Functional Interaction between the Homeotic Genes \textit{fbp1} and \textit{pMADS1} during Petunia Floral Organogenesis

Gerco C. Angenent,\textsuperscript{1} Marco Busscher, John Franken, Hans J. M. Dons, and Arjen J. van Tunen

Department of Developmental Biology, DLO-Center for Plant Breeding and Reproduction Research (CPRO-DLO), PO. Box 16, 6700 AA Wageningen, The Netherlands

The petunia MADS box floral binding protein (\textit{fbp}) gene 1 represents a class B homeotic gene determining the identity of second and third floral whorl organs. Suppression of \textit{fbp1}, which is highly homologous to the Antirrhinum gene \textit{globosa} and Arabidopsis gene \textit{pistillata}, results in the conversion of petals to sepals and stamens to carpels. In contrast to \textit{fbp1}, the petunia homeotic gene \textit{pMADS1}, encoding a protein homologous to the Antirrhinum protein \textit{DEFICIENS}, has been shown to be involved in the formation of petals only. We demonstrated that the induction of \textit{fbp1} is established independent of \textit{pMADS1}, whereas at later developmental stages, \textit{fbp1} is up-regulated by \textit{pMADS1} in petals. On the other hand, the induction and maintenance of \textit{pMADS1} expression are not affected by \textit{fbp1}. To obtain information about the functional interaction between \textit{fbp1} and \textit{pMADS1}, an \textit{fbp1} cosuppression mutant with mild phenotypic alterations was crossed with a green petals (\textit{gp}) mutant in which \textit{pMADS1} expression was abolished. Progeny plants, heterozygous for the \textit{pMADS1} gene, had flowers with a more pronounced reversion from petals into sepals than was observed for the parent \textit{fbp1} mutant. The morphology of the third whorl organs was not changed. These observations, together with expression levels of \textit{pMADS1} and \textit{fbp1} in mutant flowers, provide evidence for functional control of \textit{fbp1} by \textit{pMADS1} in vivo.

INTRODUCTION

Although flowers from angiosperms exhibit a variety of forms, the identity of the floral organs (sepals, petals, stamens, and carpels) and their arrangement in separate whorls basically coincide. This suggests that the mechanisms controlling the identity of the floral organs are highly conserved among flowering plants, raising questions about how this process is regulated and how the genes involved interact. Analysis of homeotic mutants from Arabidopsis and Antirrhinum has led to a model suggesting that the identity of the floral organs is determined by the action of three classes of homeotic genes (for review, see Coen and Meyerowitz, 1991). Mutations in these regulatory genes result in homeotic conversions in two adjacent whorls. Isolation of some of these genes from various species revealed that cognate homologs are present in a large number of dicots, such as Arabidopsis (Yanofsky et al., 1990; Ma et al., 1991; Jack et al., 1992; Goto and Meyerowitz, 1994), Antirrhinum (Sommer et al., 1990; Tröbner et al., 1992; Bradley et al., 1993), tomato (Pnueli et al., 1991), tobacco (Hansen et al., 1993), and petunia (Angenent et al., 1992, 1993, 1994; Kush et al., 1993; Tauchimoto et al., 1993), as well as in monocot species, such as maize (Schmidt et al., 1993).

For both Antirrhinum and Arabidopsis, we know two class B homeotic genes that are required to specify petal and stamen identity. The mutant phenotypes of these genes, \textit{deficiens} (\textit{defA}) and \textit{globosa} (\textit{glo}) for Antirrhinum and \textit{apetala3} (\textit{ap3}) and \textit{pistillata} (\textit{pi}) for Arabidopsis, are very similar, with organ transformations moving from petals to sepals and stamens to carpels (Sommer et al., 1990; Jack et al., 1992; Tröbner et al., 1992; Goto and Meyerowitz, 1994). Recently, we observed a similar phenotype when the petunia floral binding protein (\textit{fbp}) gene 1, which is highly homologous to \textit{pi} and \textit{glo} (Angenent et al., 1992, 1993), was cosuppressed. \textit{fbp1} mRNA could be detected throughout petal and stamen development; however, immunolocalization experiments revealed that \textit{FBP1} protein is absent at late stages of stamen development (Cañas et al., 1994). A petunia MADS box gene with a high degree of similarity with \textit{ap3} and \textit{defA} was recently isolated by van der Krol et al. (1993) and designated \textit{pMADS1}. Table 1 summarizes the class B homeotic genes from petunia and their homologs in Arabidopsis and Antirrhinum. In contrast to \textit{ap3} and \textit{defA}, \textit{pMADS1} is required to specify the identity of the second whorl only and is not involved in formation of the third whorl stamens. In the green petals (\textit{gp}) mutant (line PLV), which shows homeotic transformation of petals to sepals, the \textit{pMADS1} gene is deleted. We used another \textit{gp} mutant (line R100) that was obtained by ethyl methanesulfonate treatment of the petunia line R82 and exhibits the same homeotic reversions as line PLV (de Vlaming et al., 1984).

\textsuperscript{1} To whom correspondence should be addressed.
The products of these homeotic genes belong to the family of putative transcription factors, with the MADS box DNA binding domain at their N-terminal end (Schwarz-Sommer et al., 1990). Recently, Cañas et al. (1994) demonstrated the presence of the MADS box protein FBP1 inside the nucleus, which is in line with the function of MADS box proteins as a transcription factor. A similar observation was made for AP3 by Jack et al. (1994). Furthermore, in vitro DNA binding studies have shown that the MADS box proteins GLO and DEFA specifically bind to DNA sequences as heterodimers. Efficient binding has been observed using defA and glo promoter sequences that resemble the putative target sequence (CC[A/T]6GG) for MADS box proteins (Schwarz-Sommer et al., 1992; Tröbner et al., 1992). These data support the hypothesis that the up-regulation and maintenance of expression of both genes are established by autoregulatory control of transcription by a heterodimer formed between the DEFA and GLO proteins. A similar autoregulatory mechanism seems to control the expression of the Arabidopsis class B homeotic genes pi and ap3 (Jack et al., 1992, 1994; Goto and Meyerowitz, 1994).

Although counterparts of the Arabidopsis and Antirrhinum class B genes are present in petunia, the action and interaction seem different (van Tunen and Angenent, 1991). The defAlap3 homolog pMADS1 is functional in the second whorl only (van der Krol et al., 1993) and up-regulates the expression of fbp7 in this whorl (Angenent et al., 1992). Furthermore, two genes in petunia, fbp7 and pMADS2, share a high degree of homology with the glo and pi genes (see Table 1; Angenent et al., 1992; Kush et al., 1993). Recently, we cloned the cDNA of the MADS box gene fbp7, which appears to be identical to pMADS2 (Angenent et al., 1994). Hereafter, we refer to this gene as pMADS2. We are interested in understanding the mechanisms by which these petunia class B genes interact and regulate each other's expression. In this study, we discuss our analysis of the expression patterns of fbp7, pMADS1, and pMADS2 in the background of gp and fbp7 mutants. These data provide us with new insights into the mechanisms determining petal and stamen formation in petunia. Our emphasis is on the differences in the interactions among class B genes in petunia, Arabidopsis, and Antirrhinum.

### RESULTS

#### Expression of fbp7 and pMADS1 in Wild-Type Flowers

Expression of petunia pMADS1 is required to specify the identity of the second whorl organs (van der Krol et al., 1993), but no data are available on the temporal and spatial expression at stages when organ primordia are formed. Hence, we studied the in situ distribution of pMADS1 mRNA in young floral buds of wild-type plants (W15) and compared it with the accumulation of fbp7 mRNA. Hybridization with a pMADS1 antisense RNA probe revealed strong signals in regions of the floral meristem that give rise to petal and stamen primordia (Figure 1A). No hybridization signal was observed in the center of the floral meristem. At later developmental stages, when the second and third organ primordia were visible, high levels of pMADS1 mRNA were present in these types of organ primordia (Figure 1B). The organ primordia of the fourth whorl were not yet detectable at this stage. This distribution matched exactly the fbp7 expression pattern observed in consecutive sections of the same young floral buds (Figures 1E and 1F). This analysis of fbp7 expression by in situ hybridization precisely confirms our previously reported results using transgenic plants expressing the β-glucuronidase reporter gene under the control of the fbp7 promoter (Angenent et al., 1993). In older buds (Figures 1C, 1D, 1G, and 1H), both pMADS1 and fbp7 mRNAs were still present in the developing petals and stamens. In summary, these in situ hybridization results illustrate that the temporal and spatial expression patterns of fbp7 and pMADS1 match during early stages of organogenesis and are restricted to the second and third floral whorl.

#### Expression of fbp7 and pMADS1 in an fbp7 Cosuppression Mutant

To analyze the effect of fbp7 suppression on pMADS1 expression, in situ hybridizations were performed on sections of the severe fbp7 cosuppression plant (T8007S; Angenent et al., 1993). The flowers of T8007S plants showed sepaloid organs in the second whorl, and stamens were converted into carpels. As previously shown by gel blot analysis of RNA isolated from young floral buds, fbp7 was not expressed in this T8007S mutant. Also, in situ hybridization experiments did not show any fbp7 expression in floral buds with emerging petal and stamen primordia (Figure 1I) or in older buds (Figure 1J), confirming the RNA gel blot hybridization results. In contrast, hybridization of two sections from young T8007S floral buds (Figures 1K and 1L) with the pMADS1 probe revealed pMADS1 mRNA levels comparable to those observed in young wild-type floral buds. Hybridization signals were detectable in regions

---

**Table 1. Class B Homeotic Genes from Petunia and Their Corresponding Mutants and Homologs in Arabidopsis and Antirrhinum**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutant</th>
<th>Phenotype</th>
<th>Arabidopsis</th>
<th>Antirrhinum</th>
</tr>
</thead>
<tbody>
<tr>
<td>fbp7</td>
<td>T8007</td>
<td>Green tips on petals, stigma on anther</td>
<td>pi</td>
<td>glo</td>
</tr>
<tr>
<td></td>
<td>T8007S</td>
<td>Sepals in whorl 2, carpels in whorl 3</td>
<td>pi</td>
<td>glo</td>
</tr>
<tr>
<td>pMADS2</td>
<td>gp</td>
<td>Sepals in whorl 2</td>
<td>pi</td>
<td>glo</td>
</tr>
<tr>
<td>pMADS1</td>
<td>defA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* T8007 and T8007S are fbp7 mutants obtained by cosuppression.
* Changes in phenotype compared with wild-type petunia flowers are indicated.
Wild-Type

$pMADS1$

**fbp1 mutant**

**green petals mutant**

$pMADS1$

**fbp1**

Figure 1. Localization of $pMADS1$ and $fbp1$ mRNAs in Floral Buds from Wild-Type Plants and $fbp1$ and $gp$ Mutants.

Longitudinal sections were hybridized to antisense digoxigenin-labeled RNA probes from $pMADS1$ or $fbp1$.

(A) to (D) Distribution of $pMADS1$ transcripts during different stages of wild-type flower development.

(E) to (H) Distribution of $fbp1$ transcripts during different stages of wild-type flower development. Consecutive sections were used for the hybridization with the $fbp1$ and $pMADS1$ probe.

(I) and (J) Distribution of $fbp1$ transcripts in floral buds from the $fbp1$ cosuppression mutant (T8007S).

(K) and (L) Distribution of $pMADS1$ transcripts in floral buds from the $fbp1$ cosuppression mutant (T8007S).

(M) and (N) Distribution of $pMADS1$ transcripts in floral buds from the $gp$ mutant.

(O) and (P) Distribution of $fbp1$ transcripts in floral buds from the $gp$ mutant. A detail of a floral bud with a total length of 5 mm is shown in (P). Bright-field micrographs are shown in (D), (H), (M), (O), and (P); dark-field micrographs are shown in (A) to (C), (E) to (G), (I) to (L), and (N). Floral whorls are numbered. Bars = 0.5 mm.
Figure 2. Cross between the gp Mutant and the fbp1 Cosuppression Plant T8007.
pMADS7 is not sufficient to specify organ identity in these early stages of organ development in the gp mutant. The identity of the third whorl is determined by the expression of pMADS7 in the second and third whorls of the wild-type flowers (Figure 40). At this early developmental stage, the expression levels in the second and third whorl organs were comparable with expression of fbp7 in wild-type flowers (Figure 4F). Because no expression of pMADS7 was detectable in these early stages of organ development in the gp mutant (Figure 1M), these expression data provide evidence that the induction of fbp7 expression is established independent from pMADS1.

At later developmental stages, fbp7 mRNA was no longer detectable in the sepaloid organs of the second whorl, whereas fbp7 was still expressed in the developing stamens (Figure 1P). This observation is in line with RNA gel blot analysis showing a very low expression level in the second whorl and a high expression level in the third whorl at late stages of flower development (Angenent et al., 1992).

Interaction between fbp7 and pMADS1 in Vivo

The functional interactions between two petunia class B homeotic genes, pMADS1 and fbp7, were analyzed using a genetic approach. The recessive gp mutant (R100), in which pMADS1 expression was abolished, was crossed with a mild fbp7 mutant (T8007) obtained by cosuppression of fbp7 (Angenent et al., 1993). The phenotypes of these mutants are described in Table 2, and representative flowers are shown in Figure 2. Because the homeotic transformations in T8007 were only limited (small green tips on the petals and short style–stigma structures on the anthers), the effect of a reduction of pMADS1 expression on T8007 petal morphology could be monitored and used as a way to analyze the interaction between fbp7 and pMADS1. Because the identity of the third whorl is determined by fbp7 and not by pMADS1, the phenotype of the anthers can be employed as an internal control in the interaction experiments using fbp7/gp heterozygous double mutants.

The cross gp × T8007 resulted in two classes of offspring plants, as illustrated in Table 2 and Figure 2A. These plants were all heterozygous for pMADS1 and are designated Gg. Approximately half of the progeny plants possessed flowers with whorl two organs, with a more pronounced conversion from the petal toward the sepal. These second whorl organs were smaller and consisted of large green sectors with

<table>
<thead>
<tr>
<th>Lina/Cross*</th>
<th>Genotype</th>
<th>Phenotype Second Whorl</th>
<th>Phenotype Third Whorl</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ R82</td>
<td>GG – –</td>
<td>Petal</td>
<td>Stamen</td>
</tr>
<tr>
<td>+ gp</td>
<td>gg – –</td>
<td>Sepal</td>
<td>Stamen</td>
</tr>
<tr>
<td>T8007</td>
<td>GGF* –</td>
<td>Small green tips on petals</td>
<td>Stigma on anther</td>
</tr>
<tr>
<td>+ gp × T8007</td>
<td>Ggf –</td>
<td>Severely sepaloid</td>
<td>Stigma on anther</td>
</tr>
<tr>
<td>+ R82</td>
<td>GG – –</td>
<td>Petal</td>
<td>Stamen</td>
</tr>
<tr>
<td>+ T8007</td>
<td>GGF* –</td>
<td>Small green tips on petals</td>
<td>Stigma on anther</td>
</tr>
<tr>
<td>R82</td>
<td>GG – –</td>
<td>Petal</td>
<td>Stamen</td>
</tr>
</tbody>
</table>

*The petunia lines used were R82 (wild type), the gp mutant (R100), and T8007 (fbp7 cosuppression mutant). The genotypes refer to the green petals locus (Gg) and fbp7 cosuppression trait (F*). A representative flower of the plants designated + are shown in Figure 2.

Figure 2. (continued).

(A) Schematic presentation of the cross between gp and T8007 mutants and the F1 progeny. The gp mutant (line R100) was obtained by ethyl methanesulfonate treatment of line R82 (1). The genotype of each plant with respect to the green petals locus (GG, Gg, or gg) and the fbp7 cosuppression trait (F* – or – –) is presented. The cosuppression phenotype is inherited as a dominant trait, whereas the gp mutation is recessive. A representative flower of the wild-type R82 line is shown.

(B) Flower morphology of the gp parent plant (gg – –), a representative flower showing the T8007 phenotype in an R82 background (GGF* –), and flowers of the F1 progeny plants (Ggf* – and Gg – –). To compare the flower morphology of the T8007 cosuppression plant with that of the F1 progeny plants, a cross was made between T8007 and R82 to obtain plants with the fbp7 cosuppression trait in an R82 background (GGF* –). Bars = 1 cm.

(C) Details of the anthers and pistils from the same flowers as shown in (B). The arrows indicate the style–stigma structures on top of the anthers.
trichomes on both sides of the sepaloid petal. The anthers of these flowers were like T8007 anthers with short style-stigma structures (Figures 2B and 2C). Plants with the genotype GgF*- contained the same set of eight copies of the chimeric cosuppression gene as did their T8007 parent (results not shown). The other half of the progeny plants had flowers with no changes in organ morphology; their genotype is given as Gg- - (Table 2; Figure 2). DNA gel blot analysis revealed that these plants did not contain the cosuppression transgenes (results not shown). The same RNA samples were used to analyze the fbp1 mRNA accumulation in these plants. In wild-type R82 flowers, fbp1 is transcribed in the second and third whorls at comparable levels. In the gp mutant, the expression of fbp1 in the sepaloid petals was dramatically reduced but not completely abolished, whereas the expression in the stamens was unaffected (see also Angenent et al., 1992). In the fbp1 cosuppression plants (GGF*- and GgF*-), expression of fbp1 was almost completely abolished. fbp1 mRNA accumulation in the second whorl organs of GGF*- plants was even lower than that in third whorl organs, suggesting that a dosage effect of P-MADS1 reduces the level of remaining fbp1 expression in the second whorl. This reduction in fbp1 accumulation in the GGF*- plants reflects the more pronounced homeotic reversions observed in the second whorl of the flowers (Figure 2B). The Gg- - plant, which exhibited no phenotypic changes in the flower, expressed fbp1 at normal levels in the third whorl and only at a slightly lower level in petals.

The accumulation of the second glo/pi-like gene in petunia, pMADS2, matches exactly the expression patterns observed for fbp1 in wild-type plants and the gp mutant (R100).

Expression of MADS Box Genes in gp, fbp1, and Heterozygous Double Mutants

RNA gel blot analyses were performed to investigate the expression patterns of the MADS box genes fbp1, fbp2, pMADSI, and pMADS2 in various homeotic mutants. The fbp2 gene was transcribed in the second control because its expression is not dependent on fbp1 or P-MADS1 (Angenent et al., 1992, 1993). These data provide information on the interdependent regulation of class B homeotic genes in petunia. Figure 3 shows RNA gel blot analysis of pMADSI, fbp1, pMADS2, and fbp2 in flowers from R82 (GG- -), gp (R100; gg- -), fbp1 cosuppression mutant (T8007) in the R82 background (GGF*- -), and the F1 offspring plants (GGF*- and Gg- -) from the cross between gp and T8007. The flowers and whorl three organs of these plants are shown in Figures 2B and 2C.

Comparable hybridization signals were observed in petals and stamens of wild-type R82 flowers using a pMADSI-specific probe (Figure 3). In the gp (R100) mutant, expression of this gene was almost completely abolished. Longer exposure revealed a very weak hybridizing signal in stamens, suggesting that line R100 might not be a complete null mutant for pMADS1. In contrast to fbp1, P-MADS1 is normally expressed in the second and third floral whorl of the fbp1 cosuppression mutant (T8007; GGF*- -). This clearly demonstrates that during these stages of flower development, pMADSI transcription is not under the control of fbp1. The reduced expression levels in GGF*- and Gg- - plants might reflect the heterozygous state of the pMADSI gene in these plants.

The same RNA samples were used to analyze the expression pattern of fbp1 in these plants. In wild-type R82 flowers, fbp1 is transcribed in the second and third whorls at comparable levels. In the(gp) mutant, the expression of fbp1 in the sepaloid petals was dramatically reduced but not completely abolished, whereas the expression in the stamens was unaffected (see also Angenent et al., 1992). In the fbp1 cosuppression plants (GGF*- and GgF*-), expression of fbp1 was almost completely abolished. fbp1 mRNA accumulation in the second whorl organs of GGF*- plants was even lower than that in third whorl organs, suggesting that a dosage effect of P-MADS1 reduces the level of remaining fbp1 expression in the second whorl. This reduction in fbp1 accumulation in the GGF*- plants reflects the more pronounced homeotic reversions observed in the second whorl of the flowers (Figure 2B). The Gg- - plant, which exhibited no phenotypic changes in the flower, expressed fbp1 at normal levels in the third whorl and only at a slightly lower level in petals.

The accumulation of the second glo/pi-like gene in petunia, pMADS2, matches exactly the expression patterns observed for fbp1 in wild-type plants and the gp mutant (R100).
Figure 4. Morphology of a gp/fbpl Double Mutant Flower.

(A) A representative flower of a gp/fbpl double mutant obtained by a cross between the gp mutant and the F1 plant GgF*-+. The double mutant is homozygous for gp and contains the fbpl cosuppression trait (ggF*-).
(B) Details of the anthers and pistil from a flower similar to the one shown in (A).

Also, the expression of pMADS2 was reduced in the second whorl of R100 flowers, indicating that in the sepaloid petals the pMADS1 gene product is required for maintenance and/or up-regulation of pMADS2. In the fbpl cosuppression plant Gg/F*-, however, normal pMADS2 mRNA levels were obtained. In plants with a reduced pMADS1 level (Gg/F* and Gg- -), the accumulation of pMADS2 was also slightly reduced in the second whorl but was maintained at normal levels in the third whorl organs. A similar effect was observed for fbpl expression in these plants.

In previous reports (Angenent et al., 1992, 1993) we have shown that the expression of the MADS box gene fbp2 is independent of the identity of the floral organs and is not regulated by either pMADS1 or fbpl. The RNA gel blot analysis shown in Figure 3 confirms these data.

DISCUSSION

Phenotype of a gp/fbpl Double Mutant

To confirm the absence of an interaction between the pMADS1 gene and the fbpl gene in the third whorl organs, we investigated the phenotype of the fbpl mutant in a gp mutant background. This double mutant was raised by a cross between the gp mutant (genotype gg- -) and plants heterozygous for pMADS1 and the fbpl cosuppression trait (genotype GgF*- ).

Flowers of both plants are shown in Figure 2B. As expected, this cross resulted in four classes of offspring, with each approximately equally represented in the progeny. These classes include plants with normal flowers, two classes with flowers that phenocopied the parent flowers, and plants with sepals in the second floral whorl and style-stigma structures on top of the anthers. A representative flower of the latter plants is shown in Figure 4. DNA gel blot analysis revealed that these plants contained the same set of eight copies of the chimeric fbpl cosuppression gene as the original fbpl mutant T8007. Furthermore, no pMADS1 transcripts were detectable on RNA gel blots (results not shown), indicating that these plants are gp/fbpl double mutants (genotype ggF*+-). Second whorl sepal of this double mutant were similar to those in the gp mutant, and the anthers in the third whorl were indistinguishable from those in the primary fbpl mutant (GGF*-) and heterozygous double mutant (GgF*--). On top of the anthers, short style-stigma structures were observed. This phenotype demonstrates again that the identity of the third whorl organs is not controlled by the interaction between fbpl and pMADS1.

fbpl and pMADS1 Induction Is Established Independently

In this study, we demonstrated by in situ hybridization that fbpl and pMADS1 are simultaneously induced at positions where whorl two and three organ primordia emerge from the floral apex. In contrast to the Arabidopsis gene pi (Goto and Meyerowitz, 1994) and the Antirrhinum gene defA (Bradley et al., 1993), both petunia genes are not expressed in the center of the floral meristem. Later, during flower development, fbpl and pMADS1 are expressed in emerging petal and stamen primordia, and this spatial expression pattern persists during subsequent differentiation of these organs.

In the gp mutant in which the pMADS1 gene is mutated, the fbpl gene was transcribed at comparable levels in both petal and stamen primordia (see Figure 1O). No pMADS1 mRNA
was detectable in these young gp floral buds, which indicates that pMADSl is not involved in the induction of fbpl transcription at early developmental stages of flower development. Similarly, pMADSl expression was not affected by an absence of fbpl transcripts in emerging second and third whorl primordia from the fbpl cosuppression mutant (see Figure 1K). A possible mechanism of regulatory interaction between pMADSl and fbpl is outlined in Figure 5. In this model, the induction of the petunia B-type genes behaves like defA/glo in Antirrhinum (Tröbner et al., 1992) and ap3/pi in Arabidopsis (Jack et al., 1992; Goto and Meyerowitz, 1994), because these genes are also induced independently.

Functional interaction between fbpl and pMADSl in Petals

Although we have demonstrated that fbpl and pMADSl are independently induced, RNA gel blot analysis (Angenent et al., 1992; Figure 3) revealed an interaction between these genes at later developmental stages. fbpl expression was almost abolished in the second whorl of the gp mutant, whereas normal fbpl mRNA levels were observed in the third whorl of this mutant. This second whorl fbpl expression pattern is not in accord with results from van der Krol et al. (1993), which showed that the fbpl mRNA accumulation in the third whorl organs of a gp mutant (line PLV) was elevated compared with wild-type (line V26) stamens. However, in these experiments, RNAs were isolated from nonisogenic lines, and the developmental stages used were difficult to compare due to the homeotic transformations. Our results indicate that fbpl is transcriptionally up-regulated by the pMADSl gene product in wild-type petunia petals. This regulation might be established by a heterodimer formed between the FBPl and PMADS1 proteins. This heterodimer can bind to specific sequences (CARG motif) present in the fbpl promoter. This hypothesis is supported by in vitro DNA binding experiments showing that a heterodimer between FBPl and DEFA specifically binds to CARG motifs, whereas no binding was observed with homodimers formed by FBPl alone (W. Tröbner and Z. Schwarz-Sommer, unpublished results). A similar autoregulatory mechanism was postulated for the Antirrhinum proteins GLO and DEFA (Tröbner et al., 1992). An interaction between the petunia proteins is possible in the third whorl because the FBPl protein is absent at later stages of stamen development (Cañas et al., 1994) and, furthermore, the pMADSl gene seems to have no function in this floral whorl. In contrast to the proposed molecular mechanism controlling defA and glo expression in Antirrhinum (Tröbner et al., 1992), the interaction between fbpl and pMADSl is unidirectional (see Figure 5). The induction and maintenance of pMADSl expression in the second floral whorl is independent of fbpl.

