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Characterization of the *Antirrhinum* floral homeotic MADS-box gene *deficiens*: evidence for DNA binding and autoregulation of its persistent expression throughout flower development

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We have determined the structure of the floral homeotic *deficiens* (*def*A) gene whose mutants display sepaloid petals and carpelloid stamens, and have analysed its spatial and temporal expression pattern. In addition, several mutant alleles (morphoalleles) were studied. The results of these analyses define three functional domains of the DEF A protein and identify in the *deficiens* promoter a possible *cis*-acting binding site for a transcription factor which specifically upregulates expression of *deficiens* in petals and stamens. *In vitro* DNA binding studies show that DEF A binds to specific DNA motifs as a heterodimer, together with the protein product of the floral homeotic *globosa* gene, thus demonstrating that the protein encoded by *deficiens* is a DNA binding protein. Furthermore, Northern analysis of a temperature sensitive allele at permissive and non-permissive temperatures provides evidence for autoregulation of the persistent expression of *deficiens* throughout flower development. A possible mechanism of autoregulation is discussed.

Key words: flower morphology/gene structure/*in situ* hybridization/MADS-box/transcription factor

Introduction

Flower morphogenesis is a complex process governed by a set of regulatory genes (Meyerowitz *et al.*, 1989; Schwarz-Sommer *et al.*, 1990; Bowman *et al.*, 1991; Coen, 1991). The role of these genes in flower development becomes apparent when mutational alteration of gene function results in a morphologically altered phenotype. For example, development of a different organ in the mutant flower at a position where in the wild type the normal organ develops (Meyer, 1966) indicates the function of a homeotic gene in the process of control of floral organ identity. In *Antirrhinum* and *Arabidopsis* such homeotic genes, or their respective mutants, can be assigned to three different classes. Mutations in genes in each of these categories frequently affect differentiation of organs in two adjacent whorls of the flower. Recent molecular and genetic studies of such mutants have led to the proposal of relatively simple models of how homeotic genes, as key regulatory genes of developmental pathways, may govern floral organogenesis (Haughn and Somerville, 1988; Carpenter and Coen, 1990; Schwarz-Sommer *et al.*, 1990; Bowman *et al.*, 1991; Coen and Meyerowitz, 1991; Lord, 1991). The molecular mechanisms underlying this control are still unknown.

The *deficiens* (*def*A) gene is involved in the control of petal and stamen development (Klemm, 1927; Sommer *et al.*, 1990). Loss of the *def*A function results in sepaloid petals and carpelloid stamens in *deficiens* mutants. Sequence analysis of the *def*A cDNA clone indicated that the encoded protein (DEF A) may be a transcription factor, because DEF A contains a conserved putative DNA binding domain, the MADS-box (Schwarz-Sommer *et al.*, 1990). The MADS-box is also found in proteins known to be transcription factors: the serum response factor (SRF) in human which regulates the c-*fos* proto-oncogene in response to serum factors (Norman *et al.*, 1988), the sarcomeric CArG-binding factor CBF which is involved in the muscle-specific expression of α-actin in human (Boxer *et al.*, 1989; Sartorelli *et al.*, 1990) and the yeast regulatory proteins MCM1 and ARGR1 which are involved in cell type determination and regulation of the arginine biosynthetic pathway, respectively (Passmore *et al.*, 1988; Dubois *et al.*, 1987). The MADS-box is also present in AG, the protein product of *agamous*, a homeotic gene controlling organogenesis of stamens and carpels in *Arabidopsis* (Yanofsky *et al.*, 1990). The growing family of MADS-box proteins meanwhile comprises six AG-like homologues in *Arabidopsis* (Ma *et al.*, 1991); SRF^X, a CArG binding protein regulating somite-specific expression of the cardiac α-actin gene in early *Xenopus* embryos (Mohun *et al.*, 1991); several proteins in *Antirrhinum*, two of which are encoded by the floral homeotic genes *squamosa* and *globosa* (Schwarz-Sommer *et al.*, 1990) and five DEF-like homologues in tomato (Pnuely *et al.*, 1991). The cellular processes controlled by MADS-box proteins differ in the various organisms, but despite the differences in their specific action it seems that the function of MADS-box genes is the control of cell differentiation or to mediate the cell's response to environmental signals.

Several approaches have been applied to analyse the function of transcription factors. In the case of SRF and MCM1, where the DNA binding sites of the regulatory factors in the promoters of the target genes are known [Serum Response Element (SRE) in the promoter of c-*fos* and the P-box in promoters of **a**- and α-cell type specific genes, respectively], *in vitro* analysis of the modified proteins has revealed functional domains of the protein (Norman *et al.*, 1988; Christ and Tye, 1991). For homeotic genes like *deficiens*, naturally occurring mutations affecting the encoded protein and/or mutations which alter the gene's expression pattern can give clues about its function. We have utilized morphologically distinct mutants, so called morphoalleles, of the *deficiens* gene to obtain information on functionally important domains of the DEF A protein. Correlation of the altered gene structure with its modified function allows deeper insight into the molecular mechanisms which control *deficiens*, and the mechanisms by which *deficiens* controls organogenesis of petals and stamens.

Results

Flower morphology of mutants

Studies of morphological characteristics and temperature dependence of morphoalleles of the *deficiens* gene were conducted to get information about the role of *deficiens* in floral development. In the following the phenotype of flowers developing at high temperature (25°C) is described first, followed by the phenotype in the cold (15°C).

Wild type (defA). Wild type *Antirrhinum* flowers (Figure 1) consist of four whorls of organs i.e. the five sepals (first or outermost whorl), five petals constituting the corolla (second whorl), four stamens and the staminodium (third whorl) and two carpels (fourth or innermost whorl). In the mature flower the basal parts of the petals are fused and form a structure, while the higher parts form the upper (adaxial) and lower (abaxial) lobes. The distinct morphological features of the four types of organs and their development from organ primordia to mature organs have been described by several authors (Klemm, 1927; Bergfeld, 1956; Awasthi *et al.*, 1984; Green and Linstead, 1990; Sommer *et al.*, 1990).

Temperature has an influence on flower development. Decreasing the culture temperature from 25°C to 15°C results in enlarged, intensively coloured flowers (Figure 1). In general, flower primordia and floral organ primordia increase in size in the cold but take longer to develop into mature organs. At 15°C additional organs develop in the third whorl, which flank the filaments of stamens and often show petaloid characters (Figure 3A). The number of these structures is variable, but there are usually two petaloid organs flanking the staminodium. The growth of the upper lobe of the second whorl is promoted in the cold.

chlorantha (def^{chl}). Chlorantha flowers are smaller and their petals show weak virescence indicating sepaloid features (Figure 1). The stamens are feminized because they develop ovules on the ventral side of the anthers (directed towards the interior of the flower bud in Figure 3A). The dorsal pollen sacs (directed toward the exterior of the flower bud) are almost normal. The morphological alterations are more distinct for the upper two (adaxial) organs than for the lower two (abaxial) ones. The ring of trichomes (hair-like structures) surrounding the lower stamens in the wild type is missing in the mutant (Figure 3A). The lower two filaments are not twisted in contrast to the wild type; therefore the position of their anthers is slightly changed. Carpel development is not affected.

Development in the cold decreases the phenotypic expres-

Fig. 1. Phenotypes of *deficiens* mutants and influence of culture temperature on the phenotypic expression of the mutations. The genotype of the plants homozygous for the indicated mutant alleles (e.g. def^{chl}/def^{chl} = chl) is shown above the panels and the culture temperature is indicated at the left of each row of photographs. Wild type morphology is demonstrated by the flower of a heterozygote ($def^{gli}/defA$ = *gli* × wt) to show that decreasing the wild type gene dosage has no morphological effect. Magnifications were the same for all flowers, except for globifera flowers, which were magnified twice (upper row) and four times (lower row) as compared with the others. The photographs show flowers from an abaxial view; thus the lower lobe of the corolla is at the front of the picture, while the upper lobe is at the back. The upper part of the globifera flower (depicted in a top view, but oriented in the same way as all other flowers), grown at low temperature, was removed to demonstrate the structure of its central gynoecium. As noted in the text, temperature has no significant effect on the morphology of globifera flowers. Variability in the colour and colour intensity of petals is due to mutations in colour determining genes. Such mutations have no effect on the morphology of the flowers.

sion of the mutation. Petals are larger; the promoted growth of the upper lobe reflects the effect of low temperature on development of wild type petals (Figure 1). The feminizing effect of the mutation on stamen development is less pronounced than at 25°C (Figure 3A); the stamens do not develop ovules and the anthers contain fertile pollen, despite their slightly affected morphology.

nicotianoides (*def*^{nic}). The petals of *nicotianoides* flowers are reduced further in size and show strong green colour (Figure 1). Their form is altered and often, particularly when growing in the cold, a third petal-like organ develops between the upper two. The filaments of the stamens are broadened and their dorsal surface is covered with trichomes (Figure 3A). The ventral pollen sacs are deformed and the anthers bear few ovules. At the tip of the male organ, papillate stigmatic tissue, a female characteristic, develops. The dorsal pollen sacs are less severely affected than the ventral ones and the tendency towards feminization is more pronounced for the upper two stamens than for the lower two. Development of the carpels is hardly affected, but fusion (adnation) of the central gynoecium and parts of the filament of the upper stamens often occurs.

The influence of decreased temperature on petal development is similar to that observed for wild type or *chlorantha* flowers. Additional organs develop frequently in the third whorl that are covered with trichomes and hence resemble sepals (Figure 3A). The decreased carpellody of the stamens is similar to the temperature effect on *chlorantha* stamen development so that ovules hardly develop. Pollen, however, has not yet been obtained from *nicotianoides* mutants, although its occasional occurrence has been described (Klemm, 1927).

In summary, *chlorantha* and *nicotianoides* mutants show some common features with respect to the different strengths of the feminizing effects on stamens: (i) in both mutants the upper stamens are more severely affected than the lower stamens and (ii) the morphology of the ventral part of the anthers is more strongly distorted than that of the dorsal part. These observations indicate that the *deficiens* function is unevenly distributed across organ primordia and also between adaxial and abaxial parts of the flower. Furthermore, increasing the culture temperature increases the expression of the altered phenotypes, perhaps as a consequence of the accelerated differentiation process at the elevated temperature.

globifera (*def*^{gli}). As described in an earlier report (Sommer *et al.*, 1990), globifera 'petals' are morphologically indistinguishable from sepals, except that these sepaloid second whorl organs are larger than sepals in the first whorl and that their position in the mature mutant flower resembles the position of wild type petals of the upper and lower lobes (Figure 1). The broadened filaments fuse laterally and constitute a large central gynoecium covered with trichomes on its dorsal surface. Inside this central female organ a

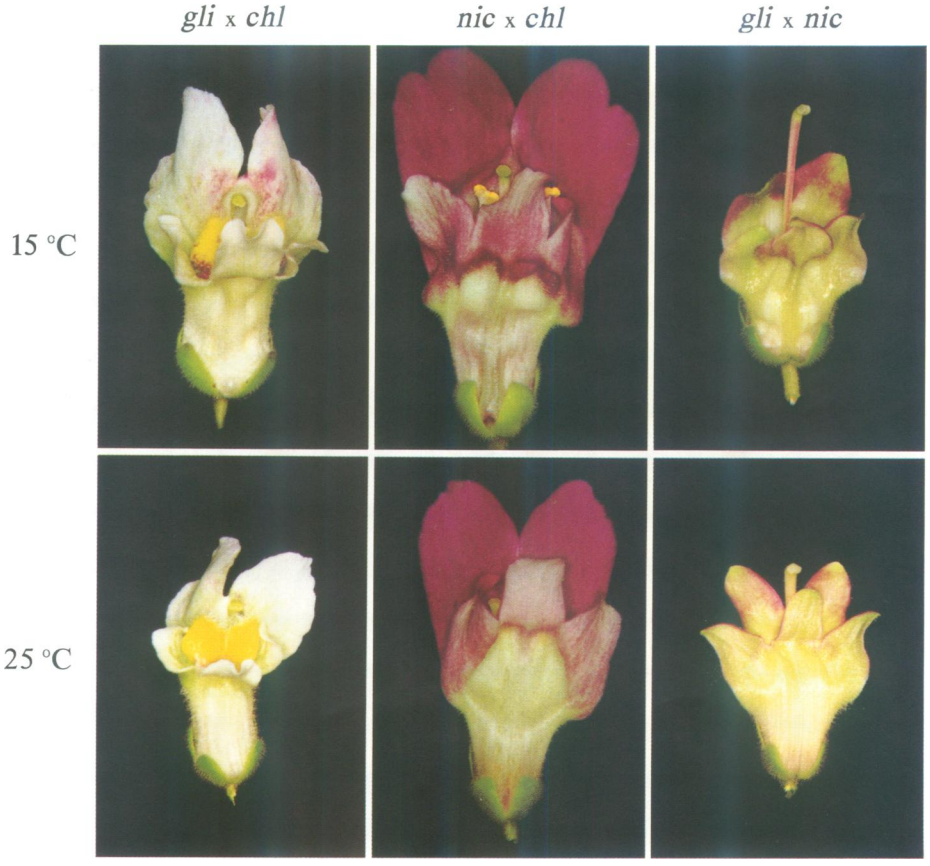

Fig. 2. Phenotypes of heterozygotes between mutant alleles of *deficiens* and the influence of culture temperature on phenotypic expression of the mutations. The crosses used to generate the allelic heterozygotes are shown above the panels and the growing temperature is indicated at the left of each row of photographs. Crosses which involved *chlorantha* plants were carried out with *chlorantha* as the male parent. To obtain *nicotianoides* × *globifera* plants, heterozygotes of each of the two mutants with the wild type *deficiens* allele were generated and these heterozygotes were subsequently crossed.

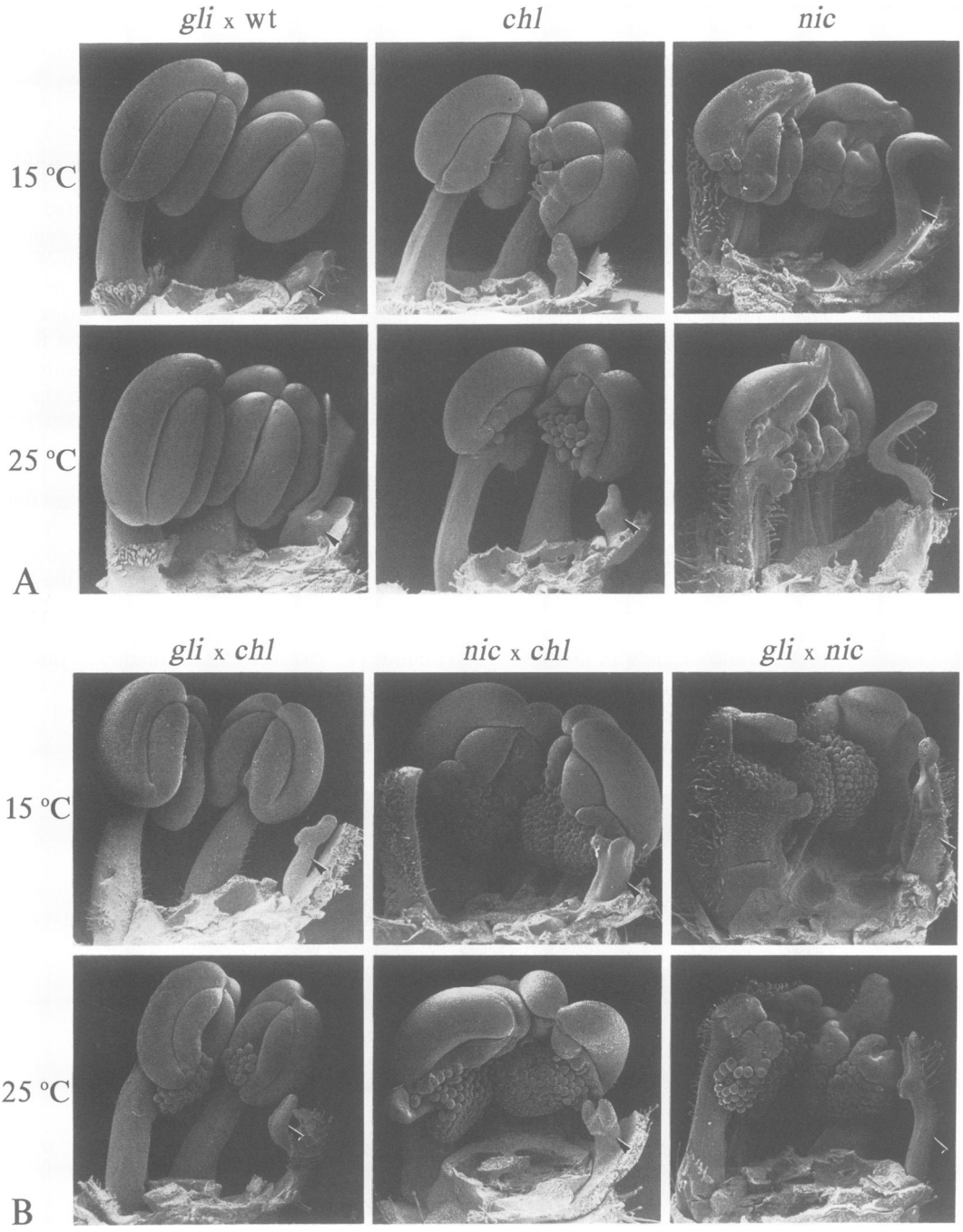

Fig. 3. Effect of mutations in the *deficiens* gene on stamen morphology and influence of culture temperature on expression of the mutations. The genotype of the plants, homozygous (**A**) or heterozygous (**B**) for the indicated mutant alleles, is shown above the panels. For scanning electron microscopy (SEM) 0.5 cm long young buds of plants grown at low and high temperature (indicated at the left of each row) were fixed and dried as described previously (Sommer *et al.*, 1990). The SEM photographs show one of the abaxial and one of the adaxial stamens after removing sepals, petals, carpels and the other pair of stamens. Additional organs were also removed unless they did not cover essential parts of stamens (see for example, *gli* × *wt* in the lower panel in part A. The staminodium (indicated by an arrowhead), located between the two adaxial (upper) stamens in the flower, is oriented to the right on each photograph.

variable number of loculi, filled with ovules, can be observed (Figure 1). The genuine gynoecium either does not develop, or it shows variable fusions with the carpelloid stamens of the third whorl. The chimney-like structure, typical of *globifera* flowers, arises by elongation of the filaments and resembles the style of a gynoecium. Occasionally a 'style within a style' structure develops, probably by development of the genuine style inside the 'chimney'.

