Conservation of Floral Homeotic Gene Function between Arabidopsis and Antirrhinum

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Several homeotic genes controlling floral development have been isolated in both Antirrhinum and Arabidopsis. Based on the similarities in sequence and in the phenotypes elicited by mutations in some of these genes, it has been proposed that the regulatory hierarchy controlling floral development is comparable in these two species. We have performed a direct experimental test of this hypothesis by introducing a chimeric Antirrhinum Deficiens (DefA)/Arabidopsis APETALA3 (AP3) gene, under the control of the Arabidopsis AP3 promoter, into Arabidopsis. We demonstrated that this transgene is sufficient to partially complement severe mutations at the AP3 locus. In combination with a weak ap3 mutation, this transgene is capable of completely rescuing the mutant phenotype to a fully functional wild-type flower. These observations indicate that despite differences in DNA sequence and expression, DefA coding sequences can compensate for the loss of AP3 gene function. We discuss the implications of these results for the evolution of homeotic gene function in flowering plants.

INTRODUCTION

Arabidopsis (Brassicaceae) and Antirrhinum (Scrophulariaceae) are widely divergent dicotyledonous species and belong to different subclasses. Based on morphological criteria and evidence from the fossil record, these species are considered to have diverged at least 70 million years ago (Cronquist, 1981, 1988; Muller, 1981). Although both species have the typical dicot arrangement of concentric whorls of floral organs, both the number and forms of these organs are quite different. The radially symmetric Arabidopsis flower, from outer to inner whorls, consists of four sepals, four petals, six stamens, and two fused carpels that form the pistil (Figures 1C and 1D). Antirrhinum flowers are subtended by a leaflike bract and consist of five sepals, five petals which are fused at the base, four stamens (a fifth stamen primordium aborts early in development), and two fused carpels that form the pistil (Figures 1A and 1B). Antirrhinum flowers are zygomorphic, and this bilateral symmetry is especially evident in the different shapes of the upper and lower petals and in the location of the aborted stamen.

Floral organ identities appear to be established by the action of similar homeotic genes in both of these species. Genetic and molecular analyses of these homeotic genes have provided the basis for the ABC model of floral development (Coen and Meyerowitz, 1991; Meyerowitz et al., 1991). In both species, mutations in the floral homeotic genes generally affect two adjacent whorls of organs; thus, three domains of function can be postulated. The A function is required for the development of sepals and petals, the B function for petal and stamen development, and the C function for stamen and carpel development. The ABC model postulates that floral organ identity is specified by the combination of A, B, or C homeotic gene functions expressed in a particular whorl. In addition, the A and C functions are postulated to regulate negatively each other’s expression. Based on similarities in DNA sequence, expression patterns, and phenotypes elicited by mutations in a number of these homeotic genes, it has been suggested that the regulatory capabilities of these homeotic gene products have been conserved through dicot evolution (Coen, 1991).

Homeotic mutations in either the Globosa (Glo) or Deficiens (DefA) loci in Antirrhinum affect the development of petals and stamens (Sommer et al., 1990; Trobner et al., 1992). In both cases, the mutant flowers display transformations of the five second whorl structures into sepaloid organs. In the third whorl, five fused carpelloid structures arise and fuse to form an enlarged gynoecium. Both the DefA and Glo genes have been cloned, and they encode proteins containing a conserved 58-amino acid region termed the MADS box (Sommer et al., 1990; Trobner et al., 1992). The MADS box–containing domains of transcription factors from several systems have been shown to bind to DNA at a consensus motif, termed the CArG box (Norman et al., 1988; Passmore et al., 1988; Christ and Tye, 1991). In vitro studies have shown that the DefA and Glo gene products can bind to specific CArG motifs as heterodimers (Schwarz-Sommer et al., 1992). One such CArG box is found in the promoter of the DefA gene, suggesting that these MADS box–containing gene products may be autoregulatory (Schwarz-Sommer et al., 1992).
Mutations in the Arabidopsis APETALA3 (AP3) and PISTILLATA (PI) genes also result in the homeotic transformation of petals into sepals, and stamens into carpelloid structures (Bowman et al., 1989; Hill and Lord, 1989; Jack et al., 1992). The AP3 gene encodes a MADS box-containing protein with a high degree of similarity with the Antirrhinum DefA gene product (Jack et al., 1992). Consistent with the mutant phenotype, AP3 transcripts are expressed early in floral development in the cells that are thought to give rise to petals and stamens (Jack et al., 1992). The PI gene appears to be the homolog of the Antirrhinum Glo gene (Goto and Meyerowitz, 1994). Both the PI and AP3 gene products are required for maintaining expression of the AP3 gene (Jack et al., 1994). This observation is consistent with the biochemical evidence from the Antirrhinum system showing that the Glo and DefA gene products form heterodimers (Schwarz-Sommer et al., 1992).

Despite these many similarities between DefA and AP3, another member of this orthologous gene family appears to be required only for petal identity. A null mutation in green petals (gp), a petunia homolog of DefA, affects the development of petals but does not affect stamens (Kush et al., 1993; van der Krol et al., 1993). Furthermore, ectopic expression of gp induces the formation of petaloid organs in place of sepals (Halfter et al., 1994). One explanation for these observations is that the regulatory function encoded by the petunia homolog has diverged from that of Antirrhinum and Arabidopsis.

Here, we describe a direct test of the hypothesis that similar Antirrhinum and Arabidopsis homeotic genes are functionally conserved. We generated transgenic Arabidopsis plants containing a chimeric AP3-DefA coding sequence driven by the Arabidopsis AP3 promoter. We also generated transgenic plants carrying a control construct consisting of the AP3

Figure 1. Antirrhinum and Arabidopsis Wild-Type Flowers.
(A) Wild-type Antirrhinum flower.
(B) Diagram of an Antirrhinum flower. Organs are, from outside to inside, the subtending bract, five sepals, five petals, four stamens (a fifth aborted stamen is indicated by an X), and two fused carpels.
(C) Wild-type Arabidopsis flower.
(D) Diagram of an Arabidopsis flower. Organs are, from outside to inside, four sepals, four petals, six stamens, and two fused carpels. The inflorescence axis is indicated by black dots in (B) and (D).
cDNA was fused to the AP3 genomic fragment at the same site. This fusion resulted in a chimeric gene that contains the AP3 promoter, followed by the AP3 MADS box coding region fused to the C-terminal portion of the DefA coding sequence.

Although the coding regions of DefA and AP3 are highly conserved, especially in the MADS box domain, the promoters have diverged significantly (Figure 3). The AP3 promoter does not contain the large inverted repeat found in the DefA promoter, nor does it contain the putative MADS box binding site CTTTTTAGG (Schwarz-Sommer et al., 1992). Instead, a very similar sequence, CTTTTTGGGG, is found at approximately the same position upstream of the translational start site. We identified several other sequences that may serve as potential MADS box binding sites in the AP3 promoter and that may function as regulatory elements (Figure 3A; S. Carr, C.D. Day, T. Hill, and V.F. Irish, unpublished data). Within the coding regions, the deduced amino acid sequences of AP3 and DefA are 92% identical (53 of 58 amino acids) through the MADS box domain and only 51% identical (87 of 169 amino acids) beyond the MADS box domain (Figure 3B).

Transgenic Arabidopsis plants were generated with all three constructs by using Agrobacterium-mediated transformation of root explants (Valvekens et al., 1988). For each construct, we generated >40 independent transgenic lines and characterized three transgenic lines for each construct (Table 1). The number of independently segregating transgene inserts was estimated by progeny tests. Despite the segregation data suggesting that all tested lines contained a single locus of insertion, DNA gel blot analyses demonstrated that most lines contained multiple, independent insertions of the transgene (Table 1). The relative level of transgene expression was assessed by RNA gel blot analysis and indicated that transgene expression in each line was roughly correlated with the number of transgene inserts (data not shown).

Transgenic plants were used as the pollen donors in crosses to plants homozygous for either ap3-1, ap3-3, or ap3-4. These mutations differed in their severity, with ap3-1 plants showing a weaker phenotype and ap3-3 and ap3-4 mutations resulting in more extreme second and third whorl homeotic transformations (Tables 2 and 3, and Figure 4; Bowman et al., 1989; Jack et al., 1992). The ap3-1 allele is temperature sensitive, and at 22°C, ap3-1 homozygous flowers display four sepals in place of petals and a conversion of the six stamens into carpelloid stamens (Figures 4B and 5C). The ap3-1 lesion is correlated with a single nucleotide change in the K-box, resulting in a conversion of lysine-153 to methionine (Jack et al., 1992). The K-box is predicted to form several amphipathic α-helices that may be required for protein–protein interactions (Ma et al., 1991; Pnueli et al., 1991; Schwarz-Sommer et al., 1992). Flowers homozygous for ap3-3 have four sepals in place of petals. The third whorl organs of ap3-3 plants have a variable phenotype, ranging from free-standing filaments to carpels fused to the central two fourth whorl carpels (Jack et al., 1992). We have rarely observed unfused carpelloid structures in the third whorl of ap3-3 plants (Tables 2 and 3, and Figure 4C). The phenotype of homozygous ap3-4 flowers is similar to that

RESULTS

Generation of Transgenic Arabidopsis Plants Containing AP3 and AP3-DefA Chimeric Genes

Three constructs were generated in the pBin19 binary transformation vector (Bevan, 1984) and are illustrated in Figure 2. pBam21 contains a 9-kb Arabidopsis genomic insert containing the entire AP3 locus, with ~6 kb 5′ and ~1.5 kb 3′ to the coding region. pla19 consists of a translational fusion of a 1.9-kb Arabidopsis genomic fragment to an AP3 cDNA at amino acid position 53, followed by a nopaline synthase translational terminator. pDef7 is similar to pla19, except that a DefA promoter driving the AP3 coding sequence. We observed that the AP3-DefA transgene can partially complement severe mutations at the AP3 locus. Furthermore, the AP3-DefA transgene can partially complement severe mutations to the wild type. These observations demonstrate that the DefA exons and introns within the transcription unit indicated by a jagged arrow. pla19 and pDef7 contain AP3 cDNA and DefA sequences, respectively, which are fused to genomic sequences that include the promoter and part of the 5′ most exon derived from AP3. A nopaline synthase translational terminator (ter) was fused to the 3′ end. AP3 coding sequences are indicated by rightward hatching, and DefA coding sequences are indicated by leftward hatching. Selected restriction sites are BamHI (B), EcoRI (E), and HindIII (H). Left border (LB) and right border (RB) sequences of the T-DNA are indicated by arrowheads.

Figure 2. Plasmid Constructs Used in This Study.

pBam21 contains a 9-kb AP3 genomic region, with the position of the exons and introns within the transcription unit indicated by a jagged arrow. pla19 and pDef7 contain AP3 cDNA and DefA sequences, respectively, which are fused to genomic sequences that include the promoter and part of the 5′ most exon derived from AP3. A nopaline synthase translational terminator (ter) was fused to the 3′ end. AP3 coding sequences are indicated by rightward hatching, and DefA coding sequences are indicated by leftward hatching. Selected restriction sites are BamHI (B), EcoRI (E), and HindIII (H). Left border (LB) and right border (RB) sequences of the T-DNA are indicated by arrowheads.
of ap3-3 (Tables 2 and 3, and Figure 4D). The ap3-3 and ap3-4 mutations appear to be caused by single nucleotide changes resulting in stop codons within the MADS box and presumably abolish function (Jack et al., 1992).

The F1 plants heterozygous for both an ap3 allele and a transgene were allowed to self-fertilize, and kanamycin-resistant progeny were recovered. For each transgenic construct, three independent lines were analyzed for their ability to complement the ap3 mutations. In all cases, plants homozygous for the ap3 allele and heterozygous for the transgenic insert were assayed for their phenotype, and the genotype was confirmed by progeny testing.

AP3 Genomic and cDNA Constructs Rescue the ap3 Mutant Phenotype

Of four transgenic constructs containing the pBam21 construct tested, three lines showed complete rescue to a wild-type floral phenotype of plants homozygous for the ap3-1, ap3-3, and ap3-4 alleles. One pBam21-containing line did not complement the ap3 mutations and was not analyzed further. Complementation of the weak ap3-7 allele by a 6-kb genomic AP3 fragment has also been demonstrated by Okamoto et al. (1994). The three lines containing the ppla19 construct also showed complete rescue of the mutant phenotypes to the wild type (Figure 4E). The transgenic complementation was confirmed by segregation analysis of progeny from candidate rescued plants.

