Development 128, 2661-2671 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
DEV0367

Epidermal control of floral organ identity by class B homeotic genes in *Antirrhinum* and *Arabidopsis*

Nadia Efremova, Marie-Christine Perbal, Alexander Yephremov, Winfried A. Hofmann, Heinz Saedler and Zsuzsanna Schwarz-Sommer*

Max-Planck Institut für Züchtungsforschung, Carl-von-Linné Weg 10, 50289 Köln, Germany
*Author for correspondence (schwarzs@mpiz-koeln.mpg.de)

Accepted 8 May 2001

SUMMARY

To assess the contribution of the epidermis to the control of petal and stamen organ identity, we have used transgenic *Antirrhinum* and *Arabidopsis* plants that expressed the *Antirrhinum* class B homeotic transcription factors *DEFICIENS* (*DEF*) and *GLOBOSA* (*GLO*) in the epidermis. Transgene expression was controlled by the *ANTIRRHINUM FIDDLEHEAD* (*AFI*) promoter, which directs gene expression to the L1 meristematic layer and, later, to the epidermis of differentiating organs. Transgenic epidermal DEF and GLO chimeras display similar phenotypes, suggesting similar epidermal contributions by the two class B genes in *Antirrhinum*. Epidermal B function autonomously controls the differentiation of *Antirrhinum* petal epidermal cell types, but cannot fully control the pattern of cell divisions and the specification of sub-epidermal petal cell-identity by epidermal signalling. This non-autonomous control is enhanced if the endogenous class B genes can be activated from the epidermis. The developmental influence of epidermal B function in *Antirrhinum* stamen development is very limited. In contrast, epidermal B function in *Arabidopsis* can control most if not all epidermal and sub-epidermal differentiation events in petals and stamens, without any contribution from the endogenous class B genes. Possible reasons for differences in the efficacy of B-function-mediated cell communication between the two species are discussed. Interestingly, our experiments uncovered partial incompatibility between class B functional homologues. Although the DEFICIENS/PISTILLATA heterodimer is functional in transgenic *Arabidopsis* plants, the APETALA3/GLOBOSA heterodimer is not.

Key words: Flower development, Epidermal chimeras, Cell communication, MADS-box protein, *DEFICIENS*, *GLOBOSA*, *FIDDLEHEAD*, *Arabidopsis*, *Antirrhinum*

INTRODUCTION

The organisation and developmental potential of meristems – the source of vegetative and reproductive organs in higher plants – has long been of great interest (for a recent review see Szymkowiak and Sussex, 1996). According to the generally accepted concept, floral meristems are organised in layers, and the three meristem layers termed L1, L2 and L3 contribute differentially to the formation of different tissues and to the development of different floral organs (Satina and Blakeslee, 1941). Organ identity becomes manifest in the number, shape, size and function of the cells that make it up. It follows that communication between neighbouring cells and cell layers is necessary to co-ordinate the pattern and rate of cell divisions and cell enlargement during organ development. Our interest is in elucidating the role of class B floral homeotic genes in this process.

In *Antirrhinum* two class B genes, *DEFICIENS* (*DEF*; Schwarz-Sommer et al., 1992) and *GLOBOSA* (*GLO*; Tröbner et al., 1992) control petal organ identity and, in combination with two class C genes, *PLENA* and *FARINELLI* (Davies et al., 1999), the development and function of stamens. In the absence of *DEF* or *GLO* expression in *def* and *glo* mutants, petals acquire a sepaloid developmental fate and stamens develop as carpels. *DEF* and *GLO* encode MADS-box transcription factors (Schwarz-Sommer et al., 1990) that are unstable when expressed alone and become stabilised by forming a heterodimer (Tröbner et al., 1992; Zachgo et al., 1995). Furthermore, DEF and GLO can form a ternary protein complex by interacