A novel gene that regulates floral meristem activity and controls floral organ number was identified in Arabidopsis and is designated FON1 (for FLORAL ORGAN NUMBER1). The fon1 mutants exhibit normal vegetative development and produce normal inflorescence meristems and immature flowers before stage 6. fon1 flowers become visibly different from wild-type flowers at stage 6, when the third-whorl stamen primordia have formed. The fon1 floral meristem functions longer than does that of the wild type: after the outer three-whorl organ primordia have initiated, the remaining central floral meristem continues to produce additional stamen primordia interior to the third whorl. Prolonged fon1 floral meristem activity also results in an increased number of carpels. The clavata (clv) mutations are known to affect floral meristem activity. We have analyzed the clv1 fon1, clv2 fon1, and clv3 fon1 double mutants. These double mutants all have similar phenotypes, with more stamens and carpels than either fon1 or clv single mutants. This indicates that FON1 and CLV genes function in different pathways to control the number of third- and fourth-whorl floral organs. In addition, to test for possible interactions between FON1 and other floral regulatory genes, we have constructed and analyzed the relevant double mutants. Our results suggest that FON1 does not interact with TERMINAL FLOWER1, APETALA1, APETALA2, or UNUSUAL FLORAL ORGAN. In contrast, normal LEAFY function is required for the expression of fon1 phenotypes. In addition, FON1 and AGAMOUS both seem to affect the domain of APETALA3 function, which also affects the formation of stamen-carpel chimera due to fon1 mutations. Finally, genetic analysis suggests that FON1 interacts with SUPERMAN, which also regulates floral meristem activity.

INTRODUCTION

Flowers develop from groups of undifferentiated cells on the flanks of the reproductive shoot apical meristem. Initially, these cells form floral meristems. The cells at different positions on the floral meristem then develop with different fates to generate the appropriate numbers of floral organs in appropriate places. Flowers of wild-type Arabidopsis consist of four concentric whorls of organs (Bowman et al., 1989): four sepals in the first whorl (the outer whorl), four petals in the second whorl, six stamens in the third whorl, and two fused carpels in the fourth whorl (the central whorl). Therefore, cells in some regions of the floral meristem must correctly interpret their positional information and then organize into specific types of organ primordia, whereas other cells are not part of the newly formed primordia. Wild-type floral meristem activity terminates after the production of a defined number of organ primordia.

Molecular and genetic studies of flower development have led to the identification and characterization of a number of genes that affect the pathway from inflorescence meristem formation to floral organ primordium formation (Ma, 1994; Weigel and Meyerowitz, 1994; Yanofsky, 1995). Among these genes, LEAFY (LFY) and APETALA1 (AP1) affect the first step of this pathway: the formation of floral meristems from the inflorescence meristem (Haughn and Somerville, 1988; Irish and Sussex, 1990; Schultz and Haughn, 1991; Huala and Sussex, 1992; Mandel et al., 1992; Weigel et al., 1992; Bowman et al., 1993). Both ify and ap1 mutants show a partial transformation of flowers into inflorescence shoots. For example, defects in LFY function cause inflorescence shoots to develop in positions normally occupied by flowers. Plants homozygous for a severe ap1 mutation display a homeotic conversion of sepals to bracts and the formation of a floral bud in the axial of each bract (Irish and Sussex, 1990). The transformation of flowers into inflorescence shoots is more complete in plants homozygous for both ify and ap1 mutations, indicating that the products of LFY and AP1 act synergistically to determine floral meristem identity. The TERMINAL FLOWER1 (TFL1) gene acts to prevent the formation of floral meristems at the center of inflorescence meristems, apparently by inhibiting LFY and AP1 expression (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992; Weigel et al., 1992; Gustafson-Brown et al., 1994).

Another class of genes that affect the floral meristem as well as other apical meristems is the CLAVATA genes: CLV1,
CLV2, and CLV3 (Leyser and Furner, 1992; Clark et al., 1993, 1995; Crone and Lord, 1993). clv mutants have similar phenotypes, and the clv1 and clv3 mutants in particular have been analyzed extensively. In clv1 and clv3 plants, vegetative, inflorescence, and floral meristems are all enlarged relative to those of the wild type. The enlarged meristems have an abnormally large pool of undifferentiated and proliferative cells, which give rise to more flowers (from inflorescence meristem) and more floral organs (from floral meristem). The phenotypes of double mutants of clv1 and fyon or ap1 mutations indicate that the CLV1 gene also contributes to the control of floral meristem identity (Clark et al., 1993). It has been proposed that CLV1 and CLV3 are required to promote the differentiation of cells at the periphery of shoot and floral meristems (Clark et al., 1995). Because, even for the strongest alleles, the phenotype of the clv1 clv3 double mutant is similar to that of the clv1 or clv3 single mutant, it has been proposed that CLV1 and CLV3 function in the same regulatory pathway (Clark et al., 1995).

The type of organ produced in each whorl depends on the activity of organ identity genes (Bowman et al., 1991b), including AP1, APETALA2 (AP2) (Haughn and Somerville, 1988; Jofuku et al., 1994), APETALA3 (AP3) (Bowman et al., 1991b; Jack et al., 1992), PISTILLATA (Pi) (Goto and Meyerowitz, 1994), and AGAMOUS (AG) (Yanofsky et al., 1990). Each of these genes is required for the specification of the identity of two adjacent whorls of floral organs (Bowman et al., 1991b; Coen and Meyerowitz, 1991; Meyerowitz et al., 1991). Genetic analysis and in situ RNA hybridization experiments have demonstrated that these flower organ identity genes act in overlapping domains of the young floral meristem to determine the fate of organ primordia, and the ABC model has been proposed to explain the function of organ identity genes (Bowman et al., 1991b; Coen and Meyerowitz, 1991; Meyerowitz et al., 1991). It has also been proposed that activation of the flower organ identity genes is regulated by the floral meristem identity genes LFY and AP1 (Weigel and Meyerowitz, 1993), with additional genes that possibly mediate between floral meristem genes and floral organ identity genes, such as UNUSUAL FLORAL ORGANS (UFO) (Ingram et al., 1995; Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). In addition, genetic characterizations of the SUPERMAN (SUP) gene have revealed that it acts to restrict the number of stamens, to promote the formation of carpels, and to limit floral meristem activity (Schultz et al., 1991; Bowman et al., 1992). Molecular studies using the cloned SUP gene have led to the proposal that it functions to maintain the boundary of third and fourth whorls (Sakai et al., 1995).

The characterization of the process from floral meristem formation to floral organ primordia formation is an important part of the analysis of flower development. However, very little is known about the mechanisms controlling how cell fates are determined in the floral meristem. Cell fate selections include whether to form an organ primordium, which type of organ to differentiate into, and when to terminate cell proliferative and organogenetic activity. Because cell fate selection in inflorescence and floral meristems is likely to be a very complex process (in addition to the known genes controlling floral meristem identity or size and those specifying organ identity), there are likely to be other genes involved in this process. Analysis of novel floral regulatory genes will provide new insights into flower development. Here, we report the isolation of mutants of a novel gene, designated FON1 (for FLORAL ORGAN NUMBER1). We present phenotypic analyses of three fon1 mutants as well as the genetic interactions between FON1 and other floral genes. Our results suggest that the FON1 gene plays a critical role in terminating floral meristem activity and in controlling stamen and carpel numbers. In addition, we describe the phenotypes of double mutants of fon1 and other floral mutations and discuss their implications.

RESULTS

Mutant Isolation and Genetic Analysis

In the course of characterizing Arabidopsis transgenic plants, we observed that a number of lines from ~1200 independent T-DNA transformants segregated plants with abnormal phenotypes. These transformants were from separate transformation experiments done by three people at different times. Three of these abnormal plants had similar floral phenotypes and were from the Landsberg erecta (Ler) ecotype. Preliminary examination showed that these three putative mutants had an increased number of reproductive organs and therefore were named fon. These three mutants were each backcrossed to the wild-type Ler. The F1 generation from those crosses all showed wild-type phenotypes, and the F2 generation segregated normal-to-mutant plants in a 3:1 ratio, indicating that the mutant phenotypes resulted from single-gene recessive nuclear mutations. Furthermore, F1 plants from crosses between the first fon mutant and each of the other two fon mutants showed similar abnormal phenotypes, indicating that the mutants were allelic; therefore, the three mutant alleles were designated as fon1-1, fon1-2, and fon1-3.

Segregation analysis of F2 plants from crosses of fon1 mutants with the wild type also showed that although fon1-1 is tightly linked to a T-DNA insertion with kanamycin resistance, fon1-2 and fon1-3 can be separated from the kanamycin resistance marker. Furthermore, DNA gel blot hybridization experiments with the original transformation vectors used as probes indicated that genomic DNA from the segregated fon1-2 and fon1-3 plants does not contain T-DNA (data not shown).

To determine the position of the fon1 locus, fon1-1 plants were crossed with lines carrying visible markers. Analysis of the F2 plants from a cross between fon1-1 and glabra1-1 (Oppenheimer et al., 1991) indicated that these genes are
The results demonstrated that FON1 is located on chromosome 3, ~15 centimorgans from GLABRA1.

**Phenotypes of fon1 Plants**

To determine when and where FON1 gene function is required during Arabidopsis development, we compared the phenotypes of wild-type and fon1 plants throughout their life cycles by using light microscopy. There were no obvious differences between the wild type and mutants in seed size and seed coat color, germination time, and cotyledon size and shape. The number and shape of rosette and cauline leaves as well as the stature of the fon1 plants were normal. We also found that there was no change in trichome and stoma distribution on the leaves of fon1 plants. The flowering time for fon1 plants was about the same as that for wild-type plants under our growth conditions for Arabidopsis (see Methods). Furthermore, as shown in Figure 1, all of the fon1 mutants—fon1-1 (Figure 1B), fon1-2 (Figure 1C), and fon1-3 (Figure 1D)—had an inflorescence structure similar to that of the wild type (Figure 1A), and fon1 flowers had all types of floral organs—sepals, petals, stamens, and carpels. An examination of the overall development of fon1 mutants indicated that the FON1 gene did not seem to affect the development of the seed and the vegetative part of the plant.

To characterize in more detail the abnormalities of the fon1 mutants, we analyzed fon1 flower phenotypes. The wild-type Arabidopsis flower (Figure 1E) has four sepals, four petals, six stamens, and two fused carpels, which are more easily seen in the silique (Figures 1I and 1M, and Table 1). In contrast, the fon1 flower showed increased stamina and carpell number, with six to 12 stamens and two to four carpels (Figures 1F to 1H, 1J to 1L, and 1N to 1P, and Table 1). Among the three fon1 mutants, fon1-1 and fon1-3 had more severely abnormal phenotypes than did fon1-2. The early-appearing flowers of the fon1-1 plants had an average of 8.5 stamens and 3.5 carpels, and fon1-3 flowers had 8.6 stamens and 3.5 carpels (Table 1). Furthermore, the sepals and petals of some fon1-1 (Figure 1F; other data not shown) and fon1-3 (Figure 1H) flowers had variable sizes. In the less severely abnormal fon1-2 plants, the early-appearing flowers contained an average of 6.7 stamens and 2.8 carpels, with a higher frequency of gynoecia having two or three carpels (Figures 1K and 1Q, and Table 1). The early-appearing flowers of all three fon1 mutants tended to have more severe phenotypes than did the later-appearing flowers. For example, the average stamina and carpell numbers from 85 late-appearing fon1-1 flowers (positions 11 to 37) were 8.4 ± 0.7 and 3.1 ± 0.5, respectively, in comparison to 8.5 ± 1.9 stamens and 3.5 ± 0.5 carpels from 64 early-appearing flowers (positions 1 to 10). Some fon1 flowers, especially those among the very late ones, had a normal number of stamens and carpels. These results indicate that the FON1 gene functions to specifically control floral organ numbers in third and fourth whorls, particularly during early inflorescence development.

In addition to increased floral organ number, fon1 flowers had other abnormalities. For example, some carpels of fon1 flowers failed to fuse on one side (17 of 79 fon1-1 flowers) or both sides (seven of 79 fon1-1 flowers), resulting in curled pistils that were sterile or minimally fertile (Figures 1J to 1L). Furthermore, fon1 flowers occasionally produced filamentous organs (Table 1), resembling the filaments of stamens. Similar filamentous organs were seen in clv1-4 and clv3-1 flowers (Clark et al., 1993, 1995). Also, some fon1 flowers produced stamina–carpel chimeric organs (Table 1), which were similar to those seen in a strong sup mutant, sup-1 (Bowman et al., 1992). These abnormal phenotypes of fon1 flowers suggest that FON1 may be involved in regulation of the stamina and carpell identity.

**Early Flower Development of fon1 Mutants**

To determine more precisely the earliest time during flower development when FON1 is required, we analyzed the early development of fon1-1 and fon1-3 flowers by using scanning electron microscopy (SEM), as shown in Figure 2. SEM revealed that there was no difference in either inflorescence meristem shape or size between wild-type (Figure 2A) and fon1-1 (Figure 2B) or fon1-3 (Figure 2C) plants. The floral primordia and young flowers before stage 6 of both wild-type and fon1 plants also appeared to have the same morphology. The first difference between fon1 and wild-type flowers was seen at stage 6. In the wild type, at the beginning of this stage, all four types of organ primordia have formed. The opposing developing sepals have come into contact with each other, but the petal primordia are still small. The stamen primordia are already quite prominent, and the central floral meristem has already terminated its activity and formed a dome-shaped gynoecium primordium that is surrounded by the stamen primordium (Figure 2D; Bowman et al., 1989; Smyth et al., 1990). The gynoecium primordium has developed into a cylinder-like structure with an elliptic hole in the center at stage 7 (Figure 2G; Bowman et al., 1989; Smyth et al., 1990).

In fon1 flowers, although sepals, petals, and the first six stamens were formed normally, the development of the center portion of floral meristem was dramatically altered. In subsequent discussions, we use floral meristem center (FMC) to refer to the central portion of the floral meristem interior to the third-whorl organ primordia. Instead of establishing carpels, the fon1 FMC still maintained meristem activity. From a top view of stage 6 flowers, we observed that whereas the wild-type FMC formed a nearly round gynoecium primordium (Figure 2D), the fon1-1 FMC (Figure 2E) and fon1-3 FMC (Figure 2F) had irregular shapes. The top
Figure 1. Phenotypes of Arabidopsis Wild-Type Plants and fon1 Mutants.

(A) An Ler primary inflorescence.
(B) A fon1-1 primary inflorescence.
(C) A fon1-2 primary inflorescence.
(D) A fon1-3 primary inflorescence.
(E) A wild-type flower showing the first-whorl sepals (s), second-whorl petals (P), third-whorl stamens (st), and a central gynoecium with fused carpels (c).
(F) A fon1-1 flower showing that the four types of organs are still present and that the number of stamens is increased.
(G) A fon1-2 flower showing a similar but a less severe phenotype than that of fon1-1.
(H) A fon1-3 flower showing a phenotype similar to fon1-1.
(I) A wild-type silique with two fused carpels.
(J) Two fon1-1 siliques: one straight and one curled.
(K) Three fon1-2 siliques exhibiting different phenotypes: a curled silique with more than two carpels (top), a straight silique with three carpels (middle), and a straight one with two carpels (bottom).
(L) Two fon1-3 siliques similar to those of fon1-1.
(M) A section of a wild-type silique showing two fused carpels.
(N) A section of a fon1-1 silique containing four carpels.
(O) Sections of two fon1-2 siliques: one with three carpels and the other with two carpels.
(P) A section of a fon1-3 silique having four carpels.

Flowers in (E) to (H) are all the second flowers on primary inflorescences. All siliques in (I) to (L) are from plants ~10 days after pollination, except the middle and bottom siliques in (K), which are from plants ~8 days after pollination. (A) to (D) are of the same magnification; (E) to (H) are of the same magnification, and (I) to (L) are of the same magnification. In (A), (E), and (I), bars = 1 mm; in (M) to (P), bars = 0.25 mm.
surface of the fon1 FMC was uneven, with protrusions. Just before (Figure 2H) or at (Figure 2I) stage 7, the protrusions on the fon1 FMC became more distinct and eventually enlarged to form additional stamen primordia.