The pla19 construct contains 1.7 kb 5' of the transcriptional start site and 0.24 kb of 3' untranslated sequence, but it does not contain any intron sequences. Therefore, it appears that essentially all of the spatial and temporal regulatory signals required for proper function of the AP3 gene are contained within this 5' region. Surprisingly, a fusion of the AP3 coding sequence to the constitutive cauliflower mosaic virus 35S

Table 1. Transgenic Lines Used in This Study

<table>
<thead>
<tr>
<th>Transgenic Line</th>
<th>Kan/Kan&lt;sup&gt;a&lt;/sup&gt; Segregation</th>
<th>Number of Inserts&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>B6</td>
<td>160:58 (3:1)</td>
<td>4</td>
</tr>
<tr>
<td>B8</td>
<td>66:27 (3:1)</td>
<td>1</td>
</tr>
<tr>
<td>B16</td>
<td>72:27 (3:1)</td>
<td>1</td>
</tr>
<tr>
<td>A6</td>
<td>26:9 (3:1)</td>
<td>2</td>
</tr>
<tr>
<td>A7</td>
<td>45:18 (3:1)</td>
<td>3</td>
</tr>
<tr>
<td>A10</td>
<td>88:36 (3:1)</td>
<td>2</td>
</tr>
<tr>
<td>D3</td>
<td>71:26 (3:1)</td>
<td>2</td>
</tr>
<tr>
<td>D6</td>
<td>94:36 (3:1)</td>
<td>3</td>
</tr>
<tr>
<td>D11</td>
<td>73:17 (3:1)</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Lines designated with B are transgenic for the pBam21 construct; with A, for the pla19 construct; and with D, for the pDela7 construct.

<sup>b</sup> The best fit (x², P > 0.05) segregation of progeny derived from a self of heterozygous plants is given in parentheses. Kan<sup>b</sup>, kanamycin resistant; Kan<sup>a</sup>, kanamycin sensitive.

<sup>c</sup> Number of inserts is estimated from number of bands hybridizing on DNA gel blots.
Table 2. Summary of Second Whorl Homeotic Transformations in Control and Transgenic Plants

<table>
<thead>
<tr>
<th>Genotype (N)</th>
<th>Second Whorl Sepaloid Petals (%)</th>
<th>Second Whorl Petaloid Sepals (%)</th>
<th>Second Whorl Sepals (%)</th>
<th>Average Number of Second Whorl Organs per Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>ap3-1 (18)</td>
<td>0</td>
<td>100</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>ap3-3 (16)</td>
<td>0</td>
<td>100</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>ap3-3 D6 (21)</td>
<td>52</td>
<td>48</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ap3-3 D11 (15)</td>
<td>0</td>
<td>67</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>ap3-4 (17)</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>ap3-4 D3 (21)</td>
<td>73</td>
<td>58</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>ap3-4 D6 (22)</td>
<td>73</td>
<td>73</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>ap3-4 D11 (18)</td>
<td>0</td>
<td>67</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

The percentage of different organ types recovered and the average number of organs per whorl scored in dissected flowers from each genotype are listed. N, number of flowers scored; the number given in parentheses.

Complementation of \textit{ap3} Mutant Phenotypes by the Chimeric AP3-DefA Construct

Transgenic plants containing the pDef7 construct were also crossed to plants homozygous for the \textit{ap3-1}, \textit{ap3-3}, or the \textit{ap3-4} allele. For all three of the transgenic lines examined, similar results were obtained. The pDef7 construct is sufficient to rescue plants homozygous for \textit{ap3-1} to a wild-type phenotype (Figure 4F). These pDef7 \textit{ap3-1} flowers are fertile and have normal seed set.

The pDef7 transgene partially complements the strong \textit{ap3-3} or \textit{ap3-4} lesions. The phenotype produced by \textit{ap3-3} plants carrying the pDef7 transgene is less severe than that of \textit{ap3-3} homozygous mutants. The pDef7 \textit{ap3-3} plants have second whorl organs that are generally petaloid sepals or sepaloid petals (Table 2 and Figure 4G). The epidermal cells in these second whorl organs have a shape and size very similar to these of wild-type petal cells but do not appear quite as round or regular (Figure 5E). Furthermore, these transgenic second whorl organs do not appear to be mosaic organs of different cell types. In pDef7 \textit{ap3-3} flowers, the most frequent third whorl phenotype consists of freestanding carpelloid stamens, often with distinct locules, that occasionally have ovules along the lateral edges of each organ (Table 3 and Figures 5G to 5I).

In the pDef7 \textit{ap3-4} flowers, we observed a phenotype that is milder than that of \textit{ap3-4} flowers (Tables 2 and 3, and Figures 4H and 5J). These phenotypes are similar to those produced by pDef7 \textit{ap3-3} plants. In addition, we observed a slight difference in the frequency of these phenotypes with different pDef7 lines (Tables 2 and 3). Plants homozygous for \textit{ap3-3} or \textit{ap3-4} carrying the D6 transgene showed slightly less severe phenotypes than those carrying the D3 or D11 transgene.

Some Transgenic Lines Show a Cosuppressed Phenotype

Approximately 10% of the transgenic lines that we generated displayed a dominant cosuppressed phenotype. These included second whorl organs have a shape and size very similar to these of wild-type petal cells but do not appear quite as round or regular (Figure 5E). Furthermore, these transgenic second whorl organs do not appear to be mosaic organs of different cell types. In pDef7 \textit{ap3-3} flowers, the most frequent third whorl phenotype consists of freestanding carpelloid stamens, often with distinct locules, that occasionally have ovules along the lateral edges of each organ (Table 3 and Figures 5G to 5I).

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Table 3. Summary of Third Whorl Homeotic Transformations in Control and Transgenic Plants

<table>
<thead>
<tr>
<th>Genotype (N)</th>
<th>Third Whorl Carpelloid Stamens (%)</th>
<th>Third Whorl Stamened Carpels (%)</th>
<th>Third Whorl Fused Carpels (%)</th>
<th>Third Whorl Filaments (%)</th>
<th>Average Number of Third Whorl Organs per Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>ap3-1 (18)</td>
<td>84</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>5.6</td>
</tr>
<tr>
<td>ap3-3 (16)</td>
<td>0</td>
<td>3</td>
<td>89</td>
<td>8</td>
<td>3.9</td>
</tr>
<tr>
<td>ap3-3 D6 (21)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5.6</td>
</tr>
<tr>
<td>ap3-3 D11 (15)</td>
<td>89</td>
<td>0</td>
<td>1</td>
<td>10</td>
<td>4.7</td>
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<td>ap3-4 (17)</td>
<td>0</td>
<td>0</td>
<td>61</td>
<td>39</td>
<td>4.1</td>
</tr>
<tr>
<td>ap3-4 D3 (21)</td>
<td>30</td>
<td>70</td>
<td>0</td>
<td>0</td>
<td>4.7</td>
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<tr>
<td>ap3-4 D6 (22)</td>
<td>53</td>
<td>44</td>
<td>0</td>
<td>3</td>
<td>5.5</td>
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<tr>
<td>ap3-4 D11 (18)</td>
<td>21</td>
<td>51</td>
<td>2</td>
<td>26</td>
<td>5.4</td>
</tr>
</tbody>
</table>

\(^a\) Notations are as given in Table 2.
Figure 4. Phenotypes of ap3 Mutant and Transgenic Arabidopsis Flowers.
(A) Wild-type flower.
(B) to (D) ap3-1, ap3-3, and ap3-4 homozygous mutant flowers, respectively.
(E) Flower from a homozygous ap3-3 mutant plant carrying the A6 transgene shows complete rescue of the mutant phenotype.
(F) Flower from a homozygous ap3-1 mutant plant carrying the D3 transgene also shows complete rescue.
(G) Flower from a homozygous ap3-3 mutant carrying the D6 transgene shows partial rescue, as compared with the flower in (C).
(H) Flower from a homozygous ap3-4 mutant carrying the D3 transgene also shows partial rescue, as compared with the flower in (D).
Scale bar in (A) = 0.5 mm for (A) to (H).

three pBam21, four pla19, and five pDef7 lines. The severity of the cosuppressed phenotype varied between lines as well as within individual plants of any one line. In the most severely affected lines, the mutant phenotype resembled that produced by a strong ap3 allele. Lines showing moderate phenotypes displayed sepaloid petals and partially transformed stamens. Some lines were weakly fertile, had nearly normal organ development, and showed some defects only in the size and shape of the stamens. These were characterized as having weakly cosuppressed phenotypes. In addition, most of the cosuppressed lines had flowers that showed a reversion to a wild-type phenotype. This reversion would often extend through several flowers along an inflorescence.

DISCUSSION

Arabidopsis and Antirrhinum Homeotic Gene Functions Are Conserved

We showed that coding sequences from the Antirrhinum DefA gene can functionally complement the Arabidopsis ap3-1 mutation. This functional test demonstrated that the chimeric DefA gene product that we used can interact appropriately with various Arabidopsis proteins to confer a normal developmental pattern to the Arabidopsis flower. However, the pDef7 transgene did not completely complement the strong ap3-3 and ap3-4 mutations. Plants carrying both the pDef7 transgene and a strong ap3 mutation showed defects in both second and third whorl organs, but these transformations were much milder than those produced by strong ap3 mutations alone. In fact, one pDef7-containing line showed almost complete rescue of strong mutations at the AP3 locus (D6; see Tables 2 and 3). The D6 transgenic line produced an almost full restoration of the second whorl to a wild-type phenotype and partial restoration of third whorl organs (Figures 4G and 5G to 5I).

The chimeric pDef7 construct that we used to rescue mutant ap3 plants contains essentially all of the MADS box domain derived from AP3, with other sequences, including the K-box, derived from the DefA coding region. Thus, the lack of complete rescue seen in the complementation experiments with strong ap3 alleles is due to sequence differences outside the MADS box. This observation indicates that specificity of MADS box gene function is modulated by sequences outside the MADS box. However, the regulatory specificity of homeobox-containing genes in Drosophila appears to depend primarily on the specificity of the homeodomain (Kuziora and McGinnis, 1989; Mann and Hogness, 1990). Thus, the mechanisms by which the regulatory specificities of MADS box-containing and homeodomain-containing homeotic genes are specified appear to be distinct.

The partial rescue of strong ap3 mutations by the pDef7 transgene can be explained in at least two ways. The pDef7 transgenic product may be able to activate completely only a subset of downstream genes. This model is supported by
Figure 5. Cellular Phenotypes of Wild-Type, Mutant, and Transgenic Arabidopsis Flowers.

(A) to (E) Abaxial surface view of first or second whorl organs. In (A), wild-type (Landsberg erecta) second whorl petal cells are shown. In (B), wild-type first whorl sepal cells are shown. In (C), $ap3-1$ second whorl cells at 22°C resemble sepal cells. Note elongated epidermal cells and stomata. In (D), $ap3-3$ second whorl cells with sepal-like characteristics are shown. In (E), second whorl cells produced by an $ap3-3$ plant carrying the D6 transgene are shown. The size and shape of epidermal cells are similar to petal cells; note the lack of stomata.

(F) to (J) Third whorl organs. In (F), a wild-type stamen during anthesis is shown. In (G) to (I), third whorl organs with increasingly carpelloid characteristics from transgenic $ap3-3$ plants containing the D6 transgene are shown. In (J), a filamentous third whorl organ from a transgenic $ap3-4$ plant containing the D3 transgene is shown.

Scale bar in (A) = 10 μm for (A) to (E). Scale bar in (F) = 100 μm for (F) to (J).

the observation of intermediate cellular phenotypes in both the second and third whorls. These mosaic organs do not appear to be a mixture of different tissue types, as seen with some of the floral homeotic mutations (Bowman et al., 1989; Irish and Sussex, 1990); rather, the cells themselves appear to have an intermediate shape and size. One interpretation of this intermediate cellular phenotype is that the pDef7 transgene can appropriately regulate some but not all of the downstream genes required to elaborate the cellular architecture. Another possible explanation of the partial rescue results is that the chimeric transgene can participate as readily in heterodimer formation (Schwarz-Sommer et al., 1992; Trobner et al., 1992). The lack of complete rescue of strong mutant $ap3$ alleles by the pDef7 transgene suggests that this transgene may not be able to participate as readily in heterodimer formation. Because the AP3 protein is thought to be stabilized by heterodimerizing with PI through the K-box domain (Goto and Meyerowitz, 1994; Jack et al., 1994), it seems possible that the sequence differences we have introduced have attenuated the stability of such heterodimeric complexes.

Conservation of AP3 and DefA Target Sequences

The DefA domain used in these experiments includes the K-box, which is thought to be required for heterodimer formation (Schwarz-Sommer et al., 1992; Trobner et al., 1992). The AP3 and DefA gene products are thought to autoregulate and so presumably bind to sequences present in the respective promoter regions (Schwarz-Sommer et al., 1992; Jack et
Cloning and Sequencing

DNA manipulations were performed according to standard techniques. Four APETALA3 (AP3) genomic clones were recovered by screening a JFX genomic Landsberg erecta library (a gift from B. Hauge, Massachusetts General Hospital, Boston, MA) with pDef1B (kindly provided by Z. Schwarz-Sommer, Max-Planck-Institut für Züchtungsforschung, Cologne, Germany) (Sommer et al., 1990). Hybridizations were performed at low stringency with washes at 60°C in 0.3 M NaCl, 20 mM sodium phosphate, 2 mM EDTA, 0.5% SDS, pH 7.0. A 1.9-kb HindIII fragment corresponding to the MADS box and 5' upstream region of AP3 was subcloned into pBluescribe (Stratagene) to give pV15. This 1.9-kb region was sequenced using a combination of XbaI or SacI subclones in pBluescript II SK+ (Stratagene) and specific primers. Both strands were sequenced in double-stranded sequencing reactions using Sequenase (U.S. Biochemical). The AP3 promoter sequence has GenBank accession number of U30729.

Plasmid Constructions

pBam21 was generated by subcloning a BamHI genomic fragment containing the AP3 gene into pBin19 (Bevan, 1984). Three AP3 cDNA clones were obtained by screening a Landsberg erecta inflorescence cDNA library (Yamamoto et al., 1995) at high stringency with washes at 0.1 x SSC (1 x SSC is 0.15 M NaCl, 0.015 sodium citrate), 0.1% SDS at 65°C. One full-length AP3 cDNA, pla, was completely sequenced, and the coding region is identical to that published by Jack et al. (1992). The pla19 construct was generated by subcloning a HindIII-Smal pla fragment corresponding to the 3' end of the AP3 cDNA into a HindIII-Smal cut pBin19 cauliflower mosaic virus 35S promoter/
nopaline synthase terminator vector (kindly provided by M. Conkling, North Carolina State University, Raleigh, NC). This intermediate construct was then cut with HindIII, and the 1.9-kb genomic HindIII fragment corresponding to the promoter and 5' end of the AP3 gene was inserted to give pDef1B. pDef7 was generated in a similar manner, except that a HindIII-EcoAV fragment of the pDeflB clone was used for subcloning into the pBin19/35S/nopaline synthase terminator vector.

Generation and Analysis of Transformants

Constructs generated in the binary pBin19 vector were transferred to Agrobacterium tumefaciens LBA4404 by electroporation. Arabidopsis thaliana wild-type (Nossen ecotype) roots were transformed using standard procedures (Valvekens et al., 1986). Kanamycin-resistant plantlets were rooted, and seed was collected from putative transformants. The presence of the transgene was confirmed by segregation of the kanamycin resistance trait to progeny, as well as by DNA gel blotting with probes corresponding to AP3 or Deficiens (DefA) sequences. RNA gel blot analyses were performed using standard methods, with total RNA extracted using Trizol (Gibco BRL). Transgenic plants were used as pollen donors in crosses to ap3 mutant plants. Tissue was prepared for scanning electron microscopy as previously described (Irish and Sussex, 1990).

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Conservation of floral homeotic gene function between Arabidopsis and antirrhinum.

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