the alleles *ufo-3* (*S1340*), *ufo-4* (*S1341*), and *ufo-5* (*S1747*) were gifts of D. Smyth (Monash University, Clayton, Australia). The lines W100 (*angustifolia [an], apetala1 [ap1], pyrimidine requiring [py], erecta1 [er1], long hypocotyl [hy2], glabrous1 [gl1], eceriferum2 [cer2], brevipedicellus [bp], male sterile [ms], transparent testa [tt3]*; Koornneef et al., 1987) and MSU7 (*eceriferum5 [cer5], distorted trichomes2 [dis2], gibberellic acid resistant [ga4], er1*; Koornneef and Hanhart, 1983), and lines segregating Agamous-1 (Ag-1), Ap1-1, Apetala2-1 (Ap2-1), Pistillata-1 (Pi-1), and Apetala3-1 (Ap3-1) were gifts from M. Koornneef (Wageningen Agricultural University, Wageningen, The Netherlands). Lines Clavata1-1 (NW45) and Clavata2-1 (NW46) were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus, OH). Other mutants used were SAS 1-3-7 (Ap2-6; Kunst et al., 1989), Leafy-1 (Lfy-1; Haughn and Somerville, 1988; Schultz and Haughn, 1991), Leafy-2 (Lfy-2; Schultz and Haughn, 1991), and Terminal Flower1 (Tfl1-14; Schultz and Haughn, 1993).

Seeds were germinated at 4°C for 3 days in 5-inch–diameter pots containing Terra-Lite Redi Earth prepared soil mix (W.R. Grace and Co. Canada Ltd., Ajax, Ontario, Canada) and then transferred to growth chambers at 22°C under continuous light (CL; 90 to 120 $\mu E\ m^{-2}\ sec^{-1}$) or short-day (SD; 10-hr light/14-hr dark at 130 $\mu E\ m^{-2}\ sec^{-1}$) conditions.

For floral morphology studies, plants were grown at a density of ~50 to 70 per pot. Plants grown for inflorescence structural analysis had a density of 14 per pot. For SD experiments, seven plants were grown in each pot.

Genetic Analysis

Linkage analysis was performed using the LINKAGE-1 computer program (Suiter et al., 1983). The *ufo-1* mutation was mapped initially to chromosome 1 by examining the phenotypes of 316 plants from the cross *ufo-1/ufo-1* × *W100*. *ufo-1* was then crossed to the chromosome 1 marker line MSU7 (*cer5, dis2, ga4; er1*). Of 535 F_2 plants, none showed a recombination event between *ufo-1* and the *dis2* marker, indicating that these two loci were closely linked (maximum 9.50 map units ±4.28 map units). From individual Dis2 plants in this population, 100 lines were scored for segregation of the Ufo phenotype. A single line segregated Ufo Dis2 Ga4 triple mutants, indicating that the map distance between *UFO* and *DIS2* was ~0.5 map units with the *UFO* locus between *CER5* and *DIS2*.

Light and Scanning Electron Microscopy and Preparation of Tissue

A minimum of 14 flowers from at least four plants were selected from each single or double mutant combination. Fresh tissue was dissected and examined with a dissecting microscope to determine morphological characteristics.

For scanning electron microscopy, partially dissected tissue was fixed in 3% glutaraldehyde in a 0.1 M phosphate buffer, pH 7. Fixed tissue was then dehydrated in an acetone series and critical point dried. Each sample was stuck to aluminum scanning electron microscopy stubs with double-sided tape and dissected further using pulled glass needles before being gold coated in an Edwards S150B sputter coater. Dissection of Ufo-1 Ap2-6 double mutant flowers involved fracturing the flower to generate cross-sectional views before sputter coating. Observations were made using a Phillips model 505 scanning electron microscope with an accelerating voltage of 30 kV.

Generation and Identification of Double Mutants

Double mutants were identified by analyzing segregation ratios and testcrossing. When double mutants could be identified, a minimum of 13 double mutant plants were examined. The exception was Ufo Pi, which is a double mutant that can only be identified by testcrossing. Five double mutants were confirmed in this manner. Ufo Lfy-2 plants were identified after a *lfy-2* homozygote was crossed to the linked marker line Ufo-1, Dis2, which was generated during mapping crosses (see previous discussion). F_2 plants from this cross segregated for wild type, Lfy-like, and Ufo plants. Among the Lfy-like plants were individuals showing the Dis2 phenotype, suggesting that they were also homozygous for the linked *ufo-1* marker. Such individuals were presumed to represent the double mutant class.

In Situ Hybridization

In situ hybridization was performed using an adaptation of Huijser et al. (1992). Digoxygenin labeling of probes, antibody detection, and staining with 4-nitro blue tetrazolium chloride/X-phosphate was performed using Boehringer Mannheim methods and reagents (kit No. 1175-041). The following plasmids were used: pCIT565 (*AG*; Yanofsky et al., 1990), pD793 (*AP3*; Jack et al., 1992), pKY89 (*AP1*; Gustafson-Brown et al., 1994), and pcPINX (*PI*; Goto and Meyerowitz, 1994). The plasmid pDW124 containing a portion of the *LFY* coding sequence was provided by D. Weigel (Salk Institute for Biological Studies, La Jolla, CA). Each plasmid was digested at the 5′ end of the insert (pCIT565-HindIII, pD793-BglII, pKY89-XhoI, pcPINX-NsiI, and pDW124-BamHI) and transcribed in the presence of digoxygenin-labeled nucleotides to generate an antisense transcript, which was hydrolyzed to generate fragments of ~150 nucleotides. Sections of wild-type (Col-2), Ufo-1 (Col-2), Ufo-3, Ufo-4, and Ufo-5 (Landsberg *erecta* [L*er*]) inflorescences were probed. After detection, slides were dehydrated through ethanol and xylene and mounted in Entellan mounting media (Merck). Sections were photographed through a Leitz DRB (Leica, Wetzlar, Germany) light microscope using Kodak Ektachrome 160 ASA film (Eastman Kodak, Rochester, NY).

ACKNOWLEDGMENTS

We thank Dr. Maarten Koornneef for providing mapping lines W100 and MSU7 and lines segregating Ag, Ap1, Ap2, Pi, and Ap3; Dr. Elliot M. Meyerowitz for providing Ufo-2 and the plasmids pCIT565 and pD793; Dr. Martin Yanofsky for providing the plasmid pKY89; Dr. Detlef Weigel for providing the plasmids pDW124 and pcPINX; Dr. David Smyth for the lines segregating *ufo-3,4,5*; and Dr. John Bowman for communicating unpublished results. We also thank Dr. Enrico Coen, Gwyneth Ingram, and Dr. Elizabeth Schultz for critical reading of the manuscript; Dr. Carl Douglas, Dr. Mary Berbee, Michael Weis, and

Dennis Dyck for assistance with production of the figures; and Marilyn Martin and Tamara Western for their technical assistance and support. We give special thanks to Zsuzsanna Schwarz-Sommer for valuable discussions about the role of *UFO*. In particular, she suggested the possibility that *FIMBRIATA* and *UFO* may be homologs, an idea that ultimately led to the cloning of *UFO* (see Ingram et al., 1995). This work was supported in part by the Natural Sciences and Engineering Research Council of Canada through a research grant to G.W.H. and in part by the University of British Columbia and a postgraduate scholarship to M.D.W.

Received April 25, 1995; accepted July 3, 1995.

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