In fon1 flowers at approximately stage 8 (Figure 2J), some of the extra stamen primordia separated from the remainder of the fon1 FMC and developed into individual stamens, whereas other stamen primordia remained associated with and developed together with the central region that became the gynoecium primordium. Whether the extra stamens developed with the gynoecium or separately seems to depend largely on when and where the stamen primordia initiated. Usually, those extra stamens that occupied positions just interior to the third whorl were phenotypically normal (Figures 2K and 2L); these stamens were likely to give rise to extra stamen primordia and later to more than the normal number of carpel primordia. In addition, fon1 mutants exhibit abnormal stamen and carpel development; in particular, some stamens fail to separate from the gynoecium. These results indicate that in fon1 flowers, the FMC remains active for a longer period than normal and that development of the reproductive organs is affected.

### Phenotypes of clv fon1 Double Mutants

Previously, it was shown that the CLV1, CLV2, and CLV3 genes regulate the meristem activities and floral organ numbers in Arabidopsis (McKelvie, 1962; Leyser and Furner, 1992; Clark et al., 1993, 1995; Crone and Lord, 1993). In clv1 and clv3 plants, the vegetative shoot apical meristem, inflorescence meristem, and floral meristems are all enlarged. Flowers of clv1 and clv3 mutants have increased numbers of organs in all four whorls (Figures 3A, 3B, 3D to 3H, and Table 2; Clark et al., 1993) and also produce additional whorls not present in wild-type flowers, whereas clv2-1 plants have a weaker phenotype, with an increased number of carpels (Figures 3C, 3G, and 3K, and Table 2). clv1 clv3 double mutants have the same phenotype as strong clv1 or clv3 single mutants, suggesting that CLV1 and CLV3 function in the same pathway (Clark et al., 1995).

We were interested in determining whether FON1 acts in the same regulatory pathway as CLV1 and CLV3. In addition, we also wanted to test for the possible interaction between FON1 and CLV2. Therefore, we constructed the clv1-1 fon1-1, clv1-4 fon1-1, clv2-1 fon1-1, and clv3-1 fon1-1 double mutants by crossing fon1-1 with corresponding clv mutants and characterized their phenotypes. Double mutants of fon1-1 and each of the clv mutations had many similar phenotypes (Figure 3). The sizes of the inflorescence meristems of clv1-1 fon1-1 (Figure 4B) and clv1-4 fon1-1 (Figure 4F) were indistinguishable from those of the clv1-1 (Figure 4A) and clv1-4 (Figure 4E) single mutants. These inflorescence

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Plants</th>
<th>Sepal</th>
<th>Petal</th>
<th>Stamen</th>
<th>Carpel</th>
<th>F.O.</th>
<th>C.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>40</td>
<td>4.0 ± 0</td>
<td>4.0 ± 0</td>
<td>6.0 ± 0</td>
<td>2.0 ± 0</td>
<td>0.9 ± 0</td>
<td>0.9 ± 0</td>
</tr>
<tr>
<td>fon1-1</td>
<td>64</td>
<td>4.0 ± 0.2</td>
<td>4.1 ± 0.3</td>
<td>8.5 ± 1.9</td>
<td>3.5 ± 0.5</td>
<td>0.1 ± 0.2</td>
<td>0.8 ± 0.9</td>
</tr>
<tr>
<td>fon1-2</td>
<td>71</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>6.7 ± 0.9</td>
<td>2.8 ± 0.3</td>
<td>0.1 ± 0.2</td>
<td>0.8 ± 0.7</td>
</tr>
<tr>
<td>fon1-3</td>
<td>76</td>
<td>4.0 ± 0.4</td>
<td>4.0 ± 0.4</td>
<td>8.6 ± 1.6</td>
<td>3.6 ± 0.3</td>
<td>0.1 ± 0.2</td>
<td>0.8 ± 0.9</td>
</tr>
</tbody>
</table>

*The first 10 flowers on each given plant were analyzed.

*F.O., filamentous organs that replace stamens.

*C.S., chimeric stamens that grow in association with carpels.

*Mean ± SD.
Figure 2. SEM of Wild-Type and *fon1* Flowers.
meristems were enlarged in comparison to those of the wild type or the *fon1* single mutants. The floral organ numbers in the first and second whorls of the double mutants were similar to or only slightly greater than those of the corresponding *clv* single mutants (Table 2).

On the other hand, the numbers of stamen and carpels of *clv fon1* double mutants were clearly greater (Figure 3 and Table 2) than those of *fon1* and *clv* single mutants, although stamen and carpel numbers were variable between individual flowers of the same double mutant plant, as in single mutants. Furthermore, in double mutant flowers, the frequency of stamen-carpel chimeric organs and filamentous organs was also increased in comparison to those in *fon1* and *clv* single mutants (Tables 1 and 2). The gynoecia of the double mutants were composed of multiple carpels that varied in size, and only some of them set seed (Figures 3U to 3X). At the top of the pistils, double mutant flowers had much larger stigmas (Figures 3M to 3P) than those in either *fon1* or *clv* single mutants (Figures 1F to 1H and 3A to 3D).

Some carpels of double mutants, unlike those in *clv* single mutants (Figures 4C and 4G), were not properly fused due to stamen-carpel chimeric growth (Figures 4D and 4H). Some siliques of double mutants, like those in *fon1* mutants, were also curled (Figures 3Q, 3S, and 3T). Furthermore, some flowers of the *fon1 clv* double mutants showed exaggerated phenotypes. For example, in the *fon1-1 clv1-4* double mutant, in ~5% of flowers the extra internal carpels developed to the extent that they forced through the primary carpel walls (Figure 3R, right). In ~5% of flowers of the *fon1-1 clv3-1* double mutant, there was a dramatic proliferation of tissues that broke through the carpel walls (Figure 3T, right). These flowers tended to appear late during inflorescence development. Unlike the extra carpels seen in the *fon1-1 clv1-4* double mutant, the massive proliferative tissue of the *fon1-1 clv3-1* double mutant was composed mainly of cells that were not well differentiated, although there were also patches of stigmatic tissue (Figure 3T, right).

We have also constructed double mutants between *fon1-3* and *clv1-1*, *clv1-4*, *clv2-1*, or *clv3-1*. The phenotypes of the double mutants were the same as those of the *fon1-1 clv* double mutants (data not shown). These results indicate that *fon1 clv* double mutants have phenotypes more severe than any of the single mutants have. Although the *fon1* mutations do not further increase the inflorescence meristem size of the *clv* mutants, *fon1* mutations resulted in more stamen and carpel numbers as well as longer periods of active floral meristems.

### Double Mutants of *fon1* and Other Mutations Affecting the Floral Meristem

The *TFL1*, *LFY*, and *AP1* genes are known to control floral meristem identity. To test for the possible genetic interactions...
Figure 3. Phenotypes of clv Single Mutants and clv fon1-1 Double Mutants.

The siliques shown in (E) to (H) are from plants 8 days after pollination.

(A) A clv1-1 flower.
(B) A clv1-4 flower.
(C) A clv2-1 flower.
Table 2. Number of Flower Organs in clv Mutants and clv fon1-1 Double Mutants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Plants</th>
<th>Sepal</th>
<th>Petal</th>
<th>Stamenb</th>
<th>Carpels</th>
<th>F.O.</th>
<th>C.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>clv1-1</td>
<td>74</td>
<td>4.1 0.2</td>
<td>4.1 0.3</td>
<td>6.7 0.7</td>
<td>4.1 0.3</td>
<td>0.1 0.3</td>
<td>0</td>
</tr>
<tr>
<td>clv1-1 fon1-1</td>
<td>82</td>
<td>4.4 0.6</td>
<td>4.2 0.5</td>
<td>8.8 2.0</td>
<td>NC 1.7</td>
<td>1.7 1.2</td>
<td>0.9 1.1</td>
</tr>
<tr>
<td>clv1-4</td>
<td>76</td>
<td>4.7 0.7</td>
<td>4.6 0.7</td>
<td>8.6 1.7</td>
<td>4.6 0.6</td>
<td>0.4 0.5</td>
<td>0</td>
</tr>
<tr>
<td>clv1-4 fon1-1</td>
<td>74</td>
<td>5.0 0.8</td>
<td>4.9 0.7</td>
<td>10.6 2.7</td>
<td>NC 1.6</td>
<td>1.6 1.1</td>
<td>1.2 0.8</td>
</tr>
<tr>
<td>clv2-1</td>
<td>76</td>
<td>4.0 0.7</td>
<td>4.0 0.7</td>
<td>6.0 0.9</td>
<td>4.0 0.7</td>
<td>0.1 0.2</td>
<td>0</td>
</tr>
<tr>
<td>clv2-1 fon1-1</td>
<td>74</td>
<td>4.1 0.3</td>
<td>4.1 0.2</td>
<td>10.1 2.9</td>
<td>NC 1.0</td>
<td>1.0 0.9</td>
<td>1.2 1.0</td>
</tr>
<tr>
<td>clv3-1</td>
<td>75</td>
<td>4.4 0.6</td>
<td>4.4 0.5</td>
<td>8.0 1.6</td>
<td>4.5 0.5</td>
<td>0.3 0.5</td>
<td>0</td>
</tr>
<tr>
<td>clv3-1 fon1-1</td>
<td>74</td>
<td>4.5 0.5</td>
<td>4.4 0.6</td>
<td>10.2 2.0</td>
<td>NC 1.3</td>
<td>1.3 1.0</td>
<td>1.2 0.8</td>
</tr>
</tbody>
</table>

a Only the first 10 flowers on any given plant were analyzed.
b The difference of stamen number between each clv mutant and the corresponding clv fon1 double mutant was significant by t tests because the probability of the difference due to chance was <1%.
c F.O., filamentous organs that replace stamens.
d C.S., chimeric stamens that grow in association with carpels.
e * Mean ± so.
f NC, not counted. The carpels in clv fon1 flowers are compacted in the center (see Figure 3) and are very difficult to count.

between FON1 and these genes, we constructed tfll-2 fon1-1, lfy-5 fon1-1, lfy-6 fon1-1, and ap1-1 fon1-1 double mutants. The tfll mutations cause early flowering and limit the development of the normally indeterminate inflorescence by promoting the formation of a terminal floral meristem. The phenotypes of tfll single mutants suggest that normal TFL1 function may be to inhibit floral meristem initiation at the inflorescence apex (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992; Schultz and Haughn, 1993). Similar to the tfll-2 single mutant (Figure 41; Alvarez et al., 1992), the tfll-2 fon1-1 double mutant was early flowering (data not shown) and produced a terminal flower (Figure 4J). In addition, the double mutant flower had increased stamen and carpel numbers (Figure 4J).

Figure 3. (continued).

(D) A clv3-1 flower.
(E) A clv1-1 silique.
(F) A clv1-4 silique.
(G) A clv2-1 silique.
(H) A clv3-1 silique.
(I) A section of a clv1-1 silique.
(J) A section of a clv1-4 silique.
(K) A section of a clv2-1 silique.
(L) A section of a clv3-1 silique.
(M) A clv1-1 fon1-1 flower.
(N) A clv1-4 fon1-1 flower.
(O) A clv2-1 fon1-1 flower.
(P) A clv3-1 fon1-1 flower.
(Q) clv1-1 fon1-1 siliques.
(R) clv1-4 fon1-1 siliques. Approximately 5% of gynoecia (at right) produced internal fifth-whorl carpels that grew through the fourth-whorl carpel wall.
(S) clv2-1 fon1-1 siliques.
(T) clv3-1 fon1-1 siliques. Within the gynoecia of ~5% of flowers (at right), the central portion continues to proliferate and generate undifferentiated cells with patches of stigmatic tissue.
(U) A section of a clv1-1 fon1-1 silique.
(V) A section of a clv1-4 fon1-1 silique.
(W) A section of a clv2-1 fon1-1 silique.
(X) A section of a clv3-1 fon1-1 silique.

a, carpel; p, petal; s, sepal; st, stamen; ud, tissues composed of undifferentiated cells; 4c, the fourth-whorl carpels; 5c, the fifth-whorl carpels. (A) to (D) are of the same magnification; (E) to (H) are of the same magnification; (I) to (L) are of the same magnification; (M) to (P) are of the same magnification; (Q) to (T) are of the same magnification; and (U) to (X) are of the same magnification. In (A), (E), (M), and (Q), bars = 1 mm; in (I) and (U), bars = 0.25 mm.
Figure 4. SEM of Double Mutants between fon1 and Mutants with Mutations Affecting Meristems.

(A) clv1-1 inflorescence and floral primordia.
(B) clv1-1 fon1-1 inflorescence and floral primordia. The inflorescence meristem sizes of clv1-1 and clv1-1 fon1-1 are similar.
(C) A clv1-1 flower with the outer two floral organs removed.
(D) A clv1-1 fon1-1 flower. The abnormal gynoecium development was accompanied by carpel-stamen chimeric growth.
(E) clv1-4 inflorescence and flower primordia showing an enlarged inflorescence meristem.
(F) clv1-4 fon1-1 inflorescence and flower primordia. The inflorescence meristem size of the double mutant is the same as that in the clv1-4 single mutant.
(G) A clv1-4 flower with the outer two floral organs removed.
(H) A clv1-4 fon1-1 flower. The FMC of the double mutant produced severely affected carpels with many associated stamens. The huge stigmatic tissue on the top of the carpels contained some undeveloped organs.
(I) tfl1-2 terminal flowers.
(J) tfl1-2 fon1-1 terminal flowers. The stamen number is increased, and carpels are unfused.
(K) A mature ap1-1 flower.
(L) A mature ap1-1 fon1-1 flower. The flower has an increased number of stamens, and carpels are unfused.
(M) A stage 10 ify-5 flower.
The LFY and AP1 genes have been shown to be important in establishing floral meristem identity. Mutations in either gene cause a partial conversion of the flower to a shoot (Irish and Sussex, 1990; Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992; Bowman et al., 1993). Flowers of a strong ify mutant, ify-6, are often subtended by floral bracts, which are similar in morphology to the cauline leaves subtending secondary inflorescence shoots (Weigel et al., 1992). In ify-6 mutants, usually four but occasionally three or five organs are present in the first whorl, and a variable number of organs arise in a slightly spiral pattern in the interior (Weigel et al., 1992). Flowers of weak ify mutants, such as ify-5, have some nearly normal petals and stamens (Weigel et al., 1992). The fon7-1 ify-5 double mutant had morphology similar to that of ify-5, including the inflorescence structure. However, stamen numbers in the double mutant were greater (Figure 4N) than those in the ify-5 single mutant (Figure 4M). Many fon7-1 ify-5 flowers produced stamen-carpel chimeric organs, and carpel development in the double mutant was often affected by the production of additional stamens. The ify-6 fon7-1 double mutant (Figure 4P), however, was indistinguishable from the ify-6 single mutant (Figure 4O) with respect to inflorescence structure, meristem and organ identities, and floral organ number.

In flowers of the severe ap1-1 mutant (Irish and Sussex, 1990; Figures 4K and 4L), four or fewer bracts develop instead of sepals, and petals are absent. In the axils of the bracts, secondary flowers are formed. In addition, an average of approximately five stamens are produced in an ap1-1 primary flower, which is only slightly fewer than the normal six stamens. The primary ap1-1 gynoecium is normal. The overall phenotype of the fon7-1 ap1-1 double mutant was similar to that of ap1-1, except that the double mutant had more stamens and carpels. In contrast to the ap1-1 single mutant (Figure 4K), the flowers of the double mutant (Figure 4L) contained unfused carpels, which were often associated with the additional stamens. Therefore, the ap1-1 fon7-1 double mutant had the additive phenotype of the ap1-1 and fon7-1 single mutants.

Double mutant analysis of fon7 with tfl1, ify, or ap1 indicates that FON7 does not exhibit genetic interaction with TFL1 and AP1, suggesting that FON7 acts in a pathway separate from those mediated by TFL1 or AP1. However, some LFY function may be required for the expression of fon7 phenotypes.

Double Mutants of fon7 with Floral Organ Identity Mutations

The phenotypes of fon7 flowers suggest that FON7 may also regulate floral organ identity. To determine whether FON7 interacts with any floral organ identity genes, we have constructed a number of double mutants, including ag-1 fon7-1, ap2-1 fon7-1, ap2-9 fon7-1, ap3-1 fon7-1, and ap3-4 fon7-1. Because the ufo mutation affects the second- and third-whorl organ identity, we also constructed the ufo-1 fon7-1 double mutant. The phenotypes of these double mutants are described below and shown in Figure 5.

ag-1 fon7-1

As described earlier, ag mutations cause the conversion of reproductive organs into perianth organs and indeterminate growth of the floral meristem (Bowman et al., 1989; Yanofsky et al., 1990; Figure 5A). Whereas the outer two whorls of ag flowers are normal, six petals develop in the third whorl at positions normally occupied by stamens. At the center, the gynoecium is replaced by another ag flower. This pattern repeats many times, resulting in the formation of a large number of whorls.

We found that flowers of the ag-1 fon7-1 double mutant had a range of phenotypes. Most of the early-appearing flowers (26 of 30 flowers at positions 1 to 5) consisted of sepals in the first whorl followed by a large number of petals (Figure 5B). This phenotype appears to be similar to that of the sup-1 ag-1 double mutant (Bowman et al., 1992). Some late-appearing ag-1 fon7-1 flowers produced sepals in the first whorl and a variable number of sepals interior to the third-whorl petals. The number of interior sepals was usually smaller than that in the ag-1 single mutant (Figure 5C). Occasionally, the ag-1 fon7-1 double mutant produced flowers similar to ag-1 flowers (Figure 5D). As mentioned above, some late-appearing fon7 flowers seemed normal, with six...
Figure 5. Phenotypes of Double Mutants between fon1 and Mutants with Mutations Affecting Floral Organ Identity.

(A) An ag-1 flower.
(B) to (D) ag-1 fon1-1 flowers.
stamens and two carpels. Similarly, in the ag-1 fon1-1 double mutant, some late-appearing flowers might not have been affected by the fon1-1 mutation and exhibited only the ag-1 single mutant phenotypes. The absence of sepals in early-appearing flowers of the ag-1 fon1-1 double mutant suggests that these two genes may interact genetically.

**ap2-1 fon1-1, ap2-2 fon1-1, and ap2-9 fon1-1**

AP2 is required for specifying the identity of the first- and second-whorl organs (Komaki et al., 1988; Bowman et al., 1989, 1991b, 1993; Kunst et al., 1989; Jofuku et al., 1994). In a weak mutant, ap2-1 (Figure 5E), the first whorl is occupied by leaflike organs that may have carpelloid features, and the second-whorl organs have features of both stamens and petals. The ap2-1 third- and fourth-whorl organ identities are normal, although the number of stamens is reduced. In severe ap2 mutants, ap2-2 and ap2-9 (Figures 5G and 5I), the outer whorls have carpelloid and leaflike organs, and the second-whorl organs are missing. The third whorl is occupied by a reduced number of stamens, and the fourth whorl has two or three usually unfused carpelloid organs (Figures 5G and 5I).

The ap2-1 fon1-1 double mutant exhibited an essentially additive phenotype of the two single mutants, with the outer two whorl organs (Figure 5F) similar to those of the ap2-1 single mutant. In addition, the number of stamens in ap2-1 fon1-1 flowers was increased (Figure 5F). In an ap2-2 flower, there are usually four to six stamens, with an average of 5.3 (197 stamens per 37 flowers), whereas in ap2-1 fon1-1 flowers, there were five to 10 stamens, with an average of 7.4 (274 stamens per 37 flowers). The most significant difference between ap2-1 and ap2-1 fon1-1 was observed at the central region of the flower. ap2-1 flowers usually produced a gynoecium with two fused carpels, but ap2-1 fon1-1 flowers produced more carpels (an average of 2.9, with 106 carpels per 37 flowers) that were usually unfused (Figure 5F).

As in ap2-1 fon1-1 flowers, the outer two-whorl organs in ap2-2 fon1-1 and ap2-9 fon1-1 flowers (Figures 5H and 5J) were similar to those of the ap2-2 and ap2-9 single mutants. However, there was an important difference in these organs between the double mutants and the ap2 single mutants: the double mutant first-whorl carpelloid organs often had some stamen features (106 stamen-carpel chimeras per 39 flowers). The central regions of ap2-2 fon1-1 and ap2-9 fon1-1 flowers were highly variable in terms of organ number as well as organ shape (Figures 5H and 5J). Stamens, gynoecia, and irregularly shaped organs were associated with each other in the central region, which differed substantially from those in the corresponding single mutants (Figures 5G and 5I). Because fon1 single mutants exhibited an increased number of reproductive organs, stamen-carpel chimeras, and unfused carpels, the ap2 fon1 double mutant phenotypes can be regarded as the additive phenotypes of the single mutants.

**ap3-1 fon1-1 and ap3-4 fon1-1**

AP3 is required for determining the second- and third-whorl organ identities (Bowman et al., 1991b; Jack et al., 1992, 1993). In the weak mutant (Figure 5M) grown under our conditions, the first whorl was occupied by four normal sepals. The second whorl of ap3-1 flowers consisted of four petals, although they were smaller in size than those of wild-type flowers. The ap3-1 third-whorl organs developed as stamenlike organs that generally were shorter than those in the wild type and without pollen, whereas the gynoecium was normal. The organs of the outer two whorls of the ap3-1 fon1-1 double mutant (Figure 5N) were almost identical to those of the ap3-1 single mutant. In the third and fourth

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**Figure 5.** (continued).

(E) An ap2-1 flower.
(F) An ap2-1 fon1-1 flower.
(G) An ap2-2 flower.
(H) An ap2-2 fon1-1 flower.
(I) An ap2-9 flower.
(J) An ap2-9 fon1-1 flower.
(K) A ufo-1 flower.
(L) A ufo-1 fon1-1 flower.
(M) An ap3-1 flower.
(N) An ap3-1 fon1-1 flower.
(O) An ap3-4 flower.
(P) An ap3-4 fon1-1 flower.
(Q) A sup-1 flower.
(R) to (T) sup-1/+ fon1-1/+ flowers.

c, carpel; ch, chimeric organ; cl, cauline leaflike organ; p, petal-like organ; s, sepal; st, stamen. (E) and (F) are of the same magnification; (G) and (H) are of the same magnification; (I) to (L) are of the same magnification; (M) and (N) are of the same magnification; (O) and (P) are of the same magnification; and (Q) to (T) are of the same magnification. All bars = 1 mm.
whorls of the double mutant, the number of stamens and carpels was increased. The average number of stamens was 5.8 (67 flowers) for the double mutant but only 4.5 for the ap3-1 single mutant. The average number of carpels was increased to 2.8 in the double mutant from 2.0 in the ap3-1 single mutant.

In the flowers of the severe ap3-4 mutant (Figure 5O; Jack et al., 1992), the two outer whorls consisted of sepals. The third whorl was occupied by either carpels or filamentous organs. The fourth whorl consisted of fused carpels, with their number ranging from two to four. Flowers of the ap3-4 fonl-7 double mutant (Figure 5P) produced two outer whorls of sepals, as in the ap3-4 single mutant. In the third whorl, ap3-4 flowers often produced filamentous organs (72 filamentous organs per 38 flowers), whereas there were hardly any filamentous organs in the ap3-4 fonl-7 double mutant. Furthermore, in between the third and fourth whorls or on the fourth-whorl carpels, ap3-4 flowers had small gynoecium-like organs (20 small gynoecia per 38 flowers), which were not seen in ap3-4 fonl-7 double mutant flowers. In addition, the ap3-4 fonl-7 double mutant produced slightly more central carpels than those in the ap3-4 single mutant (126 versus 110 carpels per 38 flowers). Finally, in fonl single mutants and in all above-mentioned double mutants whenever carpels were formed, unfused carpels were observed at the center, whereas ap3-4 fonl-1 and ap3-4 fonl-1 double mutant flowers produced a central gynoecium with fused carpels. It seems that the ap3 mutations prevent the formation of stamen–carpel chimeras, which are at least partly responsible for the formation of unfused carpels.

**ufo-1 fonl-1**

The UFO gene is involved in the control of both floral meristem identity and floral organ identity (Ingram et al., 1995; Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). ufo flowers exhibit an altered organ number in all whorls and abnormal organ identity in the outer three whorls. In particular, ufo-1 flowers produce petal–stamen chimeric organs (Figure 5K). The ufo-1 fonl-1 double mutant showed the additive phenotype (Figure 5L) of the single mutants. The double mutant inflorescence was similar to that of the ufo-1 single mutant (data not shown). The numbers of first- and second-whorl organs of the double mutant (an average of 4.2 sepals and 3.0 petal-like organs per 23 flowers) were similar to those of the ufo-1 single mutant (an average of 4.2 sepals and 3.4 petal-like organs per 21 flowers). In the third whorl, the double mutant and the ufo-1 single mutant both produced similar numbers of filamentous organs (an average of 3.5 filamentous organs in ufo-1 fonl-1 and an average of 3.4 filamentous organs in ufo-1). On the other hand, ufo-1 fonl-1 flowers contained more stamens (seven stamens per 23 flowers) than did ufo-1 flowers (two stamens per 21 flowers). At the center, double mutant flowers had more carpels (73 carpels per 23 flowers) than did ufo-1 flowers (42 carpels per 21 flowers); moreover, most of the carpels in the double mutant were unfused and associated with stamens, as in the fonl-1 single mutant. These results suggest that UFO and FON1 genes act independently to regulate floral organ development.

**Genetic Interaction between FON1 and SUP**

SUP regulates floral organ identity in the third and fourth whorls (Schultz et al., 1991; Bowman et al., 1992). sup mutations cause flowers to form multiple whorls of stamens interior to the second-whorl petals (Figure 5Q), resulting in an increased number of stamens, ranging from eight to 26 (Bowman et al., 1992). We have shown that fon1 mutations also exhibit an increase in stamen number and that FON1 maps on chromosome 3 close to the SUP locus. In addition, the F1 plants from crosses of sup-1 with fonl alleles had fonl-like phenotypes (see Methods). Therefore, it seems that fon1 mutations might be new alleles of the SUP gene. To test this possibility, we performed several experiments, as described below.

First, we compared more carefully the phenotypes of fonl and sup mutants. Strong sup mutants such as sup-1 show a greatly increased number of stamens and usually lack carpels (Schultz et al., 1991; Bowman et al., 1992; Figure 5Q), whereas fon1 mutants all have a moderately increased number of both stamens and carpels (above). Furthermore, a weak sup mutant, sup-5, exhibited a normal number of stamens and fused carpels under our growth conditions. In contrast, all fon1 mutants, including the weak fonl-2 mutant, had unfused carpels. Therefore, fon1 and sup mutant phenotypes are similar in some aspects and different in others.

Second, we analyzed the F2 progeny from crosses between fon1 and sup mutants to identify possible recombinants. Because fon1 and sup are closely linked, if there were no recombination between fon1 and sup, we expected that approximately one-quarter of the F2 progeny would be fon1 homozygous mutants and another one-quarter would be sup homozygous mutants. The remaining plants should be double heterozygous for the fon1 and sup mutations, having the same genotype as the F1 plants. Because the F1 plants had a fon1-like phenotype, these double heterozygous F2 plants should also be fon1-like. The distribution of different phenotypic classes among F2 progeny was very similar for all three crosses. The F2 plants included a majority of fon1 or fon1-like plants and approximately one-quarter of sup plants; furthermore, slightly >10% of the F2 plants were normal (see Methods). The appearance of normal plants in the F2 generation suggests strongly that the FON1 and SUP can be separated genetically. These normal plants should have the genotypes of SUP/SUP FON1/Fon1 or SUP/sup FON1/FON1. Because the SUP gene has been cloned, we used a polymerase chain reaction (PCR) assay to determine the SUP genotypes of a subset of normal plants that were still
available. We found that six of 26 plants tested had the SUP/SUP genotype, supporting the idea that they carry a recombinant chromosome. The fact that more of these normal plants were SUP/sup suggests that some of them might be double heterozygous and exhibited complementation. These results indicate that FON1 and SUP are closely linked different genes.

To further confirm that FON1 and SUP are distinct genes, we performed additional molecular experiments. We found that the SUP gene was expressed at the same level in Ler, fon1-1, and fon1-3 flowers by RNA gel blot hybridizations (data not shown). We further determined the SUP sequences from Ler, fon1-1, and fon1-3. The SUP gene was isolated by PCR based on the published sequence (Sakai et al., 1995). The entire SUP protein coding region plus 203 bp of the 5' untranslated region were sequenced (data not shown). In addition, 254 bp of the 3' region were determined for fon1-3 (data not shown). The SUP sequences from either fon1 mutant matched the wild-type sequence. Therefore, both SUP expression and sequence are normal in two fon1 mutants.

These results clearly demonstrate that FON1 and SUP are different genes. On the other hand, the phenotypes of F1 and F2 plants strongly suggest that these two genes interact genetically. In the F1 generation, double heterozygous plants from fon1 × sup-1 crosses showed fon1-like phenotypes (Figures 5R, 5S, and 5T). The number of outer two-whorl organs was close to that of wild-type flowers (an average of 4.1 sepals and 4.1 petals per 36 flowers). However, stamens and carpels were increased (an average of 7.4 stamens and 3.4 carpels per 36 flowers). Like those in the fon1-1 and fon1-3 single mutants, petals of many flowers were unevenly distributed (Figures 5R and 5S). Similarly, segregation ratios of the F2 plants (see Methods) were consistent with the double heterozygous plants exhibiting fon1-like phenotypes. This was further confirmed by the findings that approximately two-thirds of the fon1-like F2 plants were heterozygous for sup and the remaining one-third were homozygous for SUP.

**DISCUSSION**

**FON1 Is Involved in the Termination of Floral Meristem Activity**

We have shown that fon1 mutants produce flowers with an increased number of stamens and carpels. SEM indicated that during early floral development in fon1 mutants, the FMC fails to terminate its meristematic activity properly and continues to initiate extra stamens and carpel primordia. These results demonstrate that the FON1 gene is required for the termination of floral meristem activity during normal flower development.

Additional evidence supporting our hypothesis comes from the the clv1-4 fon1-1 and clv3-1 fon1-1 double mutant phenotypes. CLV genes are proposed to restrict both shoot meristem and floral meristem activities (Clark et al., 1993, 1995; see below for more thorough discussion). In clv1 single mutants, extended floral meristem activity results in extra carpel primordia being initiated inside the gynoecium. However, in clv1-1 or even in the mutant carrying the strongest allele, clv1-4, the growth and development of the extra internal carpels are limited such that these carpels do not extend outside the gynoecium wall (Clark et al., 1993). Under our growth conditions, the clv1-4 internal carpels failed to break through the fourth-whorl carpels (data not shown). We found that the clv1-4 fon1-1 double mutant exhibited much more extensive growth of the internal carpels than did the clv1-4 single mutant. Some of the clv1-4 fon1-1 internal carpels, after breaking through the gynoecium wall, eventually completed differentiation and developed into additional pistils. Therefore, the clv1-4 fon1-1 double mutant clearly has a longer floral meristem activity than does either the fon1 or clvl single mutants. In the wild type, FON1 and CLV1 both function to terminate the floral meristem activity after floral organ primordia have been formed.

In the severe clv3-2 mutant, analysis of maturing flowers revealed that the central dome continues to proliferate and generate additional organ primordia on its flank (Clark et al., 1995). In some siliques of the clv3-2 single mutant, the meristem domes can grow through the carpel walls (Clark et al., 1995). However, in the clv3-1 single mutant, which is weaker than clv3-2, this phenomenon was not observed under our growth conditions. In the clv3-1 fon1-1 double mutant, the cells inside of the gynoecia of some late flowers proliferated extensively. Rather than forming extra carpels, as seen in clv1-4 fon1-1, these cells formed a large mass of undifferentiated cells. Therefore, the phenotypes of the fon1 single mutants and clv1 fon1 double mutants indicate that normal FON1 function is required to terminate the floral meristem.