To analyze a possible in vivo interaction between fbpl and pMADSl, we have used a genetic approach and studied the expression patterns of these genes in fbpl, pMADSl, and heterozygous double mutants. These results provided supporting evidence for a functional interaction between these petunia class B homeotic genes in petals. The flowers from the heterozygous double mutants obtained by a cross between the gp and fbpl mutants had second whorl organs with a stronger reversion toward sepals than was observed in the fbpl cosuppression mutant. This indicates that a reduction in the amount of pMADSl expression results in a more severe fbpl mutation, suggesting that the transcriptional control of fbpl by PMADS1 is essential for proper petal formation. Because fbpl is up-regulated by pMADSl only at later stages of organogenesis, the persistent and precisely regulated expression of fbpl might be required to maintain the identity of the organs in the second whorl. Also, the formation of somatic revertant sectors observed in a genetically unstable defA background (Tröbner et al., 1992) and experiments with a temperature-sensitive ap3 mutant (Bowman et al., 1989) support the hypothesis that persistent expression of B genes is a prerequisite for organ identity.

The phenotype of the third whorl organs remained unaffected by the reduction of PMADS1 in the heterozygous and homozygous double mutants, demonstrating the absence of a functional interaction between fbpl and pMADSl in this whorl.

B Function in Petunia and Its Relation to Arabidopsis and Antirrhinum

To date, only two types of genes have been found in Arabidopsis and Antirrhinum that facilitate the B function required for
the correct formation of petals and stamens. In petunia, this function seems to be established by a combination of at least three genes. Based on amino acid sequence homology deduced from the cDNAs, homologs are present in the three species (see Table 1). Recently, we demonstrated that fbpl is required for petal and stamen development, analogous to glo and pi (Angenent et al., 1993). Also the (in vitro) DNA binding properties of FBP1 are comparable to those of GLO (W. Tröbner and Z. Schwarz-Sommer, unpublished results).

In contrast to defA and ap3, the petunia pMADS1 gene is involved in petal formation only and seems to have no effect on the identity of the third whorl organs. However, van der Krol et al. (1993) hypothesized that pMADS1 also has minor effects on the third whorl because petaloid tissue was observed on top of the anthers of the gp (line PLV) mutant. Indeed, occasionally we noticed these structures in the gp mutant R100. However, these structures were also found in the ancestral line R82, indicating that this phenomenon is not a result of the mutation in pMADS1. At this moment, it is unclear why pMADS1 mRNA accumulates in the third whorl at early and late developmental stages because, as yet, no function can be assigned to pMADS1 in this whorl.

Another difference between Arabidopsis and Antirrhinum, on the one hand, and petunia, on the other, is the presence of two pi and glo homologs, fbpl and pMADS2, both of which are expressed in whorls two and three. The overall amino acid sequence similarity between FBP1 and PMADS2 is 68%. Thus far, no information is available on the function of the pMADS2 gene. The expression patterns observed in wild-type and gp flowers matched exactly those found for fbpl, suggesting that fbpl and pMADS2 might play a similar role in floral organogenesis. As shown previously (Angenent et al., 1993), fbpl is essential for petal and stamen formation because suppression of fbpl results in transgenic plants with homeotic conversion in the second and third floral whorls. The expression of pMADS2 is normal in these plants when compared with its expression in wild-type plants, indicating that this gene is not controlled by fbpl. It also indicates that pMADS2 cannot complement the fbpl mutation. In this connection, attempts to suppress pMADS2 function by sense inhibition were not successful (van der Krol et al., 1993), suggesting that the role of pMADS2 might be redundant.

The interdependence of fbpl and pMADS1 is not mutual because the induction and maintenance of pMADS1 expression throughout flower development have been established in the absence of FBPI. Also, pi does not seem involved in the control of ap3 transcription in the second whorl of Arabidopsis flowers (Jack et al., 1992). In contrast to petunia and Arabidopsis, the regulation of both Antirrhinum B-type genes is strongly interdependent (Tröbner et al., 1992).