Except for their increased size, globifera flowers are not altered morphologically when developing in the cold.

defA-23. *Def*A-23 flowers (Figure 4) display greenish petals with a distorted shape. The carpelloid stamens form a chimney-like structure comparable with the central female organ of *globifera* flowers. Additional petal-like, elongated structures often develop between the second and third whorls.

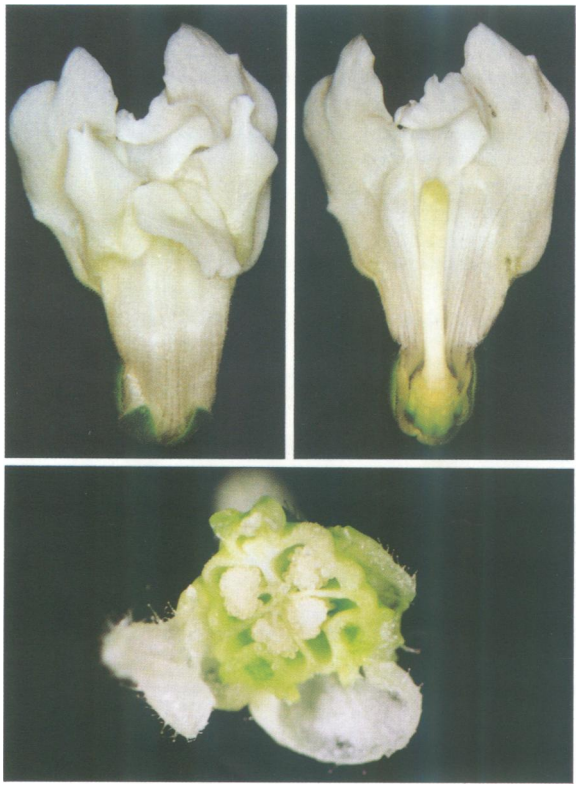

Fig. 4. Phenotype of flowers of the *def*A-23 mutant. The photographs at the top show the flower from the abaxial side. The altered structure of the reproductive organs is documented at the right where the lower lobe of the flower and additional petal-like structures were removed. The inner structure of the central gynoecium is depicted at the bottom.

They probably represent displaced parts of the filaments and often fuse with the petals or with the central gynoecium.

Besides stronger growth, decreased temperature has no visible effect on the morphology of *def*A-23 flowers.

defA-101. The second whorl organs of *def*A-101 flowers (Figure 5) are sepaloid and strongly resemble those of *globifera* flowers. The broadened filaments in the third whorl are generally shorter than the comparable organs of *globifera* or *def*A-23 flowers; their lateral fusion, resulting in loculi, is often incomplete. The central female organ is complex in structure (Figure 5). Placentae with ovules develop at the base of the fused filaments. The number of placentae is variable, although the number of loculi seems to be five. Three long, incompletely fused, rod-like structures which resemble the genuine style of the gynoecium are located in the centre of the flower.

At a lower culture temperature, *def*A-101 plants develop flowers which often possess six deformed petals in their second whorl (Figure 5). The third whorl organs are also deformed, but represent morphologically almost normal stamens with broadened filaments which carry anthers with dorsal and ventral pollen sacs. Mutant plants grown in the cold produce fertile pollen. The flower contains several additional sepaloid and petaloid structures which all seem to be lateral protrusions of the filaments in the third whorl. The dorsal part of filaments is often split at the tip. The gynoecium contains usually more than two loculi with placentae and the shape of the stigma also indicates that more than two carpels may constitute the female organ.

Fig. 5. Phenotype of flowers of the *def*A-101 mutant. Photographs in the upper row were made from the side of intact flowers. Photographs in the lower row show the structure of the reproductive organs after removing the lower lobe of the flower or after dissection of the central female organ (at the right). The growing temperature is indicated below the panels.

In the glasshouse, under intermediate temperature conditions, *def*A-101 flowers show an intermediate phenotype (Figure 5). Preliminary temperature-shift experiments indicate that, after initiation of development at 25°C, petals ultimately become restored to a certain extent when the plant is returned to the cold. Restoration of third whorl organs is less pronounced.

The effects of temperature on flowers of plants carrying the *def*A-101 allele suggest that *def*A-101 is a temperature sensitive allele. In contrast, all other *deficiens* morphoalleles display normal temperature effects on development, like wild type flowers.

Dominance relationship between different deficiens morphoalleles

All morphoalleles described above are recessive to wild type but some alleles, when combined as heterozygotes, reveal incomplete dominance relations among each other.

In general, the *chlorantha* allele dominates over all other mutant alleles with respect to petal development because the heterozygotes resemble the *chlorantha* homozygote (see *nic* × *chl* in Figure 2). The *globifera* × *chlorantha* heterozygote, however, is closer to wild type morphology than the *chlorantha* homozygote (compare curvature of upper and lower petals of the corresponding flowers in Figures 1 and 2). The modification seems to be specific for this allelic combination because combination of *chlorantha* with *nicotianoides* or *globifera* with *nicotianoides* does not seem to have a similar effect on petal development. Also, phenotypically and genotypically wild type revertants of the germinally unstable *globifera* allele are indistinguishable from other wild type forms. Since the unexpected phenotype

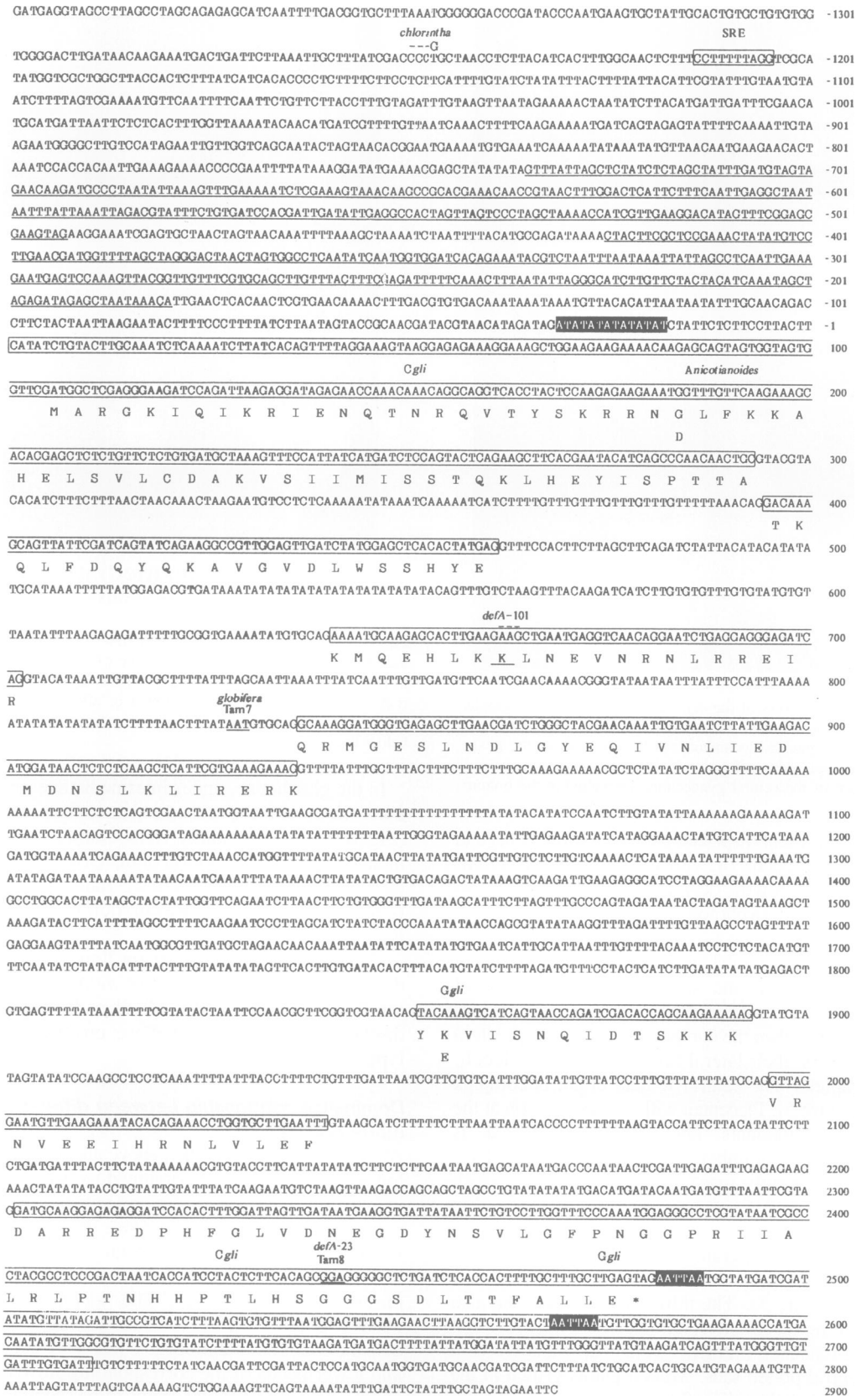

Fig. 6. Structure of the *deficiens* transcription unit. The sequence respresents part of a 7 kb *Eco*RI fragment containing the entire wild type *def*A locus (this sequence has been deposited in the EMBL data library under the accession number X62810). The exons are in boxes and the encoded amino acids are shown below the DNA sequence. Consensus sequences, such as the TATA box and polyadenylation signals are in inverted boxes. Both polyadenylation signals are functional, because cDNAs polyadenylated at two different sites were found. The inverted repeat upstream of the TATA box is underlined and a consensus SRE motif is indicated. Nucleotide exchanges and deletions found in *deficiens* mutants are shown above the nucleotide sequence and those which generate amino acid exchanges or deletions are indicated below the amino acid sequence. The insertion sites of the transposons Tam7 and Tam8 are indicated by the three underlined nucleotides which are duplicated in the mutants and flank the inserts.

of *globifera* × *chlorantha* heterozygotes was observed in a population of about ten thousand individual F1 heterozygotes, it seems that the (dominant) modifying effect on *chlorantha* is linked to the *globifera* allele.

With respect to stamen development the dominance relationships between alleles are complex. In general, allelic heterozygotes display some phenotypic characteristics of the more severely feminizing allele (Figure 3B). For example, combinations of *chlorantha* with *globifera* and *nicotianoides* possess ovules whose development persists in the cold. The morphology of third whorl organs in the *globifera* × *nicotianoides* heterozygote is interesting: at 25°C the lateral parts of the broadened filaments show a tendency to form a tube-like structure, indicating the formation of loculi which become completely closed in *globifera* flowers (Figure 1).

Our analyses of allelic combinations with *def*A-101 and *def*A-23 are not yet complete. Preliminary results indicate that *chlorantha* dominates both alleles with respect to petals. The combination *globifera* × *def*A-23 reveals increased sepalody of the petals whose morphology is still distinctively different from those of the globifera flowers.

The structure of the deficiens transcription unit

We have determined the structure of the genomic *deficiens* transcription unit by sequencing cDNA clones and ~5.6 kb of a genomic wild type clone comprising 3 kb of upstream region and 2.6 kb containing the coding region (Figure 6). Similarly, sequence analysis of genomic clones of five mutant alleles of *deficiens* was carried out to identify the sites of mutations in the respective alleles. To exclude ambiguities the entire 5.6 kb of genomic sequence, shown in part in Figure 6 for wild type, was also determined for all mutant alleles, except for *def*A-23.

Consensus sequences, such as exon−intron boundaries, start of translation, polyadenylation signals and the high AT content of introns correspond to rules obtained from other eukaryotic genes (Messing *et al.*, 1983; Joshi, 1987).

The deficiens promoter region. S1 mapping and primer extension experiments (data not shown) revealed several transcription initiation sites. The strongest signal, although not experimentally proven to represent the genuine start of transcription initiation, was arbitrarily designated as position +1 in the sequence shown in Figure 6. At position −19 to −32 an unusually long putative TATA box is found whose location, with respect to the site of transcription initiation, corresponds to that found in other plant genes (Messing *et al.*, 1983; Joshi, 1987). Further upstream, the *def*A promoter region contains a 244 bp, almost perfect inverted repeat (Figure 6). The significance of this potential stem−loop structure in the transcriptional regulation of the gene is not known.

A search for consensus binding sites of known transcription factors revaled a CArG-box [$CC(A/T)_6GG$, see SRE box in Figure 6] 1.2 kb upstream of the transcription start. This motif represents the consensus of the binding sites for MADS-box containing transcription factors (Passmore *et al.*, 1989; Boxer *et al.*, 1989). In the *chlorantha* allele, a mutation affecting four bases (CGG instead of CCCCTG) is found 32 bp upstream of this box (Figure 6), indicating the functional importance of this region for regulation of *def*A gene expression. Because the site affected in the *chlorantha* allele (CCCCTG) slightly resembles the consensus binding sequence of MyoD (CANNTG), it is interesting to note that in the promoter of the cardiac α-actin gene several CArG-boxes, representing the binding site of an SRF-like factor (CBF), are located near the binding site of MyoD1 (Gustafson and Kedes, 1989). Two of the CArG-boxes, positioned 50 and 90 bp upstream of the CANNTG-box, as well as the binding site for MyoD1 (and Sp1) were shown to be required for muscle-specific expression of the gene (Sartorelli *et al.*, 1990).

The deficiens coding region and the DEF A protein. The 294 bp first exon contains the MADS-box whose features, such as the presence of a potential phosphorylation site for calmodulin-dependent kinases, a hydrophobic putative dimerization domain and the overall conservation of a stretch of 57 amino acids, also found in the known transcription factors MCM1 and SRF and in other floral MADS-box proteins (Yanofsky *et al.*, 1990; Ma *et al.*, 1991), have been described in detail elsewhere (Sommer *et al.*, 1990; Schwarz-Sommer *et al.*, 1990). In the *nicotianoides* allele a G to A substitution at position 185 (Figure 6), within the DNA binding domain of the MADS-box, results in a glycine to aspartic acid exchange.

The short third and fourth exons of *def*A contribute to the K-box, a region with some homology to keratin (Ma *et al.*, 1991). A small deletion of three base pairs at the N-terminal end of the putative K-box causes loss of a lysine in the protein of the mutant *def*A-101 allele (Figure 6).

Analysis of the *def*A-23 allele (Figure 4) revealed the insertion of the Tam8 transposable element (see below) within the last large exon of the *def*A gene (Figure 6). Sequence analysis of the cDNA derived from *def*A-23 mRNA showed that, at the 3′ end ~300 bp of the wild type cDNA sequence is replaced in the mutant by a sequence derived from the Tam8 element. As a consequence, the last 12 amino acids of DEF A (GGSDLTTFALLE*) are replaced by seven element-encoded amino acids (HYNKNGL*) in the mutant protein. Co-transcription and co-translation of plant genes with insertion elements was also observed in *Zea mays* (Gierl *et al.*, 1985; Schwarz-Sommer *et al.*, 1987).

In the *globifera* allele a transposon, Tam7 (Sommer *et al.*, 1990), is inserted into the third intron of the *def*A gene (Figure 6). In addition, the allele contains several point mutations in exons (Figure 6) and numerous small alterations in the introns (not shown), in comparison with wild type and all other sequenced *deficiens* mutant alleles. The high number of differences in the *globifera* allele is not surprising because this mutant was isolated from a line with different genetic background (see Materials and methods). Because *globifera* is a genetically unstable allele which reverts germinally to wild type (Sommer *et al.*, 1990), amino acid exchanges in the DEF A protein encoded by this allele define positions not important for wild type functions. Such a point mutation is observed in the fourth exon, resulting in a lysine to glutamine exchange (Figure 6). The positive charge of the lysine residue at this position thus does not seem to be essential for proper wild type DEF A function.

Transposable elements. The altered phenotypes of flowers of *globifera* and *def*A-23 plants are due to transposable element insertions, Tam7 and Tam8, respectively, into the *def*A gene. The insertion sites of the elements (Figure 6), and in part their sequence (not shown), have been deter-

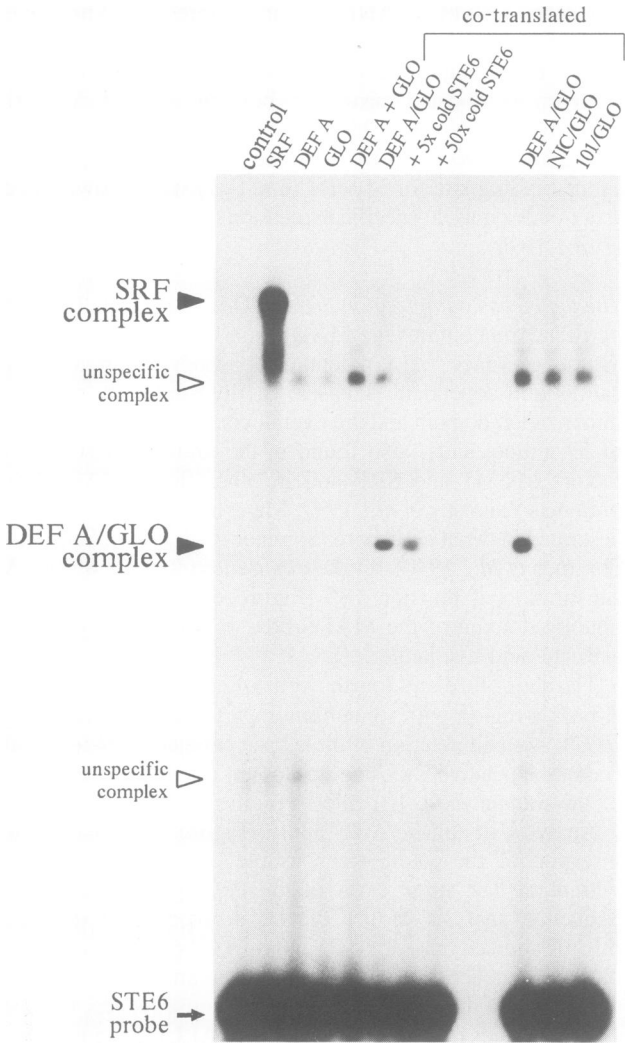

Fig. 7. DNA binding of the *in vitro* translated DEF A protein in gel retardation assays. In all experiments the labelled STE6 promoter probe (see Materials and methods) was incubated with *in vitro* translation products as indicated above the lanes. In the control experiment no mRNA was added to the reticulocyte lysate. The binding assays shown in lanes 7 and 8 contained 2.5 ng (5-fold excess) and 25 ng (50-fold excess) of unlabelled STE6 DNA, respectively. The arrowheads indicate the DNA–protein complexes. As demonstrated by the experiment in the first lane, non-specific binding occurs in proteins present in the reticulocyte lysate. Slower migration of the SRF–DNA complex is due to the larger size of the SRF protein translated from the SRF construct as compared with DEF A and GLO proteins (see Materials and methods). NIC and 101 are proteins translated from the cDNAs of the *nicotianoides* and *def*A-101 alleles, respectively.

mined. Both insertions generate 3 bp duplications of the target site, and both possess 13 bp perfect terminal inverted repeats (CACTACAAAAAAA for Tam7 and CACTACAACAAAA for Tam8). These features are common to several transposons of the CACTA family of elements detected in *Antirrhinum* and in maize (for review see Gierl *et al.*, 1989). The sequenced portions of the two new elements show no further internal homology to known transposons.

Preliminary genetic analysis indicates that Tam7 and Tam8 are defective elements not capable of autonomous transposition. However, Tam7 apparently can be mobilized by a function supplied *in trans* by a corresponding autonomous element. Together with this autonomous element Tam7 constitutes a mobile two-component system, like Ac–Ds and En(Spm)–I in *Zea mays* (for review see Fedoroff, 1989).

In vitro binding studies with DEF A

The conserved MADS-box domain shared by DEF A and other known transcription factors like SRF and MCM1 suggests that the protein is capable of binding to DNA. Gel retardation assays were performed to investigate whether the *in vitro* translated DEF A protein may bind to specific DNA sequences. No information on such target sequences for DEF A binding was available. Because the binding sites of MADS-box proteins are structurally related (Boxer *et al.*, 1989; Sartorelli *et al.*, 1990; Mohun *et al.*, 1991) and functionally interchangeable (Hayes *et al.*, 1988; Passmore *et al.*, 1989; Taylor *et al.*, 1989) the binding sequence of *MCM1* in the *STE6* promoter (the P-box; Keleher *et al.*, 1988) was used. As documented in Figure 7, DEF A does not bind to the P-box of the STE6 promoter.

A similar assay was carried out with the GLO protein, *in vitro* translated from mRNA transcribed from the cDNA of the floral homeotic gene *globosa* (*glo*) of *Antirrhinum majus* (Schwarz-Sommer *et al.*, 1990; Tröbner,W., Ramirez,L., Hue,I., Lönnig,W.-E., Saedler,H., Sommer,H. and Schwarz-Sommer,Zs., in preparation). The GLO protein also belongs to the MADS-box family and mutants of *globosa* display similar phenotypes to those of *deficiens*. It was therefore conceivable that the two proteins act together in a combinatorial manner. Like DEF A, the GLO protein alone was non-functional in the gel retardation assay under the conditions employed. However, the DEF A and GLO proteins obtained by *in vitro* co-translation of the *def*A and *glo* mRNAs revealed a specific band shift when incubated with the labelled *STE6* promoter motif (Figure 7). No specific binding was detectable when the proteins were translated separately *in vitro* and combined afterwards, perhaps because homodimers have formed during *in vitro* translation and heterodimerization of the proteins is no longer possible. The intensity of the signal decreased when unlabelled *STE6* oligonucleotide was added as a competitor to the assay. The co-translated complex does not bind to the binding site of the unrelated DNA binding protein TnpA (see Gierl *et al.*, 1989) and this unrelated binding motif cannot compete with the *STE6* promoter motif for binding to the DEF A–GLO heterodimer (data not shown). These results suggest that binding of the DEF A–GLO heterodimer to the *STE6* motif is specific.

No band shift was detectable in gel retardation assays, using the *STE6* promoter motif as artificial binding site, when the wild type *deficiens* cDNA was replaced by the cDNA of the mutant alleles *nicotianoides* and *def*A-101 during *in vitro* co-translation with the *glo* cDNA (Figure 7). Thus, the structural alterations in these mutant proteins seem to prevent DNA binding of the heterodimer and/or abolish the capability of the mutant DEF A proteins to form heterodimers with GLO. However, *in vivo*, the presence of accessory proteins that interact with the heterodimer may partially restore binding and function of the mutant complex. We also cannot rule out the possibility that *in vivo*, the DEF A and GLO homodimers can bind to specific sites in the promoters of target genes.

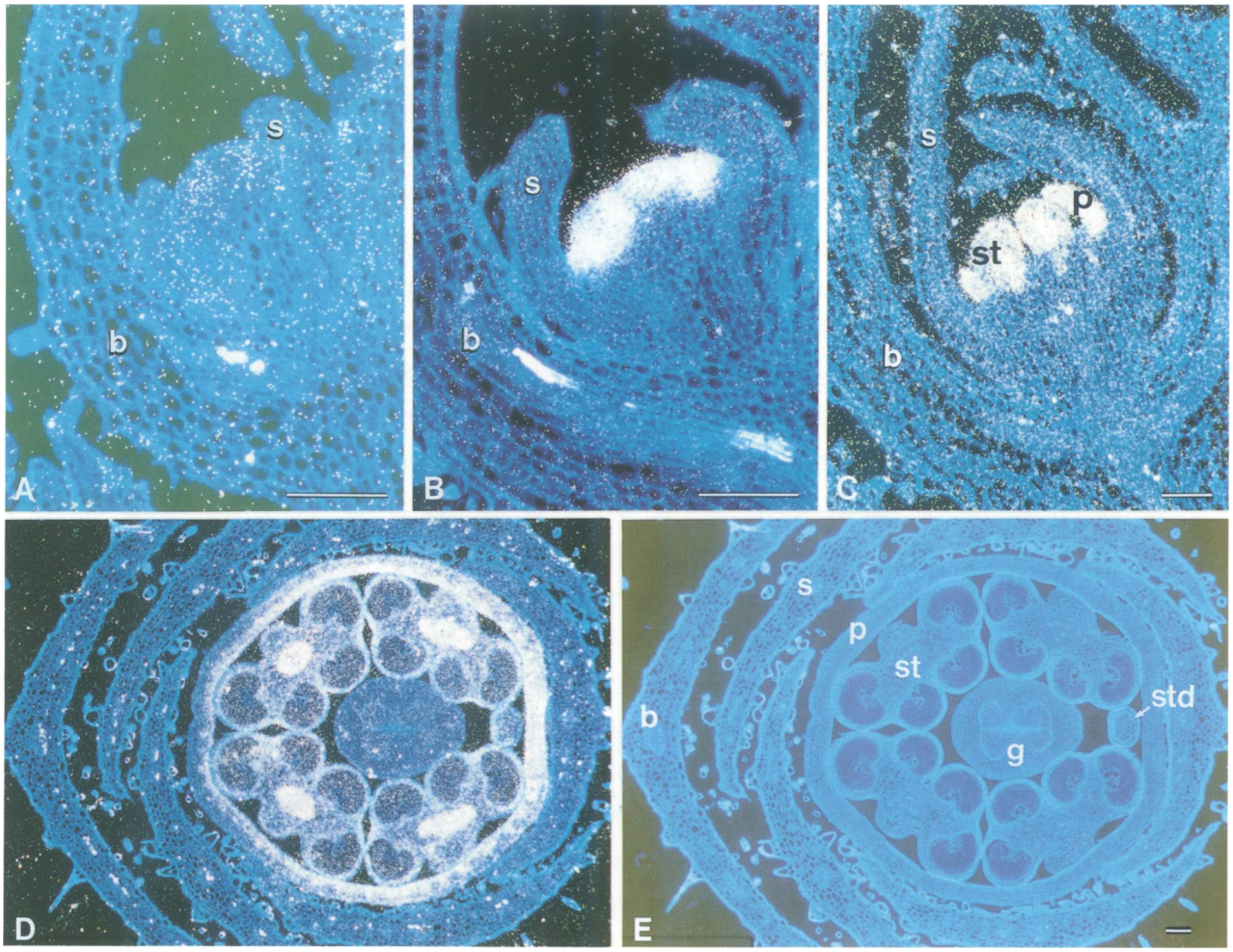

Fig. 8. Spatial and temporal expression pattern of the *deficiens* gene. Longitudinal (**A**–**C**) and cross (**D**–**E**) sections through developing flower buds before (A), during (B) and shortly after (C) initiation of petal and stamen primordia of the wild type. Sections were hybridized with radioactively labelled antisense RNA derived from the 3′ end of the *def*A cDNA (Sommer *et al.*, 1990), not containing the conserved MADS-box domain. The distribution of silver grains was studied by dark field microscopy (A–D). Epifluorescence was used to visualize the underlying tissue (A–E). A control experiment with sense RNA as probe showed only background hybridization (not shown). b, bract; s, sepal; p, petal; st, stamen; std, staminodium; g, gynoecium. Bar = 100 μm.

Temporal and spatial expression patterns of deficiens

The wild type. The temporal expression pattern and spatial distribution of *def*A mRNA was studied by *in situ* mRNA hybridization to longitudinal and cross sections of developing flower buds. As shown in Figure 8A, the beginning of sepal organ differentiation is the earliest stage at which *deficiens* mRNA becomes detectable. At the times of the appearance of morphologically detectable petal and stamen primordia, strong hybridization signals are visible in the corresponding regions of the young flower bud (Figure 8B and C). It seems that *def*A gene expression is restricted to the organ primordia and does not occur uniformly in the entire whorl area. In later stages of organogenesis unequal distribution of *def*A mRNA can be observed within the differentiating organs. Cross sections show that *def*A expression in petals is stronger in the upper lobe than in the lower and that it is more pronounced toward the ventral surface of the organ than toward its dorsal surface (Figure 8D). In stamens the *def*A hybridization signal is stronger in (if not exclusive to) the non-sporogenic tissue, such as the filament, connective tissue and the epidermis and endothecium of the anthers. Expression of *def*A in the developing gynoecium is weak but significant. The weak hybridization signal in young sepals (not shown) disappears in later stages of development (Figure 8B–D), perhaps due to elongation of cells and thus dilution of the *def*A mRNA.

Northern blot hybridization experiments with mRNA of dissected floral organs reveal that *def*A expression is very strong in petals and stamens, but is also detectable in the gynoecium and very weakly in sepals (Figure 9B). As reported previously (Sommer *et al.*, 1990), after its establishment, expression of *deficiens* is maintained throughout the whole period of flower development, at a more or less constant level. The Northern data are in agreement with the *in situ* hybridization experiments.

Deficiens expression in mutant alleles. Expression of *def*A in flowers of mutant alleles was studied by Northern blot hybridization to mRNA extracted from flower buds (Figure 9A). We collected flowers at comparable stages of development (between ~0.5 and 1 cm in length), but since the mutations affect the size of the flower the differences obtained

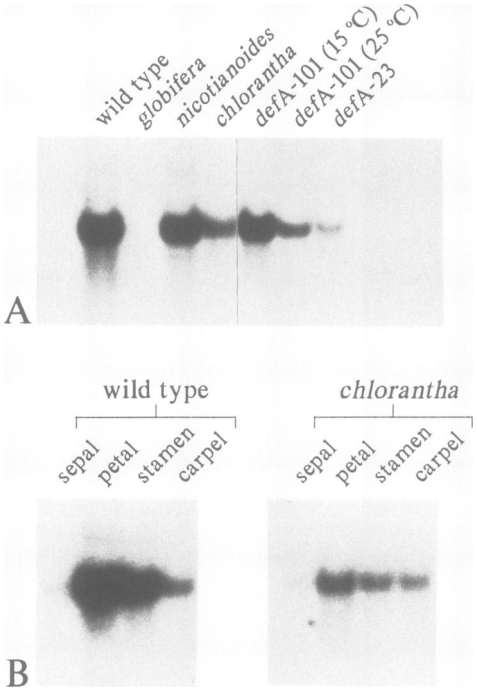

Fig. 9. Expression of *deficiens* in flowers of different *def*A mutants. (**A**) Northern blot analysis of poly(A)$^+$ RNA (1.5 μg/lane) isolated from 0.5−1 cm long young flower buds of plants carrying different mutant *def*A alleles (genotype indicated above the lanes). All plants except *def*A-101 were grown in the glasshouse. The growing temperature for *def*A-101 plants is indicated above the corresponding lanes. (**B**) Northern blot analysis of *def*A gene expression in dissected organs of 1 cm long wild type (left) and *chlorantha* (right) flowers. For all Northern hybridization experiments the 3′ end of the *def*A cDNA (Sommer *et al.*, 1990), not containing the conserved MADS-box domain, was used as a radioactive probe. To test for equal loading of RNA the same Northern blots were hybridized with an actin probe. Comparable hybridization intensities were observed for all lanes (not shown).

in the intensity of hybridization signals reflect only roughly the differences in *def*A expression *in vivo*. Nevertheless, it is clear that *def*A expression is slightly decreased in flowers of *nicotianoides* and *def*A-101 plants grown at low temperature (Figure 9A). *Def*A expression is abolished in *globifera* flowers and is greatly decreased in flowers of *chlorantha*, *def*A-23 plants and also in *def*A-101 grown at high temperature (Figure 9A). The strict temperature dependence of *def*A expression in *def*A-101 was confirmed by *in situ* hybridization of longitudinal sections of inflorescences of *def*A-101 plants (not shown).

The mRNA level in *chlorantha* flowers is much less than in wild type (Figure 9A). Northern blot experiments with dissected *chlorantha* flower organs show that the mutation specifically reduces *def*A expression in petals and stamens, but not in the gynoecium and in sepals (Figure 9B). These results demonstrate that *deficiens* expression in the wild type is upregulated organ-specifically in stamens and petals.

Discussion

The morphological and molecular studies performed with five *deficiens* morphoalleles address several aspects of the control of organ identity and organogenesis by this homeotic gene. These aspects are discussed in the context of both the genetic control of *deficiens* expression and of its functions in two different organs.

Mutations define functional domains of the deficiens protein

The DEF A protein encoded by the homeotic *deficiens* gene contains the MADS-box, a conserved DNA binding domain also found in other known transcription factors (see Introduction), at its N-terminal end. Gel retardation assays presented in this report prove that DEF A is a DNA-binding protein. The *deficiens* protein may thus function as a transcriptional regulator in the control of developmental processes during floral organogenesis.

The MADS-box domain of MCM1 in yeast is responsible not only for DNA binding but also for transcriptional activation, or co-repression, of a subset of non-cell type specific or **a**-cell type specific genes (Christ and Tye, 1991). A second, acidic domain, located carboxy-terminal to the MADS-box, is necessary for both positive and negative regulation of α-cell type specific genes, and thus possibly for the interaction of MCM1 with the α2 protein. No such detailed information on functional domains of DEF A, or other floral MADS-box proteins, is yet available. But the two mutations in the *nicotianoides* and *def*A-101 alleles define two spatially separated domains in DEF A, because no further sequence differences from the wild type were found in the two alleles. The altered morphology of the flowers of the mutants and the decreased ability of the altered proteins to bind, together with GLO, as heterodimers to an artificial binding motif in gel retardation assays suggest that both domains are involved in *deficiens* function, either in DNA binding or in dimerization.

The point mutation which results in a glycine to aspartic acid exchange in *nicotianoides* alters one of the few strictly conserved amino acids present at this position in all the MADS-box proteins of the family (for compilation see Schwarz-Sommer *et al.*, 1990; Ma *et al.*, 1991, Pnuely *et al.*, 1991) and causes loss of DNA binding to the P-box of the *STE6* promoter *in vitro*, as shown by gel retardation assays with GLO and the *nicotianoides* protein. These findings provide evidence that the MADS-box is a functional DNA binding domain of the *deficiens* protein.

The lesion in the *def*A-101 allele occurred in a region carboxy-terminal the MADS-box. In contrast to the acidic domain in MCM1, this region is rather basic in DEF A. According to Ma *et al.* (1991) this region, called the K-box, is conserved in all AG-like proteins and also in DEF A. The K-box shows weak homology to keratin and may possibly form two amphipathic helices. Computer analysis of the putative K-box of DEF A did not convincingly demonstrate that the first amphipathic helix is likely to be formed (Pnuely *et al.*, 1991). Nevertheless, the mutant phenotype of *def*A-101 flowers is caused by deletion of a lysine residue, a positively charged amino acid. This clearly defines this region as a functionally important domain of DEF A. At present the precise function of this domain is not known, although temperature sensitivity of the mutant allele may suggest that protein−protein interaction of DEF A with another regulatory factor (for instance GLO) is affected.

However, *nicotianoides* and *def*A-101 plants develop flowers whose morphology is less strongly altered than the null allele *globifera*, where no *deficiens* protein is produced. Thus, *in vivo*, the mutation in the MADS-box of the *nico-*

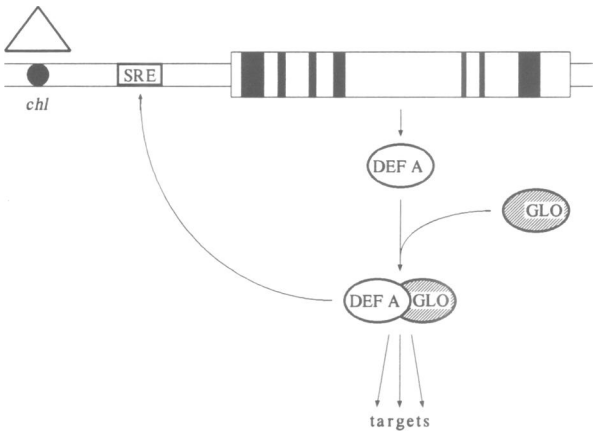

Fig. 10. Possible mechanism of autoregulation of *deficiens* expression. The *deficiens* gene with its promoter and coding region is depicted schematically at the top. The dot in the upstream regulatory region symbolizes the *chlorantha* (*chl*) mutation, which defines the *cis*-acting binding site for a regulatory protein (triangle). SRE is a potential binding site for MADS-box proteins. Open and shaded circles represent the DEF A and GLO proteins, respectively.

tianoides protein and in the K-box of the *def*A-101 protein do not abolish *deficiens* function(s) completely, suggeseting (i) that the *in vitro* assay does not precisely reproduce the *in vivo* binding conditions and (ii) that the altered morphology of mutant flowers may be due to the altered DNA binding affinity of the mutant DEF A protein to, or specificity for, binding sites within the promoter region of some of the target genes.

Deficiens controls different sets of genes in stamens and petals

Several morphological observations indicate that the function of *def*A in petals and in stamens is dissectable. For example, reduction of gene dosage of the *nicotianoides* allele in combination with a null allele, *globifera*, increases feminization of stamens but hardly affects nicotianoides petal morphology. Further, the phenotype of *def*A-23 flowers indicates that a single mutation in the *def*A gene may differentially affect petal and stamen morphogenesis. While the precise molecular mechanism underlying such organ-specific differences is not yet known, the ability of *def*A to act differentially in petal and stamen development seems to be apparent (Sommer *et al.*, 1991). As pointed out earlier (Sommer *et al.*, 1990; Schwarz-Sommer *et al.*, 1990), post-translational modification, such as phosphorylation, and combinatorial interaction with other proteins may specifically modify the function of the DEF A protein in organogenesis. As a consequence, DEF A function(s) in the two organs and at different stages of development may differ. Such temporal and spatial aspects of *deficiens* function in the control of petal and stamen organogenesis could be analysed in the future by utilizing the temperature sensitive allele *def*A-101 in temperature shift experiments.

The *def*A-23 mutation may allow access to the molecular basis of differential function of *def*A. The exchange of amino acids at the carboxy terminus of the DEF A protein could indicate a functional domain essential for DEF A function in stamens but almost dispensible for its function in petals. Unfortunately, the mutation is too complex at the molecular level, with functional consequences that are not easy to predict; for example, the stability of the altered protein product may be decreased, the stability of the *def*A-23 mRNA may be affected in the mutant or the transport of the protein into the nucleus could be hampered. Thus, quantitative changes in the amount of the protein could be the reason for the differential effects in two organs, when the affinity of DEF A for the various target sequences differs. It will be possible to analyse these aspects in the future when specific antibodies directed against various epitopes of the DEF A protein become available.

Induction of deficiens expression and its upregulation in stamens and petals are two independent processes

A single mutation in the *def*A promoter in *chlorantha* specifically decreases *def*A expression in petals and stamens, but expression in sepals and carpels is not altered. This observation points to several interesting aspects of the genetic control governing *def*A expression during organogenesis. Firstly, induction of *def*A expression in the flower and its organ-specific regulation of expression seem to be independent processes which possibly are controlled by factors binding to different sites of the *def*A promoter. Secondly, *def*A is specifically upregulated in petals and stamens; thus the low level of expression in the sepals and carpels is not due to specific suppression of gene expression in these organs. Thirdly, because a single mutation concomitantly affects expression of *def*A in petals and stamens, the factor controlling *def*A expression seems to be common to both organs. Although the molecular nature of this factor is not known, one may speculate that it is a transcription factor whose *cis*-acting binding site is affected in the *chlorantha* mutant.

In addition to a common regulatory factor of *def*A expression in petals and stamens, organ-specific regulation of the gene may exist as well. An indication for the existence of such a factor(s) is the 'improved' petal morphology of *globifera* × *chlorantha* heterozygotes, as compared with the *chlorantha* homozygotes. 'Improved' morphology of the flowers of the heterozygote is unexpected, because *globifera* is a null allele; thus its combination with *chlorantha* in a heterozygote decreases the dosage of the *chlorantha* allele. As argued in the Results section, the presence of a modifying factor in the *globifera* plant genome may be responsible for such effects. This modifier could be a petal-specific protein whose interaction with the altered promoter site in *chlorantha* is mutationally improved. On the other hand, the feminizing effect of all *deficiens* morphoalleles decreases when mutant plants are grown at low temperature. This suggests that factor(s) interacting with *deficiens* in the third whorl may accumulate when the developmental phase of stamens is prolonged due to slower growth.

Autoregulation may be involved in elevated deficiens expression in petals and stamens

Autoregulation as the molecular basis of persistent gene expression during development has been proven for several patterning genes in *Drosophila* (Jiang *et al.*, 1991; Regulski *et al.*, 1991; for review see Serfling, 1989). It has been suggested that after induction of gene expression by a transient external signal (the primary positional information), autoregulation maintains this developmental decision (Kuziora and McGinnis, 1988; Serfling, 1989). Also for *def*A there is some evidence that the high level of gene expression during petal and stamen develoment may be

achieved and maintained, at least in part, by an autoregulatory mechanism.

The temperature sensitive mutant *def*A-101, when growing under non-permissive conditions, not only affects the morphology of the flowers, but also shows dramatically reduced transcription of the mutant *def*A gene (Figure 9A). In *def*A-101 the site of mutation is located within an exon, thus altering the protein product of the gene and not affecting the promoter region. Similarly, the altered structure of the proteins in two other mutants, *nicotianoides* and *def*A-23, also leads to decreased *deficiens* expression. These observations suggest that *def*A transcription is autoregulated by the DEF A protein, either directly or indirectly.

One possible autoregulatory mechanism of *deficiens* expression is shown schematically in Figure 10. The scheme proposes that, after initial induction of *deficiens* expression, DEF A together with the GLO protein controls maintenance of *deficiens* expression. This hypothesis is compatible with several observations. Firstly, *globosa* mutants are phenotypically indistinguishable from mutants of *deficiens* (Stubbe, 1966; Schwarz-Sommer *et al.*, 1990), suggesting that GLO and DEF A control similar, if not identical, developmental processes. One of these could be the transcriptional (auto)regulation of *deficiens* expression in petals and stamens. The scheme includes also the possibility that, in addition, the DEF A−GLO heterodimer controls the expression of target genes. Second, the SRE motif in the *deficiens* promoter (Figure 6) may represent a binding site to which MADS-box proteins like DEF A could bind as homodimer or heterodimer in combination with other protein(s). Third, the DEF A protein, in combination with the GLO protein, is capable of DNA binding to an SRE-related sequence motif *in vitro*. Preliminary binding studies indicate that the DEF A−GLO heterodimer binds also to the SRE-like motif in the *def*A promoter *in vitro* (Tröbner *et al.*, in preparation). However, it will still be necessary to show that this particular site is functional *in vivo*.

The only slightly reduced mRNA level of *nicotianoides* is in contrast to the absence of DNA binding of the mutant protein in the *in vitro* assay and to the severe phenotypic alteration of the mutant. This seems to contradict autoregulation of *def*A. However, this example only demonstrates the difficulties that arise when one tries to correlate molecular data with phenotypic effects. As argued in a previous section, the amino acid exchange in the DNA binding domain of the mutant protein may have differential effects *in vivo* which cannot be simulated in the *in vitro* binding assay. Thus, it could be that, in the presence of accessory proteins and/or chemical modification, binding of the mutant protein to the SRE motif in the *deficiens* promoter is hardly affected; therefore, the level of *nicotianoides* mRNA is only slightly reduced in the mutant. In contrast, the binding to different SRE motifs (with different nucleotide sequences) of the 'downstream' target genes may be altered or abolished completely with severe consequences for the morphology of the developing flower, as indicated by the strong phenotype of a *nicotianoides* flower.

That additional proteins are involved in the transcriptional regulation of *deficiens* expression may be indicated by the fact that mutational alteration at a site which flanks the SRE-like motif in the promoter of the *chlorantha* mutant also interferes with *def*A transcription. Since the SRE motif and the site defined by the *chlorantha* mutation are located close together, it is possible that the corresponding proteins (or protein complexes) interact in organ-specific upregulation and autoregulatory maintenance of *deficiens* expression. The similarity of the structural organization of this region in the *deficiens* promoter with that of the promoter of the cardiac α-actin gene (see Results section) supports this hypothesis. Whether spatial and temporal expression of the *deficiens* gene are influenced by binding of additional proteins within this particular region and at other sites of the promoter region remains to be demonstrated.

Materials and methods

Plant material

Plants were grown in the glasshouse at 18−25°C under additional light during the winter. To exclude the influence of differences in the genetic background on the temperature dependence of mutant phenotypes, cuttings of a plant with a given genotype were studied. The young cuttings were transferred to growth chambers adjusted to a temperature of 15°C (cold) or 25°C (warm). The light intensities and day−night regimes (16 h light and 8 h dark) of the chambers were identical. Increased light intensity had no visible effects on mutant phenotypes.

Genetic stocks

Seeds of genetic stocks of wild type *deficiens* (Sippe 50) and of *globifera*, *nicotianoides* and *chlorantha* mutants (Baur, 1924; Hertwig, 1926; Stubbe, 1966) were obtained from the Gatersleben seed collection. As described by Hertwig (1926), the three mutant alleles originated from different plants with different genetic backgrounds. Thus the wild type progenitors of the *deficiens* alleles may not be related to each other. According to our analysis (see Results section) the *globifera* mutation occurred in a different *deficiens* allele from *chlorantha* and *nicotianoides*, which apparently share a parental *deficiens* allele (probably Sippe 50).

Transposon mutagenesis

Line T53 with the genotype *niv*-53::Tam1, used for transposon mutagenesis experiments, was obtained from Rosemary Carpenter (John Innes Institute, Norwich, UK). The *def*A-23 allele was uncovered in a population of *def*A/*def*^{gli} F1 hybrids (Sommer *et al.*, 1990). The plant displayed altered floral morphology indicating a mutation in the parental wild type allele. To obtain *def*A-23 homozygotes the F1 plant was fertilized with pollen from a *chlorantha* homozygote (*def*^{chl}/*der*^{chl}) and subsequently the progeny was selfed. These plants either segregated chlorantha and globifera phenotypes in a ratio of 3:1, or chlorantha and *def*A-23 phenotypes in a ratio of 3:1. The *def*A-23 phenotypes of the latter plants is thus due to their *def*A-23/*def*A-23 genotype.

The *def*A-101 mutant was obtained after selfing a colour revertant of T53. Eight of the 34 progeny plants displayed the *def*A-101 phenotype and five plants showed a strongly compressed phenotype indicating the presence of two heritable recessive mutations in the parental T53 plant. For complementation tests the *def*A-101 mutant flowers were fertilized with *chlorantha* pollen and, since the progeny showed chlorantha phenotype, this cross proved that the new phenotype was due to a mutation in the *deficiens* gene. Genetic analysis indicated that the additional recessive mutation, which affects vegetative growth, was not linked to the *deficiens* gene. The *def*A-101 plant used for experiments in this report was a heterozygote for this mutation.

Methods

All methods, including scanning electron microscopy, preparation of plant DNA and mRNA, molecular cloning of genomic DNA and cDNA, subcloning into plasmid vectors, DNA sequence analysis, blotting techniques and hybridizations were conducted as previously described (Sommer *et al.*, 1990). Techniques applied for *in situ* hybridization will be described in detail elsewhere (Huijser,P., Klein,J., Meijer,H., Saedler,H. and Sommer,H., in preparation).

Sequence analysis of the wild type *deficiens* gene and of all mutant alleles, except for *def*A-23, was performed with genomic *Eco*RI fragments cloned into λ NM1149 phages and subcloned into pBR322. In the case of the *def*A-23 allele the structure of the cDNA and the genomic integration site of Tam8 and surrounding sequences was determined.

In vitro translations

The pT7 βΔATG construct for SRF (Norman *et al.*, 1988) was kindly provided by R.Treisman (MRC, Cambridge, UK). Wild type and mutant

*def*A cDNAs were cloned into the *Acc*I−*Eco*RI site of pT7βSal (Norman *et al.*, 1988) and the *glo* cDNA was inserted into the *Nco*I−*Eco*RI site of the same vector. Transcription with T7 polymerase was performed in the absence of m7GpppG. *In vitro* translations using rabbit reticulocytes (Promega) were done according to the manufacturer's instructions and were tested on 12.5% SDS−PAGE gels.

DNA binding assay

Binding reactions contained 0.5 ng (10^4 c.p.m.) of a 32 bp 3′ end-labelled, double-stranded oligonucleotide representing the P-box of the *STE6* promoter of yeast (Keleher *et al.*, 1988), 2 μl reticulocyte lysate and 3.5 μl of binding buffer [as in Schröter *et al.* (1987) except that no unspecific poly(dI−dC) carrier was used] in a final volume of 11 μl. After 20 min incubation at room temperature the reactions were loaded onto a 5% polyacrylamide gel in 0.5× TBE buffer.

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References

Awasthi,D.K., Kumar,V. and Murty,Y. (1984) *Bot. Mag. Tokyo*, **97**, 13−22.

Baur,E. (ed.) (1924) *Bibl. Genet.*, **4**, 101−103.

Bergfeld,R. (1956) Z. Indukt. Abst.-u. Vererbungsl., **87**, 784−797.

Boxer,L.M., Prywes,R., Roeder,R.G. and Kedes,L.J. (1989) *Mol. Cell. Biol.*, **9**, 515−522.

Bowman,J.L., Smyth,D.R. and Meyerowitz,E.M. (1991) *Development*, **112**, 1−20.

Carpenter,R. and Coen,E.S. (1990) *Genes Dev.*, **4**, 1483−1493.

Christ,C. and Tye,B.-K. (1991) *Genes Dev.*, **5**, 751−763.

Coen,E.S. (1991) *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **42**, 241−279.

Coen,E.S. and Meyerowitz,E.M. (1991) *Nature*, **353**, 31−37.

Dubois,E., Bercy,J. and Messenguy,F. (1987) *Mol. Gen. Genet.*, **207**, 142−148.

Fedoroff,N. (1989) In Howe,M. and Berg,D. (eds), *Mobile DNA*. American Society for Microbiology, Washington DC, pp. 375−411.

Gierl,A., Schwarz-Sommer,Zs. and Saedler,H. (1985) *EMBO J.*, **4**, 597−583.

Gierl,A., Saedler,H. and Peterson,P.A. (1989) *Annu. Rev. Genet.*, **23**, 71−85.

Green,P.B. and Linstead,P. (1990) *Protoplasma*, **158**, 33−38.

Gustafson,T.A. and Kedes,L.-H. (1989) *Mol. Cell. Biol.*, **9**, 3269−3283.

Haughn,G.W. and Somerville,C.R. (1988) *Dev. Genet.*, **9**, 73−89.

Hayes,T.E., Sengupta,P. and Cochran,B.H. (1988) *Genes Dev.*, **2**, 1713−1722.

Hertwig,P. (1926) *Z. Indukt. Abst.-u. Vererbungsl.*, **41**, 42−47.

Jiang,J., Hoey,T. and Levine,M. (1991) *Genes Dev.*, **5**, 265−277.

Joshi,C.F. (1987) *Nucleic Acids Res.*, **15**, 6643−6653.

Keleher,C.A., Goutte,C. and Johnson,A.D. (1988) *Cell*, **53**, 927−936.

Klemm,M. (1927) *Bot. Archiv.*, **20**, 423−474.

Kuziora,M.A. and McGinnis,W. (1988) *Cell*, **55**, 477−485.

Lord,E. (1991) *Flowering Newslett.*, **11**, 4−13.

Ma,H., Yanofsky,M.F. and Meyerowitz,E.M. (1991) *Genes Dev.*, **5**, 484−495.

Messing,J., Geraghty,D., Heidecker,G., Hu, N-T., Kridl,J. and Rubenstein,I. (1983) In Meredith,C.P., Kosuge,T. and Hollaender,A. (eds) *Genetic Engineering of Plants*. Plenum Press, New York, pp. 211−227.

Meyer,V. (1966) *Bot. Rev.*, **32**, 165−195.

Meyerowitz,M.E., Smyth,D.R. and Bowman,J.L. (1989) *Development*, **106**, 209−217.

Mohun,T.J., Chambers,A.E., Towers,N. and Taylor,M.V. (1991) *EMBO J*, **10**, 933−940.

Norman,C., Runswick,M., Pollock,R. and Treisman,R. (1988) *Cell*, **55**, 989−1003.

Passmore,S., Maine,G.T., Elble,R., Christ,C. and Tye,B.K. (1988) *J. Mol. Biol.*, **204**, 593−606.

Passmore,S., Elble,R. and Tye,B.K. (1989) *Genes Dev.*, **3**, 921−935.

Pnuely,L., Abu-Abeid,M., Zamir,D., Nacken,W., Schwarz-Sommer, Zs. and Lifschitz,E. (1991) *Plant J.*, **1**, 255−266.

Regulski,M., Dessain,S., McGinnis,N. and McGinnis,W. (1991) *Genes Dev.*, **5**, 278−286.

Sartorelli,V., Webster,K.A. and Kedes,L. (1990) *Genes Dev.*, **4**, 1811−1822.

Schröter,H., Shaw,P.E. and Nordheim,A. (1987) *Nucleic Acids Res.*, **15**, 10145−10157.

Schwarz-Sommer,Zs., Shepherd,N., Tacke,E., Gierl,A., Rohde,W., Leclercq,L., Mattes,M., Berndtgen,R., Peterson,P.A. and Saedler,H. (1987) *EMBO J.*, **6**, 287−294.

Schwarz-Sommer,Zs., Huijser,P., Nacken,W., Saedler,H. and Sommer,H. (1990) *Science*, **250**, 931−936.

Serfling,E. (1989) *Trends Genet.*, **5**, 131−133.

Sommer,H., Beltran,J.-P., Huijser,P., Pape,H., Lönnig,W.-E., Saedler,H. and Schwarz-Sommer,Zs. (1990) *EMBO J.*, **9**, 605−613.

Sommer,H., Nacken,W., Beltran,P., Huijser,P., Pape,H., Hansen,R., Flor,P., Saedler,H. and Schwarz-Sommer,Zs. (1991) *Development*, **Suppl. 1**, 169−176.

Stubbe,H. (ed.) (1966) *Genetik und Zytologie von Antirrhinum L. sect. Antirrhinum*. VEB Gustav Fischer Verlag, Jena.

Taylor,M.R., Treisman,R., Garrett,N. and Mohun,T. (1989) *Development*, **106**, 67−78.

Yanofsky,M.F., Ma,H., Bowman,J.L., Drews,G.N., Feldman,K.A. and Meyerowitz,E.M. (1990) *Nature*, **346**, 35−39.

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