UFO: An Arabidopsis Gene Involved in Both Floral Meristem and Floral Organ Development

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We describe the role of the UNUSUAL FLORAL ORGANS (UFO) gene in Arabidopsis floral development based on a genetic and molecular characterization of the phenotypes of nine ufo alleles. UFO is required for the proper identity of the floral meristem and acts in three different aspects of the process that distinguishes flowers from shoots. UFO is involved in establishing the whorled pattern of floral organs, controlling the determinacy of the floral meristem, and activating the APETALA3 and PISTILLATA genes required for petal and stamen identity. In many respects, UFO acts in a manner similar to LEAFY, but the ufo mutant phenotype also suggests an additional role for UFO in defining boundaries within the floral primordia or controlling cell proliferation during floral organ growth. Finally, genetic interactions that prevent flower formation and lead to the generation of filamentous structures implicate UFO as a member of a new, large, and diverse class of genes in Arabidopsis necessary for flower formation.

INTRODUCTION

An Arabidopsis flower has four concentric whorls, from outermost to innermost, that contain four sepals, four petals, six stamens, and two carpels that fuse to form the gynoecium (Figure 1A). Three general categories of genes have been shown to control the development of floral primordia. First, floral meristem identity genes act early in the process to specify floral identity rather than shoot identity. Second, cadastral genes act to spatially regulate the extent of the expression pattern of the floral organ identity genes. Third, the homeotic or floral organ identity genes act to specify the organ type into which an organ primordium develops.

A model known as the ABC model has been proposed to explain how the homeotic genes specify floral organ identity in Arabidopsis (reviewed in Weigel and Meyerowitz, 1994). This model has three general features. First, the genes in each of three classes (A, B, and C) act in the two whorls in which their loss-of-function mutants exhibit floral organ identity transformations. Class A genes, which include APETALA1 (AP1) and APETALA2 (AP2), act in the first and second whorls in this way (Bowman et al., 1989, 1991, 1993; Kunst et al., 1989; Irish and Sussex, 1990). Class B genes, which include APETALA3 (AP3) and PISTILLATA (PI), act in the second and third whorls (Bowman et al., 1989, 1991; Hill and Lord, 1989). The class C gene AGAMOUS (AG) acts in the third and fourth whorls (Bowman et al., 1989, 1991). Second, combinatorial expression of these genes defines the organ type that differentiates in each whorl. The first whorl sepals are specified by class A gene activity alone. The second whorl petals are specified by class A and B gene activity. The third whorl stamens are specified by class B and C gene activity. The fourth whorl carpels are specified by class C gene activity alone. Third, there is an antagonism between class A and C activity so that, in the absence of A activity, C activity expands into the first two whorls and, in the absence of C activity, A activity expands into the inner two whorls. Four of these genes encode putative transcription factors with a MADS domain (Schwarz-Sommer et al., 1990), which functions in DNA binding and protein dimerization (Yanofsky et al., 1990; Jack et al., 1992; Mandel et al., 1992; Treisman and Ammerer, 1992; Goto and Meyerowitz, 1994). In general, the RNA expression pattern for these four genes is consistent with their proposed domains of function in the ABC model. AP2 differs from the others in that it does not encode a protein with a MADS domain and the expression of its RNA is not restricted within or to the developing flower (Jofuku et al., 1994).

An important question in floral development remains to be answered: How do the floral meristem identity genes act to establish a "prepattern" within the early stages of the developing flower, which leads to the spatially restricted patterns of floral organ identity gene activity described in the ABC model? Mutations in the floral meristem identity gene LEAFY (LFY) result in a decrease in the levels of class B gene expression but not in those of class A and C gene expression (Weigel and Meyerowitz, 1993). In addition to being a homeotic gene, AP1 also functions as a floral meristem identity gene (Irish and Sussex, 1990; Weigel et al., 1992; Bowman et al., 1993; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993). A plant carrying mutations in both the LFY and AP1 genes has a severe decrease in class B and C gene activity (Weigel and Meyerowitz, 1993). Thus, LFY and AP1 can be considered...
Figure 1. Phenotypes of Arabidopsis Wild-Type and Mutant Plants.

(A) Wild-type flower.
(B) *ufo-2* flower. Visible are two petal/sepal mosaic organs (arrows) and three filaments (black arrowheads).
(C) *lfy-5* flower.
(D) *ufo-2* *lfy-5* flower.
(E) *lfy-22* flower.
(F) *ufo-2* *lfy-22* flower.
(G) *ap2-1* flower.
(H) *ufo-2* *ap2-1* flower.
(I) *fl54* inflorescence.
(J) *ufo-2* *fl54* inflorescence. Apex of the plant is on the right.
(K) *ufo-2* inflorescence with filamentous structures (arrows) in place of flowers. Apex of the plant is on the right.

global (within the flower) positive regulators for the initiation of the expression of the floral organ identity genes. These genetic interactions may not be direct, so it is likely that additional genes will be identified that participate in the steps connecting floral meristem identity gene action with floral organ identity gene action.

Here, we report on one such gene, named *UNUSUAL FLORAL ORGANS* (*UFO*) (Wilkinson and Haughn, 1994). The *UFO* gene plays a role in determining floral meristem identity and in many respects is similar to *LFY* in its action. In addition to controlling floral meristem identity, *UFO* seems to have a role in defining the boundaries between floral organs or in controlling the proliferation of cells in the developing flower. This conclusion is based, in part, on the great variability and unusualness of the floral organs produced in *ufo* mutants. Finally, we uncovered a large and diverse class of Arabidopsis genes, including *UFO*, that acts in the formation of flowers. Plants carrying mutations in two genes in this class are essentially unable
to produce flowers and, instead, generate filamentous structures likely to be default or aborted structures resulting from a very early block in floral development.

RESULTS

Characterization of ufo Mutants

We examined the phenotypes of nine ufo alleles (see Methods). One other ufo allele has been described briefly by Wilkinson and Haughn (1994). All alleles appear relatively similar in their mutant phenotypes, except ufo-6, which has a weaker phenotype, and are likely to be loss-of-function mutations because of their recessive nature. For each of the strong alleles, there is such great variation in floral phenotype that it was not practical to make distinctions among these mutants based on any small differences in the relative strengths of their phenotypes (data not shown). We chose ufo-2, a representative strong allele, for most of our genetic and molecular analyses. No vegetative defects have been observed in ufo mutant plants.

The ufo mutant phenotype includes five different defects in inflorescence development. First, there was a small, but significant, increase in the number of secondary inflorescences arising on the primary inflorescence (Table 1). This defect indicates either that there is a delay in the switch from the production of secondary inflorescences to flowers or that the earliest flowers are homeotically transformed to secondary inflorescences. Second, the first flower arising on an inflorescence often was subtended by a bract (leaf), which is similar in size to or smaller than a cauline leaf, or by a filamentous structure (Figure 2A) that might be an aborted bract (Table 1). In Arabidopsis, wild-type flowers are generally not subtended by a leaf, but wild-type shoots (secondary inflorescences) are subtended by a cauline leaf. Thus, this defect could be a partial transformation of the first flower to a secondary inflorescence. Third, occasionally a flower did not develop, and in its place a filamentous structure formed (Figures 1K and 2A). At a low frequency, ufo mutants formed "reduced flowers" that were decreased in size and contained few, if any, organs (Figures 2B and 2C). The reduced flower could be a partial manifestation of the same defect that leads to the development of a filamentous structure. Both defects appeared more frequently in positions apical to the first 10 flowers of an inflorescence and more on the secondary than on the primary inflorescence. In addition, these defects were more common in the erecta genetic background than in the erecta background. Fourth,