In summary, detailed analyses of the expression and regulation of B-type homeotic genes in petunia revealed several similarities as well as differences between the mechanisms proposed for Arabidopsis (Jack et al., 1992, 1994; Goto and Meyerowitz, 1994) and Antirrhinum (Schwarz-Sommer et al., 1992; Tröbner et al., 1992). However, despite our knowledge about the expression and interdependent regulation of class B homeotic genes in these species, the information about the target genes and the cellular processes controlled by these MADS box proteins is very limited. At this time, we do not know whether the class B homeotic genes are involved in establishing species-specific differences in floral organ development. In this respect, it would be interesting to investigate whether the functions of these B genes are interchangeable among species. In addition, such experiments may provide evidence for an evolutionary relationship between pMADS1–fbpl, ap3–pi, and defA–glo.

METHODS

Plant Material

The Petunia hybrida lines W115, R82, and R100 and the fbpl (floral binding protein gene 1) cosuppression plants T8007 and T8007S were grown under normal greenhouse conditions.

RNA Gel Blot Analysis

Second and third whorl organs were collected from different developmental stages, beginning with small floral buds (±5 mm) in which the stamen filament was just starting to elongate through to mature flowers. All stages were roughly equally represented in the final mix. Total RNA was isolated from the collected floral organs, according to Verwoerd et al. (1989). The pMADS1 probe, covering nucleotides 182 to 694 (Kush et al., 1993), was generated by polymerase chain reaction (PCR) amplification using cDNA derived from W115 petal as a template. The sequences of the 5' and 3' primers (Isogen, Bioscience) are 5'-GGAATTCATTAGTCCATCTATCACGAC-3' and 5'-GTAGTGATATCATCAGCAG-3', respectively. 3' terminal fragments of the fbpl (560 bp), fb2 (620 bp), and pMADS2 (510 bp) cDNAs were labeled by oligonucleotide priming (Feinberg and Vogelstein, 1984). All of these fragments were missing the MADS box region to avoid crosshybridization with other MADS box genes. Hybridization was performed as described previously (Angenent et al., 1992).

In Situ Hybridization

Floral buds were fixed, embedded in paraffin, and cut into 8-μm sections as described elsewhere (Cañas et al., 1994). To obtain an fbpl-specific probe, a 142-bp HindIII-SacI fragment (nucleotides 218 to 360; Angenent et al., 1992) was subcloned into the T7 transcription plasmid pSP78. For a pMADS2 probe, the PCR product described earlier was subcloned into pSP78, using the EcoRI and EcoRV restriction sites present in the PCR primers. Digoxigenin-labeled RNA transcripts were generated according to the instructions of Boehringer Mannheim. RNA transcripts were partly hydrolyzed for 10 min (fbpl) and 45 min (pMADS2). Hybridization and immunological detection were performed as described previously (Cañas et al., 1994).
ACKNOWLEDGMENTS

We thank Gerrit Stunnenberg for care of the plants and Dr. Ronald Koes for providing the petunia R82 line. We are indebted to Dr. Zsuzsanna Schwarz-Sommer for permission to use unpublished data on in vitro DNA binding experiments.

Received February 6, 1995; accepted March 3, 1995.

REFERENCES


Functional interaction between the homeotic genes fbp1 and pMADS1 during petunia floral organogenesis.
G C Angenent, M Busscher, J Franken, H J Dons and A J van Tunen
*Plant Cell* 1995;7:507-516
DOI 10.1105/tpc.7.5.507

This information is current as of July 7, 2017

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>eTOCs</td>
<td>Sign up for eTOCs at: <a href="http://www.plantcell.org/cgi/alerts/ctmain">http://www.plantcell.org/cgi/alerts/ctmain</a></td>
</tr>
<tr>
<td>CiteTrack Alerts</td>
<td>Sign up for CiteTrack Alerts at: <a href="http://www.plantcell.org/cgi/alerts/ctmain">http://www.plantcell.org/cgi/alerts/ctmain</a></td>
</tr>
<tr>
<td>Subscription Information</td>
<td>Subscription Information for <em>The Plant Cell</em> and <em>Plant Physiology</em> is available at: <a href="http://www.aspb.org/publications/subscriptions.cfm">http://www.aspb.org/publications/subscriptions.cfm</a></td>
</tr>
</tbody>
</table>