**FON1 and the CLV Genes Control Third- and Fourth-Whorl Organ Number through Different Pathways**

As mentioned above, the CLV genes are regulators of shoot and floral meristem development (McKelvie, 1962; Clark et al., 1993, 1995). Furthermore, it was proposed that the CLV1 and CLV3 genes function in the same pathway (Clark et al., 1995). It is not known whether CLV2 also acts in this pathway. The clv mutants maintain a large pool of proliferative and undifferentiated cells in both the shoot and floral meristems. It is reasonable to think that if a relatively constant number of cells in the floral meristem develop into a floral organ primordium, then the number of primordia from an enlarged meristem with more cells is likely to increase. fon1 mutants also produce more floral organs, although
the increase is limited to stamens and carpels. The mechanism of floral organ number increase in the fon1 mutant is different from that in the clv mutants. Neither the inflorescence meristem nor the floral meristem is enlarged in fon1 mutants. The mechanism of floral organ number increase in the fon1 mutant works by prolonging meristem activity of the FMC after the primordia in the outer three whorls have been formed. Unlike the clv1 clv3 double mutant, which shows a phenotype similar to those of the severe clv1 and clv3 single mutants, clv1 fon1 and clv3 fon1 double mutants displayed novel phenotypes that were much more severe than were any of the fon1 or clv single mutants. Although numbers of sepals and petals of the fon1 clv flowers were similar to or only slightly greater than those of clv single mutants, the number of stamens in double mutants were considerably greater than those in the clv1, clv2, clv3, or fon1 single mutants. The number of carpels was also dramatically increased in the double mutants when compared with those of the single mutants. Furthermore, as discussed above, meristem activity was extended more in the double mutants than in the single mutants. Thus, we conclude that CLV genes and FON1 control floral meristem activity and floral organ number through different pathways.

FON1 Is Likely to Function Later than TFL1, LFY, and AP1

The TFL1 gene regulates inflorescence meristem development (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992), and LFY and AP1 control floral meristem identity (Haughn and Somerville, 1988; Irish and Sussex, 1990; Schultz and Haughn, 1991; Huala and Sussex, 1992; Mandel et al., 1992; Weigel et al., 1992; Bowman et al., 1993). We have analyzed the phenotypes of double mutants between fon1 and tf1, ify, or ap1 mutations. The inflorescence structures of double mutants tf1-2 fon1-1, ify-5 fon1-1, ify-6 fon1-1, and ap1-1 fon1-1 are identical to those of tf1-2, ify-5, ify-6, and ap1-1 single mutants, respectively. The differences in phenotype due to fon1 appear only after the formation of the floral primordia. This is consistent with the fon1 single mutant phenotypes, which suggest that FON1 does not act in the inflorescence meristem or in early floral meristems. Therefore, FON1 seems to act later than the initial functions of the meristem genes TFL1, LFY, and AP1.

The tf1 fon1 and ap1 fon1 double mutants both showed additive phenotypes, indicating that there is no genetic interaction between FON1 and these two genes. On the other hand, the situation with ify is more complex. The ify-5 fon1 double mutant exhibited an additive phenotype of the two single mutants, whereas the ify-6 fon1-1 double mutant had the same phenotype as the ify-6 single mutant. It is possible that FON1 function or expression depends on at least some level of LFY function, which seems to be present in the weak ify-5 mutant but not in the more severe ify-6 plants. It is also possible that extended floral meristem activity in the fon1 mutant requires some LFY activity, because LFY is required for floral meristem identity.

FON1 Is Involved in the Control of Stamen and Carpel Fate Selection

The process of cell fate selection is important to the development of any multicellular organism. In Arabidopsis, several genes have been characterized that control organ primordium fate selection. According to the ABC model (Coen and Meyerowitz, 1991; Ma, 1994; Weigel and Meyerowitz, 1994; Yanofsky, 1995), AP2 is an important component of the A function, and AG is the only known C function gene. In addition, A and C functions antagonize each other such that AG is ectopically expressed in ap2 mutants (Bowman et al., 1991a; Drews et al., 1991). Furthermore, AP3, a B function gene, is required for stamen and petal identities. Our results suggest that the FON1 gene also plays a role in controlling the fate of stamen and carpel primordia. The fon1 single mutant phenotype indicates that the normal FON1 function directs the cells at the FMC to develop into gynoecium and inhibits further proliferation of the floral meristem. Furthermore, FON1 prevents the formation of additional stamen interior to the third-whorl stamens. The failure of the fon1 mutant to prevent the formation of such extra stamens seems to be related to the phenomenon of stamen–carpel chimeras.

The increased number of stamens in fon1 mutants suggests that FON1 may regulate the domain of B function. It is possible that expression of AP3 may expand in fon1 mutants toward the center. However, in fon1 single mutants, the number of carpels is either normal or increased, indicating that B function is absent from the center itself. In addition, it was observed that fon1-1 ap3-1 flowers had more staminoid organs than did ap3-1 flowers and that fon1-1 ap3-4 flowers had more carpels than did ap3-4 flowers, indicating that the FMC activity was still extended in ap3 mutant backgrounds and that AP3 is not required for the prolonged floral meristem activity caused by the fon1 mutation. Therefore, the effect of fon1 mutations on AP3 expression may be an indirect consequence of an extended FMC. Furthermore, because ufo has been proposed to affect B function, the absence of interaction between fon1 and ufo also supports the idea that FON1 does not regulate B function per se. On the other hand, the lack of stamen–carpel chimeras and unfused carpels in ap3 fon1 double mutants suggests that B function may be required for these fon1 phenotypes.

Whereas fon1 single mutant flowers still have a center lacking B function, most of the early flowers of the ag-1 fon1-1 double mutant lacked internal sepals, suggesting that they are replaced by petals and that B function has expanded to include the center. This suggests that AG may contribute to the exclusion of AP3 expression from the center, which is consistent with the idea that ectopic expression of AG in ap2 mutants inhibits AP3 expression (Jack et al.,
The phenotypes of fon1 mutants suggest that it is required only at the FMC; however, in the ap2 mutant background, the fon1-1 mutation also has a similar effect in the outer whorls. It is possible that AP2 normally prevents FON1 from being expressed in the outer whorls so that fon1 mutants show no phenotypes in these whorls. Alternatively, the effect of ap2 mutations may be mediated by ectopic AG expression, which is required for stamen and carpel identities.

Because the fon1 phenotypes have some similarities to those of sup mutants and FON1 is closely linked to SUP, it was thought that fon1 mutations might be new sup alleles. Our results from several experiments clearly indicate that FON1 is a distinct gene from SUP. Although we were able to recover the wild-type recombinant chromosome among F2 plants of fon1 × sup crosses, we did not find the sup fon1 double mutant chromosome. It is possible that the double mutant chromosome is not transmitted through the gametophytic generation or that embryos carrying both mutant copies of one gene and one mutant copy of the other were lethal. Further studies are needed to resolve these possibilities.

The fact that double heterozygous plants from both F1 and F2 generations showed fon1-like mutant phenotypes suggests that these two genes interact genetically. FON1 and SUP seem to have related functions, as supported by their similar phenotypes. Because these mutations are recessive, they are likely to be loss-of-function mutations. Therefore, it is likely that the reduction of either gene function by one-half in a single heterozygote does not cause an obvious phenotype, but reduction of both gene functions by one-half does have a phenotypic consequence. It is possible that these genes might control parallel pathways that are functionally redundant. An alternative explanation is that FON1 and SUP are subunits of a protein complex and that half of the normal complex in a single heterozygote is sufficient for function but that a quarter of the normal complex in the double heterozygote is not sufficient.

Conclusions and Prospective

We have identified and characterized mutants of a novel gene, FON1. Our results show that FON1 functions in terminating floral meristem activity after floral organ primordia are formed. The extended floral meristem activity in fon1 mutants results in increased reproductive organ numbers. Our double mutant analysis further indicates that FON1 probably acts at a stage later than the earliest stages requiring the functions of TFL, LFY, and AP1 and that FON1 functions in a pathway different from those controlled by CLV1, CLV2, and CLV3.

The fact that FON1 is required for the termination of floral meristem activity raises questions about possible interactions between FON1 and other genes that are known to be involved in the same process, such as AG and SUP. Furthermore, FON1 also seems to contribute to the control of organ identity of the inner floral primordia. Double mutant analysis suggests that FON1 seems to interact with AG and that it does interact with SUP. On the other hand, there does not seem to be an interaction between FON1 and AP2, AP3, or UFO, although there could be indirect effects. Further genetic and molecular analyses, as well as other approaches, should help to provide additional insights about this new member of an ever-growing list of genes regulating flower development.

METHODS

Plant Growth

Arabidopsis thaliana seed were planted on Metro-Mix 200 (Grace Sierra Horticultural Products Company, Marysville, OH), incubated at 4°C for 3 days, and allowed to germinate under continuous fluorescent illumination at 23°C. Plants were then grown to maturity under the same conditions.