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Bract</th>
<th>Filamentous Structure</th>
<th>Nothing</th>
<th>Avg. No. of 2° Inflorescences</th>
<th>No. of Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>ufo-2c</td>
<td>40</td>
<td>20</td>
<td>40</td>
<td>3.53</td>
<td>15</td>
</tr>
<tr>
<td>ufo-3c</td>
<td>36</td>
<td>50</td>
<td>14</td>
<td>4.73</td>
<td>22</td>
</tr>
<tr>
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<td>17</td>
<td>39</td>
<td>3.91</td>
<td>23</td>
</tr>
<tr>
<td>ufo-5c</td>
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<td>21</td>
<td>3.71</td>
<td>14</td>
</tr>
<tr>
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<td>27</td>
<td>13</td>
<td>4.20</td>
<td>15</td>
</tr>
<tr>
<td>ufo-7c</td>
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<td>25</td>
<td>25</td>
<td>4.25</td>
<td>20</td>
</tr>
<tr>
<td>ufo-8c</td>
<td>45</td>
<td>25</td>
<td>30</td>
<td>3.16</td>
<td>20</td>
</tr>
<tr>
<td>Ler 1c</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>2.58</td>
<td>12</td>
</tr>
<tr>
<td>fly-5c</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>7.08</td>
<td>13</td>
</tr>
<tr>
<td>Ler 2c</td>
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<td>94</td>
<td>2.33</td>
<td>18</td>
</tr>
<tr>
<td>ufo-9c</td>
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<td>35</td>
<td>10</td>
<td>3.25</td>
<td>20</td>
</tr>
<tr>
<td>Ler 3c</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>3.54</td>
<td>13</td>
</tr>
<tr>
<td>ufo-10c</td>
<td>50</td>
<td>8</td>
<td>42</td>
<td>4.08</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 1. Floral Meristem Identity Defects

All mutants were in a Landsberg erecta (Ler) genetic background.

a Numbers are the percentage of the total. For genotype ufo-4, the rounding of percentages led to a total of 99%.

b Average number of secondary (2°) inflorescences per primary inflorescence. Flowers subtended by bracts or filamentous structures were not counted as inflorescences. Using the Wilcoxon Rank-Sum test (one tailed), we determined that the data for each ufo mutant, except for ufo-6 and ufo-10, are significantly different (P < 0.0016 or better) from that for Ler. Although the average number of secondary inflorescences for a given genotype varied, the differences between Ler and ufo mutants were reproducible (data not shown).

c All of these plants were grown at the same time under the same conditions (see Methods).

d Possibly significantly different from Ler 1, P < 0.0246 by the Wilcoxon Rank-Sum test (one tailed).

e Numbers 1, 2, and 3 represent Ler populations grown at different times.

f ND, not determined.

g All of these plants were grown at the same time under the same conditions (see Methods).

h All of these plants were grown at the same time under the same conditions (see Methods).

i Possibly significantly different from Ler 3, P < 0.127 by the Wilcoxon Rank-Sum test (one tailed).
Figure 2. Defects in ufo Mutants.
a pair of "squamules" (small filamentous structures distinct from the other two types of aforementioned filamentous structures) sometimes flanked the base of flowers (Figures 2A and 2E)

A pair of squamules could also be found flanking a larger filamentous structure of the type observed in the absence of a flower (Figure 2A). Structures termed squamules have been observed at the base of the pedicels of *Nasturtium officinale* flowers and interpreted to be stipules for a nonexistent bract (Arber, 1931). Squamules in ufo mutants may be related in some way to stipules, which flank the base of leaves, but unlike stipules, these squamules were occasionally tipped with stigmatic tissue (Figure 2E). Fifth, ufo inflorescences could produce a terminal flower or a fusion of carpelloid leaflike organs (Figures 2F and 2G). Flowering could terminate after as few as five flowers under adverse growing conditions (including a temperature above 26°C), or they could form more than 100 flowers under favorable growing conditions (including a temperature of 16°C).

In addition to the inflorescence defects observed for ufo mutant plants, the flowers that formed exhibited a wide variety of normal and abnormal organ types. There is such great variation among ufo flowers that it is uncommon for two ufo flowers to have the same set of floral organs. However, these defects can be better understood by a detailed examination of the floral organs produced (Table 2). The first whorl of ufo flowers usually contained four sepals. Rarely were five or three sepals present, with the former more common in basal flowers and the latter more common in more apical flowers (Table 2 and Figure 2D). In addition, sepals were sometimes abnormally shaped, or two sepals were apparently fused together (Figure 2D and Table 2). Because ufo flowers have so many abnormalities, it was not always possible to assign a floral organ in the second, third, or fourth whorls to a specific whorl. In the second and third whorl, sepals, petals, stamens, carpels, filaments, or mosaic combinations of these organ types were produced instead of petals and stamens (Figures 1A and 1B).

Carpels formed from the third whorl often fused with those in the fourth whorl (Figure 2H); this defect prevented an accurate analysis of the number of carpels produced by the fourth whorl. In addition, the gynoecium sometimes failed to fuse properly. The second and third whorl defects in ufo flowers correspond to a reduction in class B gene function, that is, petals were variably transformed to sepals and stamens were variably transformed to carpels. The severity of this defect decreased acropetally as more petals and stamens were produced in more apical flowers (Table 2); similar acropetal decreases in class B gene function defects have been observed for *fly* mutants (Weigel et al., 1992). The filaments produced in the second and third whorls varied in diameter, length, and number (from 0 to 9 per flower). In this study, we use "filament" to refer to long, green, cylindrical floral organs that may or may not correspond to the stalk of a stamen. These filaments might be aborted or "confused" floral organs formed when cells in the floral primordium received insufficient information to develop normally or when an insufficient number of cells were allocated to a developing floral organ primordium. A reduction in class B gene activity may also be responsible for the development of filaments in ufo flowers because filaments form in the third whorl in place of stamens in ap3 and pi flowers (Bowman et al., 1989).

The developing organs in ufo flowers differed from those in wild-type flowers not only in identity and shape but also in the timing of their growth (Figures 2H and 2J). In wild-type flowers, third whorl organs are larger than second whorl organs throughout much of development (Smyth et al., 1990), and this whorl-specific growth pattern is maintained even in *ap3, pi, and ify-5* flowers, which have altered organ identity (Bowman et al., 1989; Weigel et al., 1992). One of the more

![Figure 2.](image-url)
Table 2. Comparison of Floral Organs

<table>
<thead>
<tr>
<th>Positions of Flowers</th>
<th>ufo-2\textsuperscript{a}</th>
<th>1-5</th>
<th>6-10</th>
<th>11-15</th>
<th>16-20</th>
<th>21-25</th>
<th>1-25</th>
<th>ufo-5\textsuperscript{b}</th>
<th>Ler\textsuperscript{c}</th>
<th>Ify-5\textsuperscript{d}</th>
<th>1-25</th>
<th>1-25</th>
<th>1-25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whorl 1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepals</td>
<td>4.04</td>
<td>4.04</td>
<td>4.00</td>
<td>4.04</td>
<td>4.00</td>
<td>4.02</td>
<td>3.99</td>
<td>4.00</td>
<td>3.97</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sepals fused</td>
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<td>0.00</td>
<td>0.08</td>
<td>0.08</td>
<td>0.00</td>
<td>0.03</td>
<td>0.03</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Whorls 2, 3, and 4\textsuperscript{e}</td>
<td></td>
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<td></td>
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<tr>
<td>Petals</td>
<td>0.04</td>
<td>0.52</td>
<td>1.25</td>
<td>1.00</td>
<td>0.76</td>
<td>0.72</td>
<td>1.54</td>
<td>3.98</td>
<td>0.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petal-like (no Se)\textsuperscript{f}</td>
<td>0.33</td>
<td>0.64</td>
<td>0.75</td>
<td>1.00</td>
<td>1.00</td>
<td>0.75</td>
<td>2.01</td>
<td>0.01</td>
<td>0.73</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sepal and sepal-like\textsuperscript{g}</td>
<td>3.29</td>
<td>1.96</td>
<td>1.50</td>
<td>1.48</td>
<td>1.52</td>
<td>1.94</td>
<td>0.03</td>
<td>0.00</td>
<td>2.29</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Filament\textsuperscript{h}</td>
<td>2.38</td>
<td>2.16</td>
<td>1.00</td>
<td>1.16</td>
<td>2.28</td>
<td>1.80</td>
<td>0.16</td>
<td>0.00</td>
<td>0.07</td>
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<tr>
<td>Stamens</td>
<td>0.67</td>
<td>0.76</td>
<td>0.67</td>
<td>0.80</td>
<td>0.48</td>
<td>0.67</td>
<td>3.31</td>
<td>5.84</td>
<td>3.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stamen-like (no P or Se)\textsuperscript{i}</td>
<td>1.13</td>
<td>0.96</td>
<td>1.75</td>
<td>1.40</td>
<td>1.40</td>
<td>1.33</td>
<td>0.13</td>
<td>0.01</td>
<td>0.09</td>
<td></td>
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</tr>
<tr>
<td>Carpel and carpel-like\textsuperscript{j}</td>
<td>0.13</td>
<td>0.16</td>
<td>0.13</td>
<td>0.08</td>
<td>0.20</td>
<td>0.14</td>
<td>0.01</td>
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<td></td>
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</tr>
<tr>
<td>Fused: P or Se\textsuperscript{k}</td>
<td>0.08</td>
<td>0.24</td>
<td>0.33</td>
<td>0.16</td>
<td>0.16</td>
<td>0.20</td>
<td>0.21</td>
<td>0.00</td>
<td>0.04</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fused: all others\textsuperscript{l}</td>
<td>0.17</td>
<td>1.00</td>
<td>0.42</td>
<td>0.16</td>
<td>0.40</td>
<td>0.43</td>
<td>0.08</td>
<td>0.00</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gynoecium: no. of carpels</td>
<td>3.67</td>
<td>3.20</td>
<td>2.92</td>
<td>2.80</td>
<td>2.84</td>
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<td>2.49</td>
<td>2.00</td>
<td>3.24</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sum of all organs\textsuperscript{m}</td>
<td>15.93</td>
<td>15.64</td>
<td>14.72</td>
<td>14.08</td>
<td>15.04</td>
<td>15.08</td>
<td>13.96</td>
<td>15.84</td>
<td>13.84</td>
<td></td>
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</tr>
</tbody>
</table>

All plants were grown under the same conditions and they are a subset of those described in Table 1. The average number of organs per flower is given.

\textsuperscript{a} A total of 123 flowers from five plants was examined. For each subset of positions, 25 flowers from five plants were examined, except for positions 1 to 5 and 11 to 15 with 24 flowers.

\textsuperscript{b} ufo-6 is a weak allele. A total of 135 flowers from six plants was examined.

\textsuperscript{c} A total of 150 flowers from six plants was examined. Ler, Landsberg erecta.

\textsuperscript{d} A total of 91 flowers from five plants was examined.

\textsuperscript{e} Whorls 2, 3, and 4 are combined because it is not possible to definitively assign each organ to a whorl. Each organ is counted in only one category.

\textsuperscript{f} Mosaic organs include petal/stamen, petal/stamen/carpel, petal/carpel, petal/filament, and petal/stamen/filament. Se, sepal.

\textsuperscript{g} Sepals and mosaic organs include petal/sepal, sepal/petal/stamen, sepal/petal/stamen/carpel, sepal/filament, sepal/stamen, sepal/carpel, and sepal/petal/carpel.

\textsuperscript{h} Filaments are formed in both whorls 2 and 3.

\textsuperscript{i} Mosaic organs include stamen/filament, stamen/carpel, and stamen/filament/carpel. P, petal; Se, sepal.

\textsuperscript{j} Carpels and carpel/filament mosaic organs not fused to the gynoecium.

\textsuperscript{k} Fused organs with some petal (P) or sepal (Se) tissue in at least one of the organs.

\textsuperscript{l} All fused organs, except those with petal or sepal or those in the gynoecium.

\textsuperscript{m} The whorl 1 fused sepals have been counted in the whorl 1 sepals category. Therefore, the sum of all organs does not include the numbers for the sepal fused row.

recognizable defects in ufo flowers is the precocious increase in size of some developing second whorl organs (Figure 2H). Another defect observed in ufo mutants is that the second and third whorl floral organs were often mosaic of two or more organ types or fusions between two adjacent organs (Figures 2I to 2L, Table 2). We have observed such fusions between organs in the same whorl or those in adjacent whorls. The phenotypes of ufo mutants and weak ify mutants show many striking similarities, and the differences observed may, in some cases, provide clues to the function of the UFO gene. Weak ify mutations, such as ify-5, have been shown to have defects in the development of inflorescence and floral structures (Huala and Sussex, 1992; Weigel et al., 1992). Similar to ufo mutants, ify mutants have (1) an increased number of secondary inflorescences on the primary inflorescence, (2) leaves or filamentous structures subtending flowers, (3) filamentous structures forming in positions where flowers are missing, and (4) premature termination of flowering with carpelloid leaflike organs at the apex of the inflorescence. The first two defects are more severe in ify-5 mutants than in any of the ufo mutations (Table 1; Weigel et al., 1992), suggesting that the ify mutations have a greater disruption in the earliest stages of floral development. The floral organs formed in ify-5 flowers were generally similar to those seen in ufo flowers (Figure 1C and Table 2). Two differences between ufo and ify-5 flowers are noteworthy. First, ufo-2 mutants have an average of 1.80 filaments per flower, whereas ify-5 mutants have very few filaments (0.07 per flower). Second, ufo flowers have fused organs,
whereas *lfy*-5 flowers rarely have fused organs (Table 2), and those organs seen in *lfy*-5 are sepal/sepal fusions or stamen/stamen fusions.

**Gene Expression Patterns in *ufo* Mutant Inflorescences**

Because of the great similarity in phenotypes between *lfy* and *ufo* mutants, we were especially interested in the expression pattern of the LFY protein in *ufo* mutant inflorescences. In experiments with anti-LFY antiserum (see Methods), no differences were detected in the timing or extent of the pattern of LFY expression between the wild type and *ufo*-2 at all stages of floral development until about stage 6 (Smyth et al., 1990; Figures 3A to 3C and 3E to 3G). In later stages, LFY protein was detected in second whorl petal primordia in wild-type flowers but not in the second whorl organ primordia of *ufo*-2 flowers (Figures 3D and 3H). These data suggest either that *UFO* acts upstream of *LFY* in later stages of floral development or that the alteration in the timing of floral organ development in *ufo* flowers may indirectly result in a change in the timing of LFY expression in second whorl organs. This change could be a consequence of these organs developing earlier or differently or these cells expressing LFY in an organ-specific rather than a whorl-specific manner.

The homeotic transformations observed in the second and third whorls of *ufo* flowers suggested that there was a reduction in the expression of class B genes (see previous discussion). RNA and protein of the class B gene *AP3* have been shown to be expressed in the second and third whorls of developing flowers (Jack et al., 1992, 1994). Using anti-AP3 antiserum, we showed that the levels of AP3 protein were reduced in *ufo*-2 early floral primordia compared directly with wild-type floral primordia at the same stages (Figure 4; see Methods). To confirm these results, we also examined the expression pattern of *AP3* RNA using in situ hybridization. There was a dramatic decrease in the early (stages 3 to 5) expression of *AP3* RNA in *ufo*-2 flowers relative to wild-type flowers (Figures 5I and 5M). In flowers at stage 6 and later, the reduction in *AP3* expression became more subtle (Figures 5J, 5K, 5N, and 5O). Previously, temperature shift experiments with the *ap3*-1 temperature-sensitive allele have shown that after stage 6, the restoration of *AP3* activity was insufficient to rescue the *ap3* mutant phenotype in the third whorl organs (Bowman et al., 1989). This experiment suggests that the low level of *AP3* expression before stage 6 is likely to be responsible for the homeotic conversions seen in *ufo*-2 flowers. In addition, a low residual level of *AP3* expression is to be expected because some class B gene function clearly remains in most *ufo*-2 flowers, which produce organs with petaloid and staminoid tissue (Table 2).

We also examined the RNA expression pattern of the other class B gene, *PI*. The wild-type expression pattern of *PI* is slightly different from *AP3* in that *PI* is transiently expressed in the fourth whorl at stage 3 (Goto and Meyerowitz, 1994). Similar to the effects on *AP3* expression, a great reduction in...
Figure 4. Expression of the AP3 Protein in Wild-Type and ufo-2 Inflorescences.

(A) and (B) Wild-type inflorescences.
(C) and (D) ufo-2 inflorescences.
Direct comparisons between wild-type and ufo-2 flowers can be made for (A) and (C) and (B) and (D). Numbers refer to stages of floral development.

PI expression occurred at the early stages of floral development in ufo-2 flowers; this difference became less dramatic in older flowers (Figures 5A to 5H).

In addition to examining the expression pattern of AP3 and PI in developing ufo-2 flowers, we looked in the filamentous structures that occasionally appear in place of flowers (see previous discussion). There was no detectable expression of AP3 and PI in these structures (Figures 5L and 5P). These observations suggest that these structures are not developing as flowers and instead may be leaves or flowers aborted before stage 3 when AP3 and PI expression begins or without the second and third whorls.

In situ hybridization experiments to examine the expression patterns of AG and API RNA in ufo-2 flowers indicated no obvious differences from wild-type flowers at the earlier stages of floral development (until about stage 6) (Figures 6A to 6F). API RNA is expressed in the entire floral primordium starting at stage 1, but disappears from the third and fourth whorls starting at stage 3 when AG RNA begins to be expressed there (Drews et al., 1991; Mandel et al., 1992). In the second and third whorl of older flowers (after approximately stage 6), the floral organ primordia in ufo-2 appeared different from those in the wild type, so the expression patterns did not appear identical. Nevertheless, the overall levels and regions of expression usually appeared comparable (Figures 6G and 6I). In the flower shown in Figures 6H and 6I, it appears that API RNA was expressed in a petal/stamen mosaic organ in the third whorl. The appearance of petal/stamen mosaic organs in ufo flowers (Table 2) suggests that there was some perturbation in the expression of one or both of these genes at the boundary between the second and third whorl. It is unclear what alterations took place because it was not possible to determine with certainty whether a given floral organ had grown in the wrong position and ended up straddling the border between the second and third whorl or if that organ was in a normal position and the expression patterns of AG and API had been perturbed.

Interactions with Meristem Identity Genes

Because the ufo mutant phenotype resembles a weak Ify mutant phenotype, we analyzed the interaction of the two genes. Ify-6, a strong Ify allele, has floral meristem identity defects stronger than but similar to those of Ify-5 (described previously) (Weigel et al., 1992). Inflorescences and flowers produced by ufo-2 Ify-6 and Ify-6 plants were indistinguishable (data not shown). There are two possible explanations for this result. One is that LFY is epistatic to (and genetically upstream of) UFO, such that, in the absence of LFY function, the presence of UFO function has no effect. Alternatively, LFY and UFO could be exhibiting an additive interaction, with the more severe Ify phenotype masking the ufo phenotype. In this case, they might have overlapping functions and act at the same step.
Figure 5. Expression of PI and AP3 RNA in Wild-Type and ufo-2 Inflorescences.

Shown are dark-field/bright-field double exposures with a red filter for the dark-field exposure, which causes the silver grains (representing RNA expression) to appear red.

(A) to (H) and (L) PI RNA expression.
(I) to (K) and (M) to (P) AP3 RNA expression.

(A) to (D) and (I) to (K) are wild type and all others are ufo-2. Direct comparisons between wild-type and ufo-2 flowers can be made for (A) and (E), (B) and (F), (C) and (G), (D) and (H), (I) and (M), (J) and (N), and (K) and (O). Filamentous structures from ufo-2 inflorescences are shown in (L) and (P). (E) shows an oblique section, but all the serial sections of this floral primordium show a consistent reduction in PI expression (data not shown). Numbers refer to stages of floral development.
To characterize further the interaction of the two genes, we constructed the ufo-2 lfy-5 double mutant. Double mutant plants were identified based on the presence of bracts subtending most flowers and the absence of petal or stamen tissue in the floral organs—a phenotype that appears identical to that of a strong Ify mutant (Figure 1D). This synergistic interaction between these two mutations provides additional evidence for the close relationship of LFY and UFO. Finally, we tested the interaction of ufo-2 with a new and very weak Ify allele, Ify-22 (T. Jack and D. Weigel, personal communication) (Figure 1E). The Ify-22 mutant phenotype includes the following five defects: the most apical secondary inflorescence on the primary inflorescence is not subtended by a cauline leaf; the first whorl floral organs have a leaflike shape and stellate trichomes, which are normally found on leaves and not sepals; an occasional secondary flower arises in the axil of a first whorl organ; there is a small but consistent increase in the number of secondary inflorescences (D. Weigel, personal communication); and there is a small reduction in the number of petals and stamens in more apical flowers. The first three of these defects have been observed for ap1 mutants (Irish and Sussex, 1990; Bowman et al., 1993). The phenotype of ufo-2 Ify-22 plants is similar to a strong Ify mutant phenotype, with an increase in the number of secondary inflorescences arising on the primary

Figure 6. Expression of AG and AP1 RNA in Wild-Type and ufo-2 Inflorescences.

(A) to (D) AG RNA expression.

(E) to (G) and (I) AP1 RNA expression.

(H) A bright-field view of (I). A third whorl organ (arrowheads in [H] and [I]) shows AP1 expression in (I). By examining serial sections of this floral primordium, we identify this organ as being in the third whorl because it is between the gynoecium and a second whorl organ (marked with an arrow). Based on its shape, this organ is likely to be a petal/stamen mosaic rather than a petal or sepal, the only organs that normally express AP1.

(A), (C), (E), and (G) are wild type and all others are ufo-2. Direct comparisons between wild-type and ufo-2 flowers can be made for (A) and (B), (C) and (D), (E) and (F), and (G) and (I). Numbers refer to stages of floral development.
inflorescence and flowers containing leaflike sepal organs in a nonwhorled pattern surrounding a gynoecium in the center (Figure 1F). This phenotype is slightly weaker than that of *fly-8*, based on the presence of some petal and stamen tissue in flowers from the double mutant. Thus, these two mutations display a strong synergistic interaction that is especially clear in the basal flowers on an inflorescence (Bowman et al., 1993) (Figures 7N and 7O). We constructed a double mutant with a strong *ap7* allele and a strong *ufo* allele. *ap7-1 ufo-2* flowers consisted of leaflike organs arranged in a spiral-like arrangement with an improperly fused gynoecium in the center (Figures 7P to 7R). UFO plays a role in maintaining the whorled pattern of the floral organs, based on the disruption of this pattern in *ap7-1 ufo-2*. Stamens were rarely present adjacent to the gynoecium (Figure 7Q). Although only the first flower on a *ufo-2* inflorescence is usually subtended by a leaf or filamentous structure, many of the other flowers on an *ap7-1 ufo-2* inflorescence were subtended by a filamentous structure. The production of secondary flowers in the axil of the first whorl organs was reduced from an average of 9.6 in the first five flowers (seven plants scored) in *ap7-1* plants to an average of 1.4 in the first five flowers (five plants scored) in *ap7-1 ufo-2* plants. In summary, the *ap7-1 ufo-2* mutant phenotype can be interpreted as a partial conversion of floral primordia to inflorescences, indicating that the two genes share a partially redundant function in floral meristem identity. Thus, *LFY* and *UFO* interact with *AP1* in a similar fashion. Finally, we constructed a double mutant with *ufo-2* and the intermediate strength allele *ap1-4* (Bowman et al., 1993) and observed a similar phenotype to that of *ap1-1 ufo-2*, except for an increase in the number of stamens in more apical flowers in *ap1-4 ufo-2* compared with *ap1-1 ufo-2* (data not shown). A synergistic interaction between *ap1-4* and *fly-5* has also been observed (Bowman et al., 1993).

The phenotypes of *ap1* mutants are greatly enhanced by mutations in *cassiflower (cal)*. Although a *cal* mutation has no visible phenotype alone, an *ap1 cal* double mutant exhibits a proliferation of multiple, undifferentiated inflorescence meristems to generate a structure resembling the head of a cassiflower (Bowman et al., 1993) (Figure 7U). It appears that the phenotype of the *ufo-2 cal-1* double mutant is identical to that of the *ufo-2* single mutant (see Methods); a similar result was observed for the *cal-1 fly-6* double mutant (Bowman et al., 1993). To analyze further the interaction of *CAL* with *UFO*, we constructed the *ap1-1 ufo-2 cal-1* triple mutant. Triple mutant inflorescences produced "flowers" with several leaves growing in a spiral-like arrangement around a gynoecium (Figures 7S and 7T). Often, secondary "flowers" grew from the axils of these leaves, and there could be increased internode elongation between leaves so that the "flowers" actually appeared much like an inflorescence shoot (Figures 7S and 7T). Because *ufo-2* single mutant inflorescences often terminated in carpels (Figures 2F and 2G), these flowers may actually correspond to inflorescences. In addition, some of the flowers were subtended by a leaf with stigmatic tissue along one margin (data not shown). In comparison with the *ap1-1 cal-1* double mutant, the triple mutant had a reduction in the reiteration of the formation of undifferentiated meristems and a decrease in the floral character of these structures; similar effects were seen in comparing *ap1-1* with *ap1-1 ufo-2* (see previous discussion). The major effect of the *cal-1* mutation in the triple mutant observed by comparing *ap1-1 ufo-2* plants with *ap1-1 ufo-2 cal-1* plants is the presence of secondary "flowers" in the axils of leaves of the latter but not the former.

**Interactions with Cadastral and Floral Organ Identity Genes**

SUPERMAN (*SUP*) is a cadastral gene that seems to be required to set the border between the third and fourth whorl. *sup* mutants produce extra stamens in the third whorl and less carpelloid tissue in the fourth whorl (Figure 7A) (Schultz et al., 1991; Bowman et al., 1992). The floral organs made by *ufo-2 sup-7* showed some variability from flower to flower (Figures 7B and 7C), as do those of *ufo-2* flowers, but the following generalizations can be made. In the fourth whorl, the amount of carpelloid tissue present was less than that in *ufo-2* flowers but more than that in *sup-1* flowers. In the third whorl, the number of stamens present was more than in *ufo-2* flowers but less than in *sup-1* flowers. Often, there were stamen/carpel mosaic organs fused to the carpelloid tissue in the center of the flower. For these two whorls, the double mutant phenotype was intermediate between the two single mutant phenotypes, suggesting that *UFO* and *SUP* act antagonistically. From these results, it remains unclear whether any direct interaction occurs between these two genes. There appeared to be a reduction in the number of organs in the second whorl of *ufo-2 sup-1* flowers (Figures 7B and 7C and data not shown). The severity of this defect increased in the more apical flowers, but quantitation of second whorl organs was complicated by a problem shared with *ufo-2* flowers, that is, it is difficult to be certain if a given organ belongs to the second or the third whorl. This effect on the second whorl was unexpected because *sup* mutants exhibited no defects in the second whorl.

We constructed the *ufo-2 pi-1* double mutant and observed a phenotype consistent with an additive interaction between these mutations. In *pi-1* mutants, the second whorl petals were converted to sepals and the third whorl stamens were converted to carpels or filaments (Bowman et al., 1989). Similar homeotic conversions were seen in *ufo* flowers (Table 2) due to a reduction in PI RNA expression (Figures 5A to 5H). *ufo-2 pi-1* flowers appeared to be like *ufo-2* flowers except for the...
Figure 7. Phenotypes of ufo-2 Double and Triple Mutants Compared with Those of the Relevant Single and Double Mutants.
absence of petaloid tissue in the second whorl and of staminoid tissue in the third whorl; that is, sepals and filaments were present in the second whorl, and filaments and carpels fused to the gynoecium were present in the third whorl (data not shown).

We constructed a ufo-2 ap2-2 double mutant. ap2-2 is a strong AP2 allele with the following phenotype: medial first whorl sepals are converted to staminoid carpels or carpels; lateral first whorl sepals are converted to leaves or carpelloid leaves or are absent; second whorl petals are absent; third whorl stamens are mostly absent; and fourth whorl organs are carpels that sometimes fail to fuse properly (Bowman et al., 1991) (Figures 7J and 7K). In ufo-2 ap2-2, the medial first whorl organs were leaflike carpelloid organs that fused with the gynoecium (Figures 7L and 7M). The appearance of stellate trichomes on these organs indicates their leaflike character. In ufo-2 ap2-2, the lateral first whorl organs were leaves, filamentous structures, carpelloid leaves, or carpels (rarely), or the lateral first whorl organs were absent (Figures 7L and 7M). Second and third whorl organs were usually absent, although there was an occasional internal carpel, presumably from the third whorl, growing inside the first whorl carpels of the gynoecium. Overall, this double mutant phenotype is additive for floral organ identity, with the exception of the stellate trichomes on the medial first whorl organs.

Although ufo-2 and ap2-2 exhibit an essentially additive interaction, the interaction of ufo-2 with ap2-1, which is a weak and unusual allele, is quite different. The interaction of ap2-1 with Ify and ap1 mutations has revealed a role for AP2 in floral meristem identity (Irish and Sussex, 1990; Huala and Sussex, 1992; Bowman et al., 1993; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993). In ap2-1 flowers, the first whorl organs are leaves, the second whorl organs are mostly stamen/petal mosaic organs, and the third and fourth whorl organs are normal (Bowman et al., 1989) (Figure 1G). ufo-2 ap2-1 flowers contained the following, from the outside to the center: leaves, carpelloid leaves, carpelloid stamens, carpels, and a gynoecium, which often failed to fuse properly (Figure 1H). Because the arrangement of these floral organs did not seem to correspond exactly to a wild-type whorled pattern, the assignment of organs to specific whorls was not possible in these flowers. Although there was some enhancement of the ufo-2 floral meristem identity defect in ufo-2 ap2-1 flowers, the phenotype was not quite as severe as that seen in ufo-2 ap1-1 flowers, which had floral organs arising in a spiral-like pattern. In comparison with ufo-2 ap2-1 plants, ap2-1 Ify-5 plants exhibit a similar phenotype, except that the whorled pattern for floral organs is converted to a spiral-like pattern and there is an increase in the number of secondary flowers formed in the

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Figure 7. (continued).

All first whorl sepals have been removed in (A) to (E), (G), (H), and (V) to (X), except that one remains in (A).

(A) sup-1 stage 12 flower.
(B) ufo-2 sup-1 stage ~10 flower.
(C) ufo-2 sup-1 stage 10 flower.
(D) ag-1 floral meristem.
(E) ag-3 second and third whorl organs.
(F) ag-1 mature flower.
(G) ufo-2 ag-3 fasciated floral meristem (outlined with black line).
(H) ufo-2 ag-3 second and third whorl organs. Arrows show filaments (F).
(I) ufo-2 ag-3 fasciated flower.
(J) ap2-2 stage 8 flower. One first whorl organ removed.
(K) ap2-2 mature flower. First whorl lateral leaflike organs are indicated by arrowheads.
(L) ufo-2 ap2-2 stage 8/9 flower.
(M) ufo-2 ap2-2 mature flower. Stellate trichomes are visible on lateral leaf/carpel organs (arrowheads) in the first whorl and on the gynoecium.
(N) ap1-1 stage 6 flower.
(O) ap1-1 mature flower.
(P) ap1-1 ufo-2 stage 6 flower. Organs arising in a spiral-like pattern are numbered from the youngest in the center outward. Two outer organs have been removed.
(Q) ap1-1 ufo-2 stage 11/12 flower. One of the two carpel/sepal organs (asterisks) is partially fused to the gynoecium. Seven outer organs have been removed.
(R) ap1-1 ufo-2 mature flower. A carpelloid organ is partially fused to the gynoecium and is topped with stigmatic tissue (arrowhead). Other floral organs are leaf/sepal organs arranged in a non-whorled pattern.
(S) ap1-1 ufo-2 cal-1 mature “flower” with secondary flowers.
(T) ap1-1 ufo-2 cal-1: higher magnification of a secondary flower from (S). Note the developing gynoecium (G) in the center.
(U) ap1-1 cal-1 “flower.”
(V) ufo-2 sup-1 ag-3 second and third whorl organs.
(W) Fasciated floral meristem (FM) in ufo-2 sup-1 ag-3 flower. One interior organ appears to face outward rather than inward (arrowhead).
(X) sup-1 ag-3 flower. Floral meristem (FM) is not fasciated.
B, bract; C, carpel, F, filament; G, gynoecium; M, floral meristem; P, petal; St, stamen. In (B) to (D), (G), (H), (J), (L), (N), (P), (T), (V), (W), and (X), bars = 10 μm; in (A), (E), (F), (I), (K), (M), (O), (R), (S), and (U), bars = 100 μm.
axils of the leaflike organs in the flowers (our observations; Huala and Sussex, 1992; Schultz and Haughn, 1993). These interactions are another indication that Ify-5 has a more severe floral meristem identity defect than ufo-2.

The UFO and AG genes interact differently in their control of floral organ identity and floral meristem determinacy. In strong ag mutants, such as ag-1 and ag-3, the third whorl stamens are converted to petals (floral organ identity defect) and the fourth whorl carpels are converted to an internal flower (determinacy defect) to produce flowers with a repeating pattern of sepals, petals, petals (Bowman et al., 1989, 1991; Figures 7D to 7F). In ufo-2 ag-3 flowers, the third whorl organs were similar to the organs formed in the second whorl of ufo-2 flowers, that is, sepals, petals, petals/sepal mosaic organs and filaments (Figures 7H and 7I); thus, there is an additive interaction with respect to floral organ identity. ufo-2 ag-3 double mutants exhibited fascination such that the floral meristem, which is usually a small circular disk of growing tissue, was enlarged to form a broader ribbon or band that generated many more medial organs than lateral organs (Figures 7G and 7I). Because ufo single mutants displayed no obvious defect in floral meristem determinacy, we concluded that these two mutations interact synergistically and that there may be an overlap in the functions of UFO and AG in their control of determinacy.

To understand better the role of UFO in floral determinacy, we constructed the ufo-2 sup-1 ag-3 triple mutant. This experiment is relevant because sup ag plants also exhibit fasciated floral meristems (Bowman et al., 1992), although the severity of the determinacy defects for sup-1 ag-3 appeared weaker than those for ufo-2 ag-3 (Figures 7X and 7I, respectively). In the triple mutant, the fascination was more extreme than in either of the double mutants (Figures 7I, 7W, and 7X). The first whorl organs were sepals, and the subsequent whorls were mostly petals and to a lesser extent filaments, sepals, and petal/sepal mosaic organs (Figures 7V and 7W).

Genetic Interactions Causing an Enhancement in the Formation of Filamentous Structures

The genetic interactions of UFO revealed the existence of a new and diverse class of genes in Arabidopsis. Mutations in these genes, either singly or in combination with a second member of this class, prevented the formation of a flower and caused a filamentous structure to grow in its place. Not every double mutant combination of genes in this large class resulted in this phenotype.

The if54 mutant phenotype is a complex and variable one that includes defects in the size, shape, and position of the floral organs (Komaki et al., 1988) (Figure 1). Most notably, if54 inflorescences often produce filamentous structures or sepal-like structures in place of flowers. Similar to UFO, FIL54 plays a role in floral meristem identity based, in part, on the observation that the first flower is often subtended by a bract or filamentous structure. In the ufo-2 if54 double mutant, the inflorescence formed a few basal flowers and then formed filamentous structures instead of flowers (Figures 1J and 8D). The surface of the epidermal cells at the distal tip of the filamentous structures was irregularly shaped with indentations (Figure 8B). The epidermal cells in the rest of the filamentous structures had a relatively uniform surface and were rectangularly shaped (Figure 8C); they appeared similar to cells in elongated pedicels or the midvein on the abaxial surface of a leaf (Bowman, 1993). Although the significance of this similarity is unclear, the presence of stellate trichomes on some of these filamentous structures indicates they may be related to leaves (data not shown). Similar to the inflorescences of both single mutants, the inflorescence terminated with several carpel-like organs that partially fused at the apex (Figure 8A).

Secondary inflorescences generally produce few, if any, flowers before commencing to make filamentous structures. In the few flowers that formed in ufo-2 if54 plants, the floral organs appeared much like those in if54 single mutants, with a decrease in the number of petals and stamens (Figure 8E). Thus, for floral organ identity, an additive interaction occurs between these two mutations; however, for floral meristem identity (flower versus filamentous structures), there is a synergistic interaction. These interactions are not allele specific because we have found similar results with a different ufo allele and with a different if54 allele (see Methods).

Similar to ufo-2 If54 inflorescences, Ify-6 If54 inflorescences produced mostly filamentous structures and terminated in a mass of carpels (our observations; D. Weigel and T. Jack, personal communication), but some of the more apical filamentous structures appeared leaflike because they were wider and flatter (Figures 8F and 8G). In addition, they sometimes had a cylindrical opening at their distal end (Figure 8H), and their surface morphology appeared similar to that of a style; thus, these characteristics indicate that these organs have some carpel-like properties. ufo Ify double mutants did not show any enhancement in the formation of the filamentous structures, providing additional evidence that UFO and LFY act in the same processes (see previous discussion).

Filamentous structures were also formed by plants with mutations in both the UFO and CLAVATA3 (CLV3) genes. clv3-1 plants have enlarged apical and floral meristems, and additional floral organs are formed, particularly in the third and fourth whorl (Clark et al., 1995). In addition, the apical meristem often fasciates, and the floral meristem can form additional whorls of carpels within the developing gynoecium in the fourth whorl. Inflorescences from ufo-2 clv3-1 plants formed a variable number of flowers (zero to more than 20) before filamentous structures appeared in place of flowers (Figures 8I and 8J). The ufo-2 clv3-1 filamentous structures appeared to be the same as those of other genotypes. Also, as in other genotypes, the number of flowers formed was greater on the primary inflorescence than on nonprimary inflorescences. There appeared to be no increase in the frequency of fascination of the inflorescence meristem in ufo-2 clv3-1 compared with clv3-1. Often the filamentous structures were flanked by two squamules that appeared roughly similar to stipules on
Figure 8. Phenotypes of Plants with Filamentous Structures.

(A) to (C) show ufo-2 fl54 plants. (D) and (E) show ufo-2 fl54 plants with a different fl54 allele (see Methods). (F) to (H) show lfy-6 fl54 plants. (I) to (L) show ufo-2 clv3-1 plants.

(A) Inflorescence with filamentous structures and terminal carpelloid organs.
(B) Distal tip of a filamentous structure.
(C) Basal portion of a filamentous structure.
(D) Developing filamentous structures and inflorescence meristem.
(E) Mature flower.
(F) Inflorescence with filamentous structures and terminal carpelloid organs.
(G) Apical leaflike organ (L) and carpel-like organs (C).
(H) Organ with a filamentous basal portion and an open cylindrical tip with tissue similar to that of a style around the opening.
(I) Fasciated secondary inflorescence meristem with filamentous structures.
(J) Higher magnification view of (K). IM, inflorescence meristem.
(K) Stage ~12 flower. Note the second whorl sepal (Se) and sepal tissue (arrowheads) on the gynoecium (G). Two first whorl sepals have been removed.
(L) Stage 11/12 flower. Note the filament/sepal mosaic organ (arrow) and filament (arrowhead). Four first whorl sepals have been removed.

In (B) to (D), bars = 10 μm; in (A) and (E) to (L), bars = 100 μm.
leaves (data not shown). The first and fourth whorls of ufo-2 clv3-1 flowers were very similar to those of clv3-1 flowers, but the second and third whorls contained only a few organs (Figures 8K and 8L). These included sepals, sepal/filament mosaic organs, filaments, and sepal/carpel mosaic organs that often partially fused to the gynoecium. This unexpected phenotype suggests that there is a nearly complete elimination of class B gene activity, in addition to a reduction in the number of organs formed. It appears that CLAVATA1 (CLV1) is also a member of this diverse class of genes, based on the observation of a similar phenotype of filamentous structures for the clv1-4 ify-6 double mutant (Clark et al., 1993a). The weaker clv1-1 allele does not exhibit this dramatic enhancement of the filamentous structure defect, in combination with the ify-6 allele.

In addition to UFO, LFY, FL54, CLV1, and CLV3, the LEUNIG (LUG) and HANABA TARANU (HAN) genes are also members of this class of genes. LUG functions as a cadstral gene to repress AG in the first and second whorls; in addition, lug mutants produce narrow leaves and floral organs (Liu and Meyerowitz, 1995). The ufo-2 lug-2 double mutant phenotype includes the formation of filamentous structures similar to those seen in the previous double mutants (Z. Liu, personal communication). In lug ify double mutants, several leaves are formed in place of flowers, and the inflorescence terminates prematurely (Liu and Meyerowitz, 1995). These data suggest that the filamentous structures in ufo-2 lug-2 plants may be aborted leaves. The han mutant phenotype includes a reduction in the number of floral organs in all four whorls, particularly in the second and third whorls (H. Sakai, personal communication). The ufo-2 han-1 double mutant produces initially a limited number of flowers, followed by numerous filamentous structures similar to those described earlier (data not shown).

To assess the role of AP1 in the formation of filamentous structures in such double mutants, we constructed the ap1-1 ufo-2 fl54 triple mutant. Similar to the ufo-2 fl54 double mutant, the triple mutant produced filamentous structures in place of flowers and terminated with several fused carpelloid structures (data not shown). From this result, we concluded that AP1 is not involved in the production of filamentous structures and that the structures may be more closely related to leaves than to flowers. The few basal flowers formed in the triple mutant have one or two leaves, a few filaments, and a central gynoecium (data not shown).

**DISCUSSION**

The complexity of the ufo mutant phenotype suggests that UFO plays a role in several processes during floral development. The earliest defects in ufo mutants relate to the function of UFO in floral meristem identity (see Figure 9). The observation of extra secondary inflorescences on the primary inflorescence and a bract or a filamentous structure subtending the most basal flower in ufo mutants implies that there is a partial conversion of flowers to shoots. In addition, UFO and AP1 show a functional redundancy in their control of floral meristem identity. Taken together, these defects imply that UFO may act early in floral development when the floral meristem is being formed, that is, prior to or at stage one (Smyth et al., 1990).

In Arabidopsis, floral meristems differ from shoot apical meristems in three characteristics that are all influenced by UFO function (Figure 9). First, the organs of a floral meristem arise in a whorled pattern, whereas shoots and flowers arise in a spiral pattern from a shoot apical meristem. Although no defect was observed in this process in ufo single mutants, the floral organs in ufo double mutants arose in a spiral-like pattern (Figures 7P to 7R). Thus, UFO seems partially redundant with AP1 for this function. Second, a floral meristem is determinate, whereas a shoot apical meristem is indeterminate. Although AG expression seems to be the primary factor controlling floral determinacy, the fasciated flowers observed in ufo ag double mutants suggest that UFO also controls the proliferation of the cells in the floral meristem. Floral fasciation has also been observed in clv1 ag and sup ag double mutants (Bowman et al., 1992; Clark et al., 1993a). The increased fasciation in the ufo-2 sup-1 ag-3 triple mutant compared with that in both the ufo-2 ag-3 and sup-1 ag-3 double mutants suggests that these genes may control cell proliferation in the developing flower through different pathways. Third, sepals, petals, stamens, and carpels are produced by the floral meristem, whereas leaves are generated by the shoot apical meristem. In the absence of function of the

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**Figure 9. UFO Roles in Floral Development.**

The floral meristem identity genes control the decision of cells in primordia flanking the inflorescence meristem to develop as flowers rather than shoots. A flower differs from a shoot in the three ways shown: organs arise in a whorled rather than a spiral pattern, growth is determinate, and floral organs are produced rather than leaves. TERMINAL FLOWER (TFL) acts to prevent the premature termination of the production of floral primordia by the inflorescence meristem (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). Other genes are described in the text. The asterisk indicates that UFO seems to act to define the boundaries between floral organs in adjacent whorls and between floral organs in the same whorl. UFO is also a positive regulator of the class B genes.
homeotic genes, floral organ identity is almost completely lost (Bowman et al., 1991). In ufo mutants, there was a reduction in class B gene function, as indicated by the decrease in AP3 protein expression (Figure 4) and in AP3 and PI RNA expression (Figure 5) and the homeotic transformations of petals to sepals and stamens to carpels (Table 2). Thus, UFO acts as a positive regulator of class B genes. No dramatic alteration in class A or C gene function was consistently observed in ufo mutants (Figure 6).

Unusual Floral Organs

The ufo floral organs show an "unusualness" not seen in those of any other Arabidopsis mutant previously described, except perhaps for f154 (Komaki et al., 1988). First, there is a high degree of phenotypic variability for a given ufo mutant such that almost every flower appears different. This variability could be explained if every ufo mutation has some residual UFO activity stochastically expressed in the developing flower. This possibility seems unlikely because this variation is observed in all nine ufo alleles examined. Phenotypic variability has been observed for other genes in other systems, for example, the Arabidopsis EMB30 (GNOM) gene (Meyer et al., 1993; Shevell et al., 1994) and the Caenorhabditis elegans lin-31 gene (Ferguson and Horvitz, 1985; Miller et al., 1993). We suggest two alternative explanations for the phenotypic variability in the abnormal floral organ development in ufo mutants. UFO could have some fundamental role in floral cell growth, but ufo mutants might not cause the same terminal phenotype for every organ in a single whorl of the flower because that process is also controlled by a gene whose function is redundant with UFO. Loss of UFO function may cause a deregulation of development, leading to the production of variable patterns of organs in each flower.

A second unusual feature of ufo mutants is that their flowers contain filaments, mosaic organs, and fused organs (Table 2). One explanation for the development of such organs is that UFO plays a role in setting the boundaries for the growth of cells in the flower and a reduction in UFO function leads to defects in the number of cells assigned to form a particular floral organ or to a loss of control in the proliferation of cells in the floral organs. We speculate that filaments might be reduced or aborted organs that have an insufficient number of cells or grow too little; similarly, fused organs might be the result of too many initial cells or too much cell division at the organ borders. Finally, it is unclear whether there is an alteration in the positions of the second and third whorl floral organs or in the underlying pattern of gene expression for class A and C genes (see Results).

Comparison with LFY

UFO shows great similarity to LFY in its mutant phenotype, its activation of class B gene expression, and its genetic interactions with other genes necessary for floral development. These observations suggest that LFY and UFO may act together and are likely to play roles in the same processes. By comparing these properties for the ufo-2 and lfy-5 mutations that have similar phenotypes, we conclude that there are also subtle differences between them. Even in lfy-5, which is a weak lfy allele, there is a much stronger defect in floral meristem identity, as seen in the production of more secondary inflorescences (Table 1) and a more complete transformation of flowers to shoots in ap3 double mutants and in ap2-1 double mutants. The expression of class B genes may be more severely affected in ufo-2 than in lfy-5, as seen by our observation of fewer stamens in ufo-2 (Table 2) and apparently less expression of AP3 and PI in ufo-2 flowers (Figure 5) than in lfy-5 flowers (Weigel and Meyerowitz, 1993). It appears that UFO and LFY differ in their control of processes in flower development, following floral meristem identity, because more filaments and fused organs are formed in ufo-2 flowers (Table 2; see previous discussion). LFY may have a role as a global positive regulator of floral development, and UFO may be important for more specific events in this process, such as maintaining boundaries between floral organs. Because the LFY expression pattern does not appear to be altered significantly in ufo-2 mutants, we conclude that UFO does not act upstream of LFY. One possibility is that UFO may be a cofactor for LFY and also act downstream of LFY. Some aspects of the lfy mutant phenotype may actually be a consequence of a reduction in UFO function.

Comparison with Antirrhinum FIM Gene

Although Arabidopsis and Antirrhinum are not closely related plants and produce flowers that appear quite different, they use homologous genetic mechanisms for floral development (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994). The Arabidopsis UFO gene is similar to the Antirrhinum FIM-BRIATA (FIM) gene not only in some aspects of its mutant phenotype (Simon et al., 1994) but also in its amino acid sequence (G. Ingram, J. Goodrich, M. Wilkinson, R. Simon, G. Haughn, and E. Coen, personal communication). Both genes seem to be positive activators of class B gene activity, because ufo and fim mutants exhibit a decrease in class B gene transcription that affects the identity of the second and third whorl organs. Although fim flowers exhibit a decrease in class C gene expression and a corresponding decrease in floral determinacy, ufo flowers do not show a dramatic decrease in class C gene expression (Figure 6) or in floral determinacy.

Filamentous Structures

One of the most intriguing aspects of the ufo mutant phenotype is the formation of filamentous structures in place of flowers. It seems likely that these structures are aborted leaves, although it is difficult to rule out that they may be aborted flowers. The first flower on ufo inflorescences is often subtended by a bract or a filamentous structure (Table 1), which is most
probably a reduced leaf. Because this structure appears indistinguishable from the more apical filamentous structures observed in the absence of a flower at a given position, the latter might also be aborted leaves. The LFY and API gene products are not required to form filamentous structures, because these structures were observed in both a fly single mutant and in an apt1-1 ufo-2 fl54 triple mutant. In addition, neither AP3 nor PI was expressed in filamentous structures in ufo-2 plants (Figure 5L and 5P). Thus, we have no evidence indicating these structures have any floral character.

Double mutants with inflorescences that produce predominantly filamentous structures provide another perspective on the origin of these structures. This phenomenon has also been described for several other double mutant combinations of genes within this class (Clark et al., 1993b; Clark and Meyerowitz, 1994). What do the genes responsible for this dramatic phenotype have in common? The mutant phenotypes of UFO, Lfy, Fl54, Han, Lugh, CLV1, and CLV3 appear quite different. Perhaps the unifying characteristic they share is simply that they contribute positional information to the developing cells in the floral primordia. Thus, cells in the earliest stages of floral development in the double mutants may not receive sufficient information from these genes and, as a consequence, fail to form a flower. The filamentous structure may be a default structure similar to an aborted leaf.

Is there any precedent for the natural formation of filamentous structures in other plants? In aquatic plants such as Myriophyllum humile, Potamogeton pectinatus, and Ranunculus longirostris, the submerged leaves are often reduced to threadlike structures (Fassett, 1960). In addition, spines produced in Crataegus and Euphorbia are modified stems (Raven et al., 1992). We are unaware of any case in a normal plant with an Apl- phenotype (probably genotype ap7-7 ufo-2 cal-1), we observed plants with an Apl-, Apl- Cal-, and Apl- Ufo- mutant phenotypes in the F3 generation. Based on this observation, the recovery of a greater number of apt1 cal-1 mutants than apt1 ufo mutants in the F2 progeny, and a weak linkage observed in other experiments between ufo and apt1 mutations (data not shown), we suspected that UFO was located between API and CAL (Koornneef et al., 1983; Bowman et al., 1993). Thus, two recombination events were necessary to generate the triple mutant, and additional generations had to be examined. From a single F2 plant with an Ap1+ phenotype (probable genotype apt1-1 ufo-2 +/apt1-1 + cal-1), we observed plants with the Ap1+, Ap1- Cal+, and Ap1- Ufo- mutant phenotypes in the F3 generation. From a single F3 plant with an Ap1- Ufo- phenotype (probable genotype apt1-1 ufo-2 +/apt1-1 + cal-1) inadvertently cross-pollinated by another F3 plant (proband genotype ap7-7 cal-4 and collected in the background may have made these plants sicker and decreased the number of secondary inflorescences formed.

Strain Constructions

Double mutants were constructed by crossing a plant homozygous for a ufo mutation with a plant homozygous for the other mutation, except heterozygotes were used for crosses with pl-1 and ag-3. All double mutant phenotypes were confirmed in the F2 generation by observing the segregation of the double mutant from an F2 parent homozygous for only one of the mutations. For several double mutant combinations, we repeated the experiment with a second ufo allele as well as with a ufo-2 for comparison; no significant differences were observed for any of the strong ufo alleles. All ufo fl54 double mutant combinations showed the same filamentous structure defect, including ufo-2 and fl54, ufo-6 and fl54, and ufo-2 and a new allele of Fl54 with a nearly identical phenotype to the original allele (T. Jack, personal communication).

To construct the apt1-1 ufo-2 cal-1 triple mutant, we crossed ufo-2 with apt1-1 cal-1 and collected F2 and F2 seed but failed to detect the triple mutant in the F2 progeny. Based on this observation, the recovery of a greater number of apt1 cal-1 mutants than apt1 ufo mutants in the F3 progeny, and a weak linkage observed in other experiments between apt1 and ufo mutations (data not shown), we suspected that UFO was located between API and CAL (Koornneef et al., 1983; Bowman et al., 1993). Thus, two recombination events were necessary to generate the triple mutant, and additional generations had to be examined. From a single F2 plant with an Ap1+ phenotype (probable genotype apt1-1 ufo-2 +/apt1-1 + cal-1), we observed plants with the Ap1+, Ap1- Cal+, and Ap1- Ufo- mutant phenotypes in the F3 generation. From a single F3 plant with an Ap1- Ufo- phenotype (probable genotype apt1-1 ufo-2 +/apt1-1 + cal-1) inadvertently cross-pollinated by another F3 plant (probable genotype apt1-1 ufo-2 +/apt1-1 + cal-1), we observed plants with the Ap1+, Ap1- Ufo+, and Ap1- Cal+ mutant phenotypes in approximately a 1:2:1 ratio in the F2
generation. From a single F4 plant with an Ap1− Cal− phenotype (genotype ap1−1 ufo−2 cal−lapl−1 + cal−1), we observed plants with the Ap1− Cal− and Ap1− Ufo− Cal− phenotypes in approximately a 3:1 ratio in the F5 generation. It seems likely that the phenotype of ufo−2 cal−1 plants is identical to that of ufo−2 plants because no new phenotype was observed in the F5 generation of the aforementioned construction or in the F3 progeny of single F2 plants with a Ufo− phenotype.

To construct the ufo−2 sup−1 ag−3 triple mutant, we fertilized ag−3/+ plants with pollen from ufo−2 sup−1 plants, collected seed from individual F1 plants, and screened for the segregation of Ag− in addition to Ufo− and Sup− phenotypes in the F2 progeny. From the F2 progeny of a single F1 plant with a Sup− phenotype (probable genotype ufo−2/+, sup−1/sup−1 ag−3/+), triple mutants in addition to both double mutants were isolated.

To construct the ap1−1 ufo−2 fl54 triple mutant, we fertilized ap1− plants with pollen from ufo−2/+ fl54/+ plants, collected seed from individual F1 plants, and screened for the segregation of Ufo− and fl54− as well as Ap1− phenotypes in the F2 progeny. From the F2 progeny of a single F1 plant with an Ap1− phenotype (probable genotype ap1−1 ufo−2/ap1−1 + fl54/+), triple mutants as well as both double mutants were isolated. Triple mutants were identified as those plants with the ufo−2 fl54 phenotype (production of filamentous structures in place of flowers).

**Scanning Electron Microscopy**

Samples were collected, fixed, mounted, coated, and photographed as described previously, except Kodak Tmax 100 film was used (Bowman et al., 1989, 1991).

**Antibody Staining**

Tissue preparation and antibody staining for AP3 were performed as described previously (Jack et al., 1994). Polyclonal rabbit anti-LFY antibodies were provided by D. Weigel and used according to his specifications. A similar protocol was used for LFY experiments, with minor modifications made in accordance with the instructions for the Vectastain Elite kit (Vector Labs, Buringame, CA) (D. Weigel, personal communication). To ensure genuine comparisons of expression levels from ufo−2 and wild-type inflorescences, tissue from both was collected, fixed, and embedded at the same time in an identical manner. Each microscope slide contained sections from both genotypes.

**RNA in Situ Hybridization**

Inflorescences were collected, fixed, embedded, sectioned, and hybridized as described previously (Drews et al., 1991). The ufo−2 and wild-type samples were processed in parallel as described for antibody staining (see previous discussion). Antisense gene-specific probes were prepared from pCIT565 for AG (Drews et al., 1991), pAM128 for AP1 (Mandel et al., 1992), pD793 for AP3 (Jack et al., 1992), and pCINIX for PI (Goto and Meyerowitz, 1994). Wild-type and ufo−2 floral primordia direct comparisons in Figures 5 and 6 are from sections on the same microscope slide.

**Image Processing**

Slides and negatives were scanned and digitized with a Nikon Coolscan machine (Melville, NY). Images were adjusted for brightness, contrast, and color and assembled for figures with Adobe Photoshop (versions 2.5 and 3.0) (Mountain View, CA). Figures were printed with a Kodak XLS 8300 Digital Printer.

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