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Parallels between *UNUSUAL FLORAL ORGANS* and *FIMBRIATA*, Genes Controlling Flower Development in Arabidopsis and Antirrhinum

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The *unusual floral organs* (*ufo*) mutant of Arabidopsis has flowers with variable homeotic organ transformations and inflorescence-like characteristics. To determine the relationship between *UFO* and previously characterized meristem and organ identity genes, we cloned *UFO* and determined its expression pattern. The *UFO* gene shows extensive homology with *FIMBRIATA* (*FIM*), a gene mediating between meristem and organ identity genes in Antirrhinum. All three *UFO* mutant alleles that we sequenced are predicted to produce truncated proteins. *UFO* transcripts were first detected in early floral meristems, before organ identity genes had been activated. At later developmental stages, *UFO* expression is restricted to the junction between sepal and petal primordia. Phenotypic, genetic, and expression pattern comparisons between *UFO* and *FIM* suggest that they are cognate homologs and play a similar role in mediating between meristem and organ identity genes. However, some differences in the functions and genetic interactions of *UFO* and *FIM* were apparent, indicating that changes in partially redundant pathways have occurred during the evolutionary divergence of Arabidopsis and Antirrhinum.

INTRODUCTION

Studies of the molecular and genetic control of flower development have led to the characterization of several sequentially acting genes in floral meristems (Schwarz-Sommer et al., 1990; Coen and Meyerowitz, 1991; Coen and Carpenter, 1993; Weigel and Meyerowitz, 1994). Meristem identity genes act early to switch on the floral genetic program, and their activity is required for the later expression of floral organ identity genes in specific domains of the meristem. Several meristem and organ identity genes have been isolated from both Arabidopsis and Antirrhinum, and a high degree of gene conservation both structurally and functionally has been observed between the two species. It is not known, however, whether the processes that act between the two groups of genes are also evolutionarily conserved. To address this issue, we compared the structure and function of two genes that act between meristem and organ identity genes, *UNUSUAL FLORAL ORGANS* (*UFO*) in Arabidopsis and *FIMBRIATA* (*FIM*) in Antirrhinum (Simon et al., 1994; Wilkinson and Haughn, 1995).

The flowers of wild-type Antirrhinum and Arabidopsis consist of four concentric whorls of organs: sepals in whorl 1 (the outer whorl), petals in whorl 2, stamens in whorl 3, and carpels in whorl 4 (the central whorl). Wild-type flowers of both species terminate after the production of a defined number of organs (apical determinacy), and no meristems are produced in the axils of the floral organs (lateral determinacy). The type of organ produced in each whorl depends on the activity of organ identity genes that can be classified into three groups, *a*, *b*, and *c*, according to their mutant phenotypes. Class *a* mutants (such as *apetala2* [*ap2*] in Arabidopsis) have carpels that replace sepals and stamens that replace petals; class *b* mutants (such as *pistillata* [*pi*] and *apetala3* [*ap3*] in Arabidopsis and *deficiens* [*def*] and *globosa* [*glo*] in Antirrhinum) have sepals that replace petals and carpels that replace stamens; class *c* mutants (such as *agamous* [*ag*] in Arabidopsis and *plena* [*ple*] in Antirrhinum) have petals that grow instead of stamens, with carpels replaced by indeterminate whorls of petal- or sepal-like organs (loss of apical determinacy). Phenotypic analysis and in situ RNA expression patterns suggest that the genes from each class act in overlapping domains of the young floral meristem to determine the fate of organ primordia.

The correct function of organ identity genes depends on the activity of meristem identity genes, such as *LEAFY* (*LFY*) and *APETALA1* (*AP1*) in Arabidopsis and *FLORICAULA* (*FLO*) and *SQUAMOSA* (*SQUA*) in Antirrhinum (Coen et al., 1990; Irish and Sussex, 1990; Schultz and Haughn, 1991; Huala and

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Sussex, 1992; Huijser et al., 1992; Mandel et al., 1992; Weigel et al., 1992). In wild-type Arabidopsis, the most basal nodes of the inflorescence produce lateral inflorescence shoots, termed coflorescences (Weiberling, 1989), which are subtended by cauline leaves or bracts. Subsequent nodes produce flowers in a racemose arrangement that are not subtended by bracts. In plants homozygous for strong *lfy* alleles, the lower flowers of the inflorescence are replaced by coflorescences, and when the upper flowers are eventually formed, they are composed mainly of sepal and carpel tissue and are subtended by bract-like structures. The wild-type Antirrhinum inflorescence is a simple raceme of flowers, each subtended by a bract. In plants homozygous for strong *flo* alleles, all flowers are replaced by inflorescence shoots. Mutations in the meristem identity genes of both species can therefore be considered to cause a loss of both apical and lateral determinacy in lateral meristems.

The *UFO* gene of Arabidopsis has a mutant phenotype that shows several features of both meristem and organ identity mutants (Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). The first few flowers of *ufo* mutants are replaced by coflorescences. Both the coflorescences and the main inflorescence of *ufo* mutants terminate in carpelloid or sepaloid structures. Above these coflorescences, flowers that show variable homeotic transformations in the first three whorls are produced. The most dramatic transformations in *ufo* mutant flowers are observed in the second and third whorls, where petals and stamens are replaced by chimeric organs composed of sepal, petal, carpel, and filament tissue. Many flowers are subtended by bracts or filamentous structures. In addition, *ufo* mutants show a decrease in floral organ number in the second and third whorl and appear to show a loss of whorl organization, sometimes making it difficult to assign organs to a given whorl. Mutants also commonly form bracts with no axillary shoots or flowers.

The *ufo* mutant of Arabidopsis has many phenotypic features in common with the *fim* mutant of Antirrhinum. Plants mutant for *fim* also show features of both meristem and organ identity gene mutants, including an increase in inflorescence characteristics in some floral meristems and homeotic organ transformations in the second and third whorls of all flowers. In addition, *fim* mutants also have disturbed whorl organization and produce nodes at which the axillary meristem fails to develop fully. Expression studies have shown that *FIM* transcripts appear later than those of meristem identity genes but earlier than those of organ identity genes. Furthermore, *FIM* expression depends on the activity of the meristem identity gene *FLO*, and *FIM* in turn regulates the expression of at least two organ identity genes (*DEF* and *PLE*), suggesting that *FIM* acts as a mediator between these two gene classes.

The phenotypic similarities between *ufo* and *fim* suggest that they might be mutations in homologous genes. We cloned the *UFO* gene and showed that it is similar to *FIM* not only with respect to mutant phenotype but also with respect to structure and expression pattern. We concluded that *UFO* is the cognate homolog of *FIM* and that the conservation observed for the meristem and organ identity genes of Arabidopsis and Antirrhinum also extends to genes mediating between the two groups.

RESULTS

Isolation of the *FIM* Homolog from Arabidopsis

To determine whether genes having sequence homology with the Antirrhinum *FIM* gene are present in Arabidopsis, genomic DNA from Arabidopsis was digested and probed with a fragment spanning most of the *FIM* open reading frame. Under low-stringency conditions, it was not possible to detect any clear bands. When a genomic library derived from the Landsberg *erecta* (*Ler*) ecotype of Arabidopsis was screened under the same conditions, 15 λ clones that hybridized to *FIM* were isolated. These fell into nine classes that were based on restriction analysis and the chromosomes to which the clones mapped. Of these nine classes, four showed particularly strong hybridization with *FIM*. To determine which one of these classes might correspond to the *FIM* homolog, we subjected the clones to a second round of screening. Analysis of the *FIM* locus had revealed a transcribed gene ~1 kb from *FIM* (R. Simon, unpublished results). This downstream gene was used as a probe for the four strongly hybridizing classes of Arabidopsis clones. One of the four showed strong hybridization to the downstream gene, suggesting that the linkage observed between the Antirrhinum genes has been preserved in Arabidopsis and that this class most likely represents the true *FIM* homolog. Our subsequent analysis was carried out using three overlapping clones from this class: λJAM2001, λJAM2002, and λJAM2003 (see Figure 1).

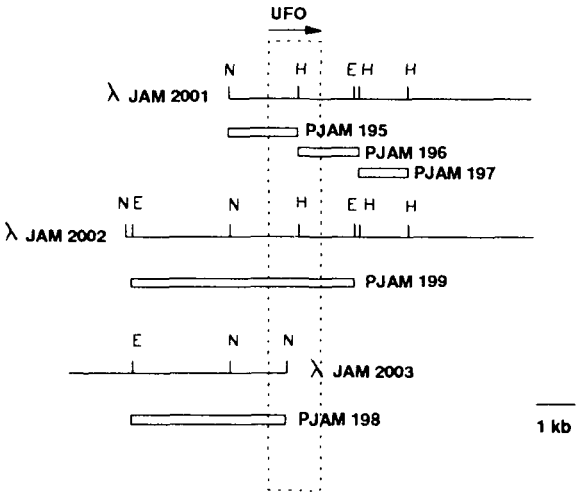

Figure 1. The *UFO* Locus.

The positions of portions of the three λ clones isolated by homology with *FIM* are shown as single lines. Subclones used during the genetic characterization of *UFO* are shown as open boxes, and the *UFO* open reading frame is indicated by an arrow. Restriction sites used in subcloning are indicated: E, *Eco*RI; H, *Hin*dIII; N, *Not*I.

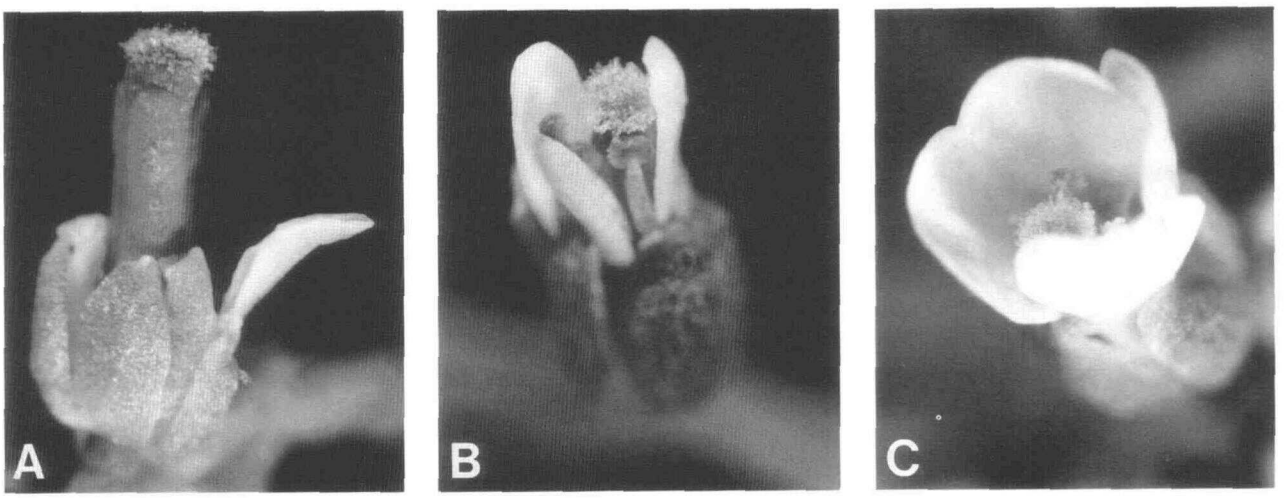

Figure 2. Complementation Analysis.

(A) Typical flower from a *ufo-1* mutant. The outer whorl of four sepals is visible and encloses two whorls of chimeric organs and sepals with a central gynoecium.

(B) Flower from a partially complemented *ufo-1* plant. Two morphologically normal petals, one petal–sepal mosaic organ, and one sepal are present in the second whorl. Four complete stamens have been produced in the third whorl.

(C) Flower from a fully complemented *ufo-1* plant. This flower has four petals and six stamens and is phenotypically indistinguishable from the wild type.

FIM and *UFO* Are Homologous

The phenotypes of *fim* mutants of Antirrhinum and *ufo* mutants of Arabidopsis share many features. To test whether the *FIM* homolog from Arabidopsis corresponded to *UFO*, we performed a restriction fragment length polymorphism (RFLP) analysis. Homozygous *ufo-1* plants in the Columbia-2 (Col-2) background were crossed to wild-type Ler plants. The F_1 generation was selfed, and 67 plants from the F_2 generation were genetically characterized as either *UFO/UFO, UFO/ufo*, or *ufo/ufo*, based on phenotype and progeny testing. DNA was collected from F_3 families, digested with HindIII, and probed with a fragment from one of the isolated clones (pJAM195; Figure 1). A RFLP that segregated with the *ufo* genotype in all the plants analyzed was detected. No recombination events were observed in a total of 134 chromosomes, indicating tight linkage to the *UFO* locus in Arabidopsis.

To confirm that we had cloned *UFO*, transformations were carried out to complement the *ufo* mutant phenotype. A 6-kb EcoRI fragment, pJAM199, which contains the entire genomic region hybridizing to *FIM* but not to the downstream gene, was inserted into a binary vector conferring kanamycin resistance (Kan^r). Agrobacterium-mediated transformation was used to introduce this plasmid directly into either *ufo-1* mutants or wild-type Arabidopsis. The first transformation was carried out with plants heterozygous for *ufo-1* and *distorted trichomes2 (dis2)*, a very closely linked marker (map distance of ~0.5 map units; Wilkinson and Haughn, 1995). The close linkage allowed the *dis2* phenotype to be used as a marker to identify homozygous *ufo-1* plants in subsequent generations. Two primary transformants were selected on Kan for the presence of the introduced T-DNA and selfed to give T_1 generations. T_1 plants sown on Kan segregated for Kan^r/Kan sensitivity (Kan^s) with a 3:1 ratio and also segregated 3:1 for wild-type/*dis2* individuals. When sown on Kan, all of the *dis2* plants from one line produced flowers with a novel phenotype. This phenotype was less extreme than that of *ufo-1* flowers (Figure 2B). When T_1 plants of this line were sown without Kan, 75% of the *dis2* plants produced the novel phenotype and 25% showed the *ufo-1* phenotype (Figure 2A). The other transformed line produced no new phenotype: all T_1 plants either were wild type or showed the *ufo-1* phenotype. We concluded that the introduced DNA was not causing any phenotypic effects in this line.

The second transformation was carried out using the wild type (Ler). Four primary transformants were selected on Kan and selfed to give four T_1 families that showed a 3:1 segregation ratio for Kan^r/Kan^s. T_1 plants that were homozygous for the T-DNA insertions were identified by progeny testing on Kan and were then crossed to homozygous *ufo-1 dis2* plants. The F_1 plants were all Kan^r and were selfed. The F_2 families gave the expected 3:1 wild-type/*dis2* segregation ratio and showed a 3:1 segregation ratio for Kan^r/Kan^s. When the F_2 families were sown on Kan, all *dis2* plants had novel floral phenotypes that were less extreme than *ufo-1*. When the F_2 families were sown without Kan, 25% of the *dis2* plants had a *ufo-1* phenotype and 75% had a less extreme phenotype.

The observed novel phenotypes fell into three classes. Two of the transformed lines had a novel phenotype that was almost like that of the wild type (class 1; Figure 2C). Plants with this phenotype show no increase in the number of basal coflorescences when compared with the wild type. The flowers usually contain four petals and six stamens, although distorted

petals were sometimes observed. Two of the transformed lines had a novel phenotype that was easily distinguishable from that of the wild type but was much less extreme than that of *ufo-1* mutants (class 2; Figure 2B). These plants produce the same number of coflorescences as do wild-type plants. Flowers show four normal sepals in the first whorl, between two and four morphologically normal petals in the second whorl, between three and six normal stamens in the third whorl, and a wild-type carpel. The proportion of normal organs observed in the second and third whorl is greatly increased in these plants as compared with *ufo-1* mutants. In addition, flowers are not subtended by the cauline leaves or filamentous structures seen in *ufo-1* plants. The novel phenotype is closer to that of the wild type in basal flowers in which the *ufo-1* mutant phenotype is normally most severe (Wilkinson and Haughn, 1995). Due to self-pollination, these transgenic plants produce large numbers of siliques as compared with the *ufo-1* mutant, which is largely infertile.

One of the transformed lines had a novel phenotype with several of the features of *ufo-1* plants (class 3; not illustrated). These included the production of extra coflorescences in some plants, the presence of filamentous structures subtending some flowers, and a considerable decrease in the number of normal second and third whorl organs produced as compared with the wild type. Petals were usually small and were often replaced by petal–sepal mosaic organs. Sepals were sometimes observed in the second whorl. Filamentous structures were often observed in the third whorl, and the number of normal stamens produced was reduced, although between two and four were usually observed. However, these plants are still significantly less extreme in their floral phenotype than are *ufo-1* individuals, and this is reflected in their relatively high frequency of self-pollination.

Because these novel phenotypes all segregate with the introduced T-DNA construct and are less extreme than the *ufo-1* mutant phenotype, we concluded that they were all due to varying degrees of complementation of the mutant phenotype by the introduced DNA sequence.

Sequence and Structure of the *UFO* Gene

Two of the overlapping clones, λJAM2001 and λJAM2003, were subcloned, and 4.8 kb of genomic sequence was obtained from the regions hybridizing to *FIM* (Figure 1). The sequence contains one open reading frame of 1326 bp (Figure 3) that has the potential to code for a 442–amino acid protein with a molecular mass of 49 kD. (The nucleotide sequence data reported in this paper have an EMBL, GenBank, and DDBJ accession number of X89224.) This protein sequence was compared with data base sequences, and it showed no significant similarity to any other protein except the FIM protein of Antirrhinum. The sequence generated also showed that the subclone pJAM199, which was used in our complementation analysis, contains the

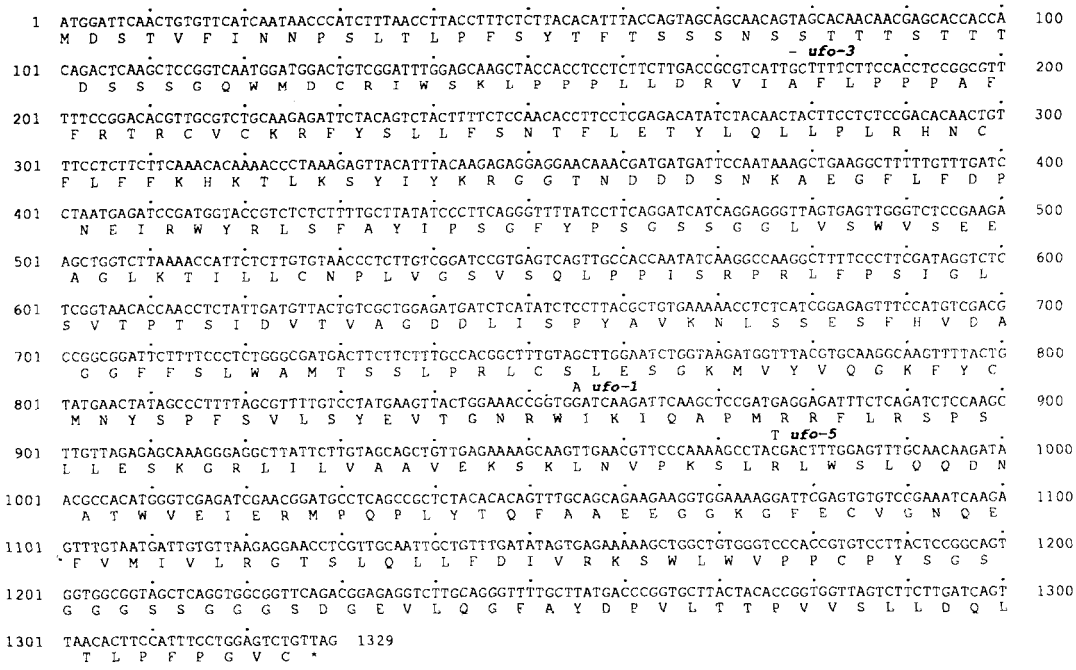

Figure 3. Sequence of the *UFO* Gene and the Derived Protein Sequence.

Amino acids are indicated in the standard one-letter code. The point mutations (G → A substitution for *ufo-1*; C → T substitution for *ufo-5*)) and deletion (deletion of G for *ufo-3*) determined for each of the *ufo* mutant alleles are indicated above the corresponding nucleotides in the wild-type sequence.

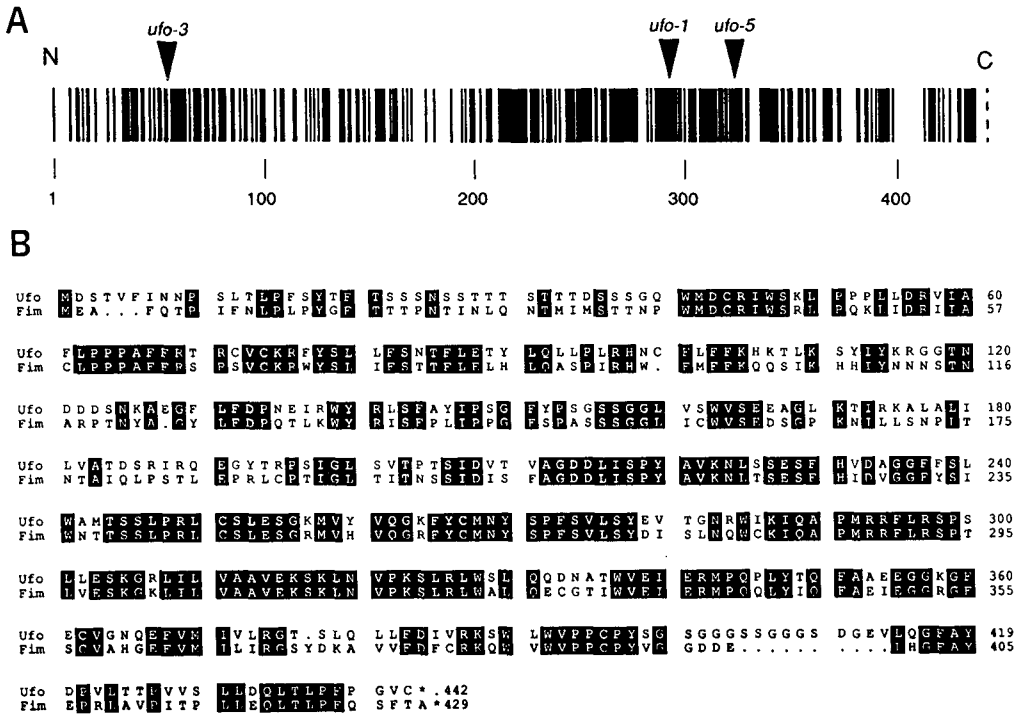

Figure 4. Molecular Similarities between UFO and FIM.

(A) Diagrammatic representation of regions of identity between the UFO and FIM proteins. Each vertical line represents an amino acid that is identical between the two proteins. The positions of the mutations in each of the three mutant alleles are indicated.
(B) Alignment of the amino acid sequences of the UFO and FIM proteins. Amino acids that are identical between the two proteins are blocked. The amino acid numbers of the aligned sequences are indicated at right. Asterisks indicate gaps in sequence due to alignment.

entire *UFO* open reading frame and ~4 kb of upstream sequence.

Homology of UFO with FIM is marked, with an overall amino acid identity of 60% and similarity of 72% (Figure 4). This degree of conservation is similar to that seen for other known homologous Antirrhinum and Arabidopsis proteins such as DEFA and AP3 (58% identity and 77% similarity; Jack et al., 1992; Schwarz-Sommer et al., 1992). However, it is lower than that seen for other conserved proteins, such as FLO and LFY (70% identity and 82% similarity; Coen et al., 1990; Weigel et al., 1992). Similarity at the N- and C-terminal ends of UFO and FIM is relatively low (27 and 29% identity, respectively). Homology is strongest in two regions of the protein: between amino acids 41 and 88 (77% identity) and between amino acids 212 and 374 (78% identity) (Figure 4). When these regions alone were compared with data base sequences, no significant similarity with other sequences was detected.

Mutant Alleles of *UFO*

To determine the molecular basis of the *ufo* mutations, three different mutant *ufo* alleles were sequenced: *ufo-1* (Col-2), *ufo-3* (L*er*), and *ufo-5* (L*er*). The region spanning the *UFO* open reading frame was amplified from plants homozygous for the mutant alleles and from wild-type Col-2 and wild-type L*er* by using polymerase chain reaction (PCR). To avoid spurious results due to PCR artifacts, three products from three independent reactions were cloned for each allele sequenced. These reactions were each carried out with DNA from an individual plant to minimize the risks from contamination. Only changes that appeared in all three clones from a given allele were considered genuine (Figures 3 and 4).

The sequence obtained from *ufo-1/ufo-1* plants showed a single G-to-A nucleotide change at base 855 that replaces the tryptophan residue at position 285 with a stop codon and therefore would result in a truncated protein. Sequences obtained from plants homozygous for the *ufo-3* and *ufo-5* alleles would also code for truncated proteins. The *ufo-3* sequence contains a single nucleotide deletion at base 178 that would lead to a frameshift at amino acid 60 and a predicted protein product of 108 amino acids. The *ufo-5* sequence contains a single C-to-T nucleotide substitution at base 976, resulting in a stop codon that would truncate the predicted protein at amino acid 326. Phenotypic observations have shown that the *ufo-3* allele is more extreme than the *ufo-5* allele (Levin and Meyerowitz, 1995). This correlates with the presence of a longer predicted protein in *ufo-5* and may indicate that gene function is not

completely abolished in this allele. It is not possible to compare the phenotype of *ufo-1* due to the difference in the background of this mutant.

Several base changes were observed between the *UFO* open reading frames from L*er* and Col-2 wild-type plants. All but one of these changes did not cause any alterations in the predicted amino acid sequence of the gene. However, a single T-to-G base change at base 130 in the Col-2 *UFO* open reading frame replaced cysteine-44 with a glycine residue. A cysteine residue is found in this position in the *FIM* open reading frame, and it is situated in a region of high similarity between the two proteins. The appearance of a nonconservative amino acid substitution in this part of the protein indicates that it is of relatively low importance with respect to gene function.

Distribution of the *UFO* Transcript

The timing and distribution of the *UFO* transcript were determined by in situ hybridization with digoxigenin-labeled antisense *UFO* RNA probes against sections of young wild-type inflorescences from Arabidopsis (Figure 5). Stages of floral development were taken from Smyth et al. (1990). To confirm that the probe used would not cross-hybridize with other sequences, a DNA gel blot of wild-type genomic Arabidopsis DNA, cut with three different enzymes, was probed with labeled template DNA at a stringency lower than that used in our in situ analysis (Wahl et al., 1987). No bands, other than those expected from *UFO*, were observed (results not shown).

UFO transcripts were detected in the earliest floral meristems examined and found as early as stage 1. At this stage, the transcript appears to be most abundant in the central cells of the meristem and is expressed three to four cell layers deep (Figure 5A). By stage 2, prior to sepal initiation, transcripts were retained in a ring approximately four cells wide but could not be detected in the center of the meristem (Figure 5C). As sepal primordia appear (stage 3), the transcript is limited to a small region just inside the sepal whorl (Figure 5B). Transcripts then become further restricted to four regions consisting of a small number of cells on the abaxial base of each petal primordium (Figure 5C). Transcripts were detectable in these cells until stage 10 or later. Transcripts were never observed in the apical meristem of the primary or lateral inflorescences or in other inflorescence tissues.

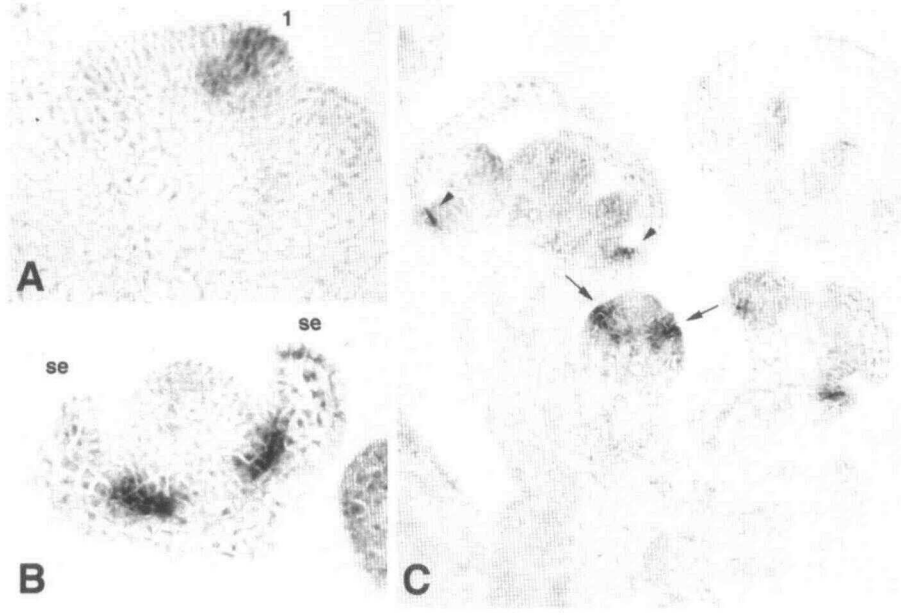

Figure 5. In Situ Hybridization of *UFO* Transcript with Sections of Wild-Type Inflorescences and Floral Meristems.

Regions expressing *UFO* stain dark on a lighter background.

(A) Longitudinal section through an inflorescence meristem. The first floral primordium (1) is indicated. The center of the apical meristem is slightly behind the section and to the left.

(B) Longitudinal section through a young flower. Only sepal (se) primordia are visible.

(C) Longitudinal section through an inflorescence meristem and several stages of floral buds. The ring of *UFO* expression in the floral primordium, which has not yet initiated sepal primordia, is indicated by arrows. Petal primordia are visible in two older floral buds. *UFO* expression is visible at the base of these petal primordia (arrowheads).

DISCUSSION

UFO and *FIM* Show Structural and Functional Similarities

We have cloned the *UFO* gene of Arabidopsis using the *FIM* gene of Antirrhinum as a probe. This is supported by three types of evidence: (1) using RFLP analysis, we found that the *UFO* clone always segregates with the *ufo* mutant phenotype, suggesting very tight linkage; (2) the *ufo* mutant phenotype was partially or fully complemented by the presumptive *UFO* clone; and (3) three mutant alleles of *UFO* showed different point mutations in the open reading frame.

The *UFO* gene is similar to *FIM* in several respects. The protein sequences of *UFO* and *FIM* show 60% amino acid identity (72% similarity), although neither protein shows similarity to other sequences in the data bases. In addition, the distribution of the *UFO* transcript in Arabidopsis shares many features with that of *FIM* in Antirrhinum (Simon et al., 1994; Wilkinson and Haughn, 1995). In both cases, the genes are expressed in young floral meristems before organ identity genes. Expression initiates in the center of the floral meristem and then resolves into a ring, leaving a region clear of expression in the center of the meristem. Later still, expression becomes restricted to small regions at the junction between petals and adjacent organs; *UFO* expression appears only at the sepal–petal junctions, whereas *FIM* expression appears at both the sepal–petal and petal–stamen junctions.

These molecular similarities are borne out by phenotypic and genetic studies on mutations of the two genes, although in this analysis, the *fim* and *ufo* alleles described have not been shown to represent complete loss of gene function. Homeotic conversions and chimeric organs are often observed in whorls 2 and 3 of *fim* and *ufo* mutants (Simon et al., 1994; Wilkinson and Haughn, 1995). These changes can include the presence of sepal tissue in the second whorl and petal, sepal, or carpel tissue in the third whorl, suggesting that in both mutants, class *b* gene function is considerably reduced. In Antirrhinum, this has been shown to reflect reduced levels of *b* gene transcription throughout flower development. In Arabidopsis, there are data both in favor of (Levin and Meyerowitz, 1995) and against (Wilkinson and Haughn, 1995) a decrease in the expression of *b* function genes in *ufo* mutants. The presence of sepaloid tissue in the third whorl of *ufo* and *fim* mutants suggests that the *c* gene function is also reduced. This has been confirmed by studying double mutants of *ufo* with *b* function genes, *ap3* or *pi*, in Arabidopsis and of *fim* with *def* or *glo* in Antirrhinum. Whereas the third whorl of single *b* mutants consists of carpels, the double mutants often have sepal tissue, indicating that *UFO* and *FIM* normally increase *c* gene activity in this whorl.

The phyllotaxy of flower organs produced by the lateral meristems of wild-type Arabidopsis and Antirrhinum inflorescences is whorled, whereas inflorescences and coflorescences a spiral phyllotaxis. In both *fim* and *ufo* mutants, there is a tendency toward producing organs in a spiral. In *ufo* mutants, we observed this as the production of structures resembling coflorescences in place of flowers. These are similar to structures seen in *lfy* mutants (Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992). In *ufo* flowers produced above the coflorescences and in most *fim* mutant flowers, spiral phyllotaxis or disruption of whorl organization only occurs after a relatively normal first whorl has been formed.

Both *fim* and *ufo* mutants also showed some loss of lateral and apical determinacy. In *ufo* mutants, basal flowers are replaced by coflorescences that show an almost total loss of both apical and lateral determinacy. This was also observed in early *fim* mutant flowers as the production of extra whorls of organs within the flower and the production of secondary floral meristems in the axils of floral organs. In both mutants, more apical flowers are less abnormal with respect to determinacy and generally terminate in a relatively normal carpel-like structure.

Both *ufo* and *fim* mutants produce nodes at which the development of the axillary meristem appears to be reduced. In *ufo* mutants, the production of "empty nodes" has been observed (Wilkinson and Haughn, 1995). These nodes have bract or filamentous structures with no axillary shoot development. We observed a similar phenomenon in *fim* mutants, although in these cases axillary shoots appear to be initiated but arrested at an early stage, giving rise to a few very small sepal-like structures in the axil of the bract. These underdeveloped meristems may indicate that *FIM* and *UFO* may play a role in stabilizing the initiation of floral development.

Genetic studies have shown that the interactions between *UFO* and the Arabidopsis meristem identity genes *LFY* and *AP1* are very similar to those observed between *FIM* and the corresponding genes *FLO* and *SQUA* in Antirrhinum. Mutations in *LFY* are largely epistatic to *ufo* mutations, suggesting that *UFO* may act downstream of *LFY*. Similarly, in Antirrhinum it has been shown that *FIM* acts downstream of *FLO*, and this is reflected by the absence of *FIM* transcripts in axillary meristems of *flo* mutants. The *ufo ap1* double mutant shows a strong enhancement of the single mutant phenotypes, leading to the production of axillary structures with coflorescence-like characters. This phenotype is similar to the phenotype seen in *fim squa* double mutants in Antirrhinum. Thus, in Arabidopsis, *AP1* appears to act in a pathway separate from *UFO*/*LFY*, similar to the way *SQUA* interacts with the *FIM*/*FLO* pathway in Antirrhinum.

Another feature shared by the *ufo* and *fim* mutants is their phenotypic variability. This variability occurs with all known alleles of both genes, between plants sharing similar genetic backgrounds as well as between adjacent flowers of the same plant. This could reflect genetic redundancy, such that loss of *FIM* or *UFO* activity does not lead to a complete inactivation of a genetic pathway. A reduction in the activity of a pathway could make plants highly sensitive to environmental and other factors.

Functional Divergence between Arabidopsis and Antirrhinum

Despite the many similarities between *ufo* and *fim* mutants, there is also evidence of considerable functional divergence between the two genes. Axillary flowers in wild-type Antirrhinum are always subtended by bracts, whereas in wild-type Arabidopsis, bracts subtend only coflorescences and are not found at the base of flowers. In *ufo* mutants, however, flowers are often subtended by bract-like organs or filaments. This loss of bract suppression has been observed in *lfy* mutants (Schultz and Haughn, 1991; Weigel et al., 1992), again suggesting that *UFO* may be acting as a downstream mediator of *LFY.* Because bracts are not suppressed in Antirrhinum, this is probably an acquired function for *UFO* that *FIM* does not possess.

The *UFO* gene appears to act slightly earlier than *FIM.* Whereas early flowers of *ufo* mutants are replaced by coflorescence-like shoots, inflorescence-like shoots in extreme *fim* mutant flowers always produce at least one whorl of organs. The earlier action of *UFO* may also correlate with a slightly earlier appearance of *UFO* transcripts in Arabidopsis compared with *FIM* in Antirrhinum.

The *UFO* and *FIM* genes may also differ in the manner in which they regulate *c* function organ identity genes. In situ hybridization analysis has shown that transcript levels of *PLE* are reduced in *fim* mutants, whereas in Arabidopsis, transcripts from *AG* are not markedly reduced in *ufo* mutants (Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). These results suggest that in Antirrhinum, normal *c* gene function depends on *FIM* expression, whereas in Arabidopsis, *c* gene function is less dependent on *UFO.* However, we observed carpel structures and relatively normal apical determinacy in the later flowers of *fim* mutants, indicating that there is also a pathway by which *c* gene function can be activated independently of *fim* in Antirrhinum. This pathway seems to be less active in Antirrhinum than in Arabidopsis, which may explain why *flo* mutants produce carpels very rarely when compared with *lfy* (Schultz and Haughn, 1991; Huala and Sussex, 1992; R. Carpenter, L. Copsey, C. Vincent, S. Doyle, R. Magrath, and E.S. Coen, unpublished data). In addition, the presence of some reproductive tissue in the second and even the first whorl of *ufo* mutants suggests there may be some ectopic expression of *c* function genes in some flowers. Thus, *UFO* may play a role as a negative regulator of *c* function genes in the first and second whorls. This phenomenon has not been observed in *fim* mutants either because *FIM* does not function as a negative regulator of *c* function genes or because the overall reduction in *c* gene transcription in *fim* mutants masks any negative regulation.

In *fim* mutants, the apical meristem of the main inflorescence is indeterminate, and some inflorescence-like axillary structures also appear to be indeterminate. In contrast, all coflorescences and the main inflorescence of *ufo* mutants eventually terminate in either carpel-like or sepal/carpel-like structures. This suggests that *ufo* may also play a role in the indeterminacy of the inflorescence meristem through negative regulation of *c* function genes. When *UFO* is not present, ectopic expression of *c* function genes can eventually lead to termination of the apex in a carpel structure. The presence of transcripts of the *c* function gene *AG* in the apical meristem of *ufo* mutants has been confirmed by in situ hybridization analysis (Wilkinson and Haughn, 1995).

Conclusion

Comparisons of *fim* and *ufo* show that in addition to having similar mutant phenotypes, they are conserved with respect to both their molecular structures and their expression patterns. The structural and functional conservation already demonstrated between meristem and organ identity genes in Arabidopsis and Antirrhinum can therefore also be found between the mediator genes active during flower development. However, despite their similarities, some differences in the functions and genetic interactions of *fim* and *ufo* are apparent. These differences may reflect changes in functionally redundant pathways that occurred during the evolutionary divergence between Arabidopsis and Antirrhinum.

METHODS

Plant Material

The wild-type *Arabidopsis thaliana* strain used in this work was Landsberg *erecta* (L*er*). The mutant *unusual floral organs-1* (*ufo-1*) was generated in the Arabidopsis ecotype Columbia-2 (Col-2). The alleles *ufo-3* and *ufo-5* (L*er*) were gifts of D. Smyth (Monash University, Clayton, Australia). The line used for complementation analysis carried both the mutant *ufo* allele *ufo-1* and a mutation in the very closely linked *DISTORTED TRICHOMES2 (DIS2)* gene in a mixed L*er* and Col-2 background (Wilkinson and Haughn, 1995).

Cloning of *UFO*

A 1.3-kb polymerase chain reaction (PCR) product containing the *FIMBRIATA* (*FIM*) open reading frame was cloned into pGEM4z (Promega) to give pJAM172 and was subsequently excised as a BamHI fragment. This fragment was labeled and used as a probe to screen DNA gel blots of Arabidopsis genomic DNA and a genomic library made from a partial Sau3A digest of Arabidopsis (L*er*) DNA in λFIXII (Stratagene) under low-stringency conditions (hybridization at 50°C with 2 × SSC [1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate], 1% [w/v] SDS, and 0.5% [w/v] dried milk powder; washing at 50°C with 2 × SSC and 0.5% SDS). The insert from a plasmid containing the cDNA of a gene situated downstream of *FIM* (pJAM126) was used as a probe for the λ clones isolated. The same low-stringency conditions were used.

Restriction Fragment Length Polymorphism Analysis

Homozygous *ufo-1* plants (Col-2 ecotype) were crossed to wild-type plants of the L*er* ecotype. Subsequent generations were selfed, and DNA from the F_3 progeny of 67 F_2 plants genotypically characterized

as *UFO/UFO, UFO/ufo*, or *ufo/ufo* was extracted. DNA was then digested with HindIII and probed with a subclone of λJAM2001 (pJAM195), which contains part of the putative *UFO* open reading frame.

Complementation Analysis

A 6-kb EcoRI fragment was subcloned from one λ clone (λJAM2002) into pBluescript SK+ (Stratagene) to give pJAM199. This fragment was excised and cloned into the binary vector pJE189 (a gift from J. English, John Innes Centre). The resulting vector was introduced into Arabidopsis roots by *Agrobacterium tumefaciens*–mediated transformation as described in Bancroft et al. (1992).

Sequencing of *ufo*

To sequence the region of interest, two HindIII fragments, pJAM196 and pJAM197 (1.6 and 1.2 kb, respectively), and one HindIII-NotI (1.8-kb) fragment, pJAM195, from one λ clone (λJAM2001), and a NotI-EcoRI fragment, pJAM198 (4.2 kb), from an overlapping clone (λJAM2003) were cloned into pBluescript SK+ (Stratagene). A sequence of 4.8 kb was obtained from these subclones by dideoxy methods using a Sequenase II kit according to the manufacturer's instructions (U.S. Biochemicals). A series of oligonucleotides was synthesized to use as primers. To sequence each of the mutants (*ufo-1, ufo-3,* and *ufo-5*), primers situated to either side of the open reading frame were used for PCR, and products from each reaction were cloned separately into pGEM4z (Promega) to give three independent PCR products from each plant for sequencing.

In Situ Hybridization Analysis

In situ hybridization was adaptated from Huijser et al. (1992). Digoxigenin labeling of RNA and detection of signal were performed using Boehringer Mannheim methods and reagents (kit No. 1175-041). A plasmid, pJAT170, containing a 530-bp PCR-amplified fragment of the *UFO* coding region was cut at the 5′ end of the insert and transcribed in the presence of digoxigenin-labeled nucleotides. The resulting antisense transcript was hydrolyzed to generate fragments of ~150 nucleotides. Sections of wild-type (Col-2) inflorescences were probed. After detection, sections were dehydrated through ethanol and xylene and mounted in Entellan mounting media (Merck). Sections were photographed through a Leitz DRB light microscope (Leica, Buffalo, NY) using Kodak (ASA 160) Ektachrome film (Eastman Kodak, Rochester, NY).

To test the specificity of the RNA probe, genomic DNA from wild-type *Ler* plants was digested separately with EcoRI, HindIII, and EcoRV, and DNA gel blots were prepared. The insert from pJAT170 was isolated and labeled using a random hexamer oligonucleotide labeling kit (kit No. 27-9250-01; Pharmacia). Hybridization was performed at 60°C with 2 × SSC, 1% SDS, and 0.5% (w/v) dried milk powder, and washing was performed at 60°C with 0.2 × SSC and 0.5% SDS.

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