Genetic Materials

The fon1 mutants were segregated from transformants generated in our laboratory. fon1-1 was from a transformant carrying a pMON530-based construct that contains the GPA1 sense cDNA encoding a G protein α subunit. fon1-2 was from a transformant made by C.A. Weiss (American Cyanamid Company, Princeton, NJ). fon1-3 was from a transformant of a construct containing the maize P gene sense cDNA, which was part of a collaboration with E. Grotewold (Cold Spring Harbor Laboratory). clv1-1 (NW 45) and clv2-1 (NW 46) mutants were obtained from the Arabidopsis Stock Centre (Nottingham, UK). clv1-4 and clv3-1 mutants were provided by S. Clark (University of Michigan, Ann Arbor). tf1-2, ap1-1, ap2-9, and ap3-4 are from the Ohio State University Arabidopsis Stock Center (Columbus, OH). Ify-5 and Ify-6 are from D. Wiegel (Salk Institute, La Jolla, CA). ag-1, ap2-1/ap2-2, ap3-1, and sup-1 are from J. Bowman (University of California, Davis), ufo-1 is from J. Levin (California Institute of Technology, Pasadena, CA), and sup-5 is from C. Gasser (University of California, Davis). All mutants used in this study were in the ecotype Landsberg erecta (Ler) background.

Double Mutant Construction

Homozygous fon1-1 plants were used to pollinate homozygous clv1-1, clv1-4, clv2-1, clv3-1, tf1-2, ap1-1, Ify-5, and Ify-6 plants, respectively. F1 progeny of those crosses were all phenotypically normal. F2 seed was collected and sown. For clv and fon1-1 crosses, double mutants were recognized among the F2 plants as those with the novel phenotype of large stigmatic tissue on the top of the pistil and more than eight carpels in one gynoecium. F2 segregation data were consistent with a 9:3:3:1 ratio, as listed below. For clv1-1 × fon1-1, the distribution of F2 plants was 73 wild type, 19 clv1-1, 14 fon1-1, and six clv1-1 fon1-1 (χ² = 4.25; P > 0.1); for clv1-4 × fon1-1, the F2 distribution was 226 wild type, 75 clv1-4, 72 fon1-1, and 24 clv1-4 fon1-1 (χ² = 0.14; P > 0.9). For clv2-1 × fon1-1, the F2 distribution was 126 wild type, 33 clv2-1, 37 fon1-1, and five clv2-1 fon1-1 (χ² =
The ftf1-2 fon1-1, ap2-1 fon1-1, and ify-5 fon1-1 double mutants were also identified from the F2 population by their novel phenotypes. These double mutants had developmental patterns and phenotypes similar to those of ftf1-2, ap2-1, and ify-5 single mutants, except that the number of stamens was increased and some carpel development was seriously affected. The segregation data were also in agreement with a 9:3:3:1 ratio. We found the following F2 distributions: for ftf1-2 × fon1-1, 79 wild type, 24 ftf1-1, 19 fon1-1, and eight ftf1-2 fon1-1 (X² = 0.92; P > 0.7); for ap2-1 × fon1-1, 57 wild type, 20 ap1-1, 16 fon1-1, and six ap2-1 fon1-1 (X² = 0.5; P > 0.9); and for ify-5 fon1-1, 60 wild type, 29 ify-5, 24 fon1-1, and eight ify-5 fon1-1 (X² = 2.81; P > 0.3). There was no novel phenotype observed among F2 plants from the cross between ify-6 and fon1-1. Thus, we planted F2 seed from eight F2 plants with fon1-1 phenotypes. The progeny of five of these eight F2 plants exhibited a segregation pattern with a 3:1 ratio of fon1-1 to ify-6 plants. These F2 plants with the ify-6 phenotype were considered to be ify-6 fon1-1 double mutants.

Double mutants between fon1-1 and ag-1, ap2-1, ap2-2, ap2-9, ap3-1, ap3-4, and ufo-1 were constructed as described below. fon1-1 was crossed as a male with an ag-1/+ heterozygote or plants homozygous for one of the other mutations, and the F1 plants were grown to maturity. For ag-1/+ × fon1-1, F2 progeny of some of the F1 plants segregated for both single mutants. All other F2 progenies also segregated for the expected single mutants. Although some of the F2 plants from several crosses appeared to show novel phenotypes, these phenotypes still resembled the respective mutants that were crossed with fon1-1. Thus, we identified the double mutants among the F3 progeny of phenotypically fon1 F2 plants. Seed from these F2 plants were collected, and their progeny were examined for novel phenotypes or phenotypes resembling the non-fon1-1 mutant of the cross. For each cross, some of the fon1 F2 plants had a 3:1 segregation ratio in the F2 generation of fon1 to novel plants or plants resembling the other mutant in the cross. For example, for fon1-1 × ag-1/+ or fon1-1 × ap2-1, one-quarter of the F2 plants appeared similar to ag-1, except for the lack of internal sepals in early flowers. For fon1-1 × ap2-2, one-quarter of the F2 plants appeared novel with properties of both fon1 and ap2-2 plants. These non-fon1 appearing plants were regarded as double mutants.

For test for allelism between fon1 and sup, three crosses were performed. The distribution of F1 and F2 plants is described as follows. For cross 1 (fon1-1 × sup-1), 16 F1 plants were planted, all being fon1-like; in the F2 generation, 161 were fon1 or fon1-like, 70 were sup, and 33 were normal. For cross 2 (sup-1/+ × fon1-1), 82 F1 plants were planted, with 44 being normal and 38 being fon1-like; in the F2 generation from the fon1-like plants that were planted, 137 were fon1 or fon1-like, 62 were sup, and 29 were normal. For cross 3 (fon1-3 × sup-1), 16 F1 plants were planted, all being fon1-like; in the F2 generation, 139 were fon1, 45 were sup, and 17 were normal.

Polymerase Chain Reaction Analyses of the SUP Gene

We have designed two primers based on the published SUP sequence (Sakai et al., 1995) for polymerase chain reaction (PCR) assays of the SUP genotype: oMC195, 5'-CCGATGTTAGTAAAGGAC-3'; and oMC196, 5'-GGCTGCGTGATCTTTTACAAG-3'. These two primers flank an Ncol site that is destroyed by the sup-1 mutation, and the PCR products were digested and separated on agarose gels to determine the SUP genotypes. In addition, we used two pairs of primers to amplify and clone genomic SUP DNA from Ler, fon1-1, and fon1-3 plants. The first set was for the entire protein coding region: oMC177, 5'-TCGAGATCCCTTTAGGATGAAGTT-3'; and oMC178, 5'-AGCCATG-GAGAGATTACACGC-3'. The second set was for the entire published SUP sequence, including both 5' and 3' untranslated regions and an intron: oMC200, 5'-GGATATAACATAATTAAAGGAC-3'; and oMC201, 5'-ATCTCTCCTCCTCCTTCTTAAAGA-3'. The PCR products were cloned, and two or three clones from each PCR product were sequenced by the chain termination procedure.

Light and Scanning Electron Microscopy

Fresh inflorescences, flowers, and siliques of wild-type and mutant plants were examined using a Zeiss Stemi SV6 dissecting microscope (Carl Zeiss, Thornwood, NY). Siliques were sectioned manually with industrial razor blades. Light micrographs were taken on Kodak films. For scanning electron microscopy (SEM), primary inflorescences of the wild type or mutants at different stages were fixed in 3% glutaraldehyde in 0.025 M SP buffer (sodium phosphate, pH 7.0) at 4°C overnight and then transferred to 1% osmium tetroxide in SP buffer at 4°C overnight. The specimens were washed three times in SP buffer and dehydrated through a graded alcohol series of 40, 60, 75, 85, 95, and 100% ethanol, changed to 100% ethanol once, and then stored in 100% ethanol. Before critical point drying, the 100% ethanol was changed once more. The specimens were critical point dried in liquid carbon dioxide. Individual flowers were removed from inflorescences and mounted on SEM stubs. Organs were dissected from individual flowers with glass needles. The mounted specimens were sputter coated with gold and examined with a scanning electron microscope (model JSM 5300; JEOL U.S.A. Inc., Peabody, MA) at an accelerating voltage of 3 to 10 kV. The images were photographed on Polaroid 55 film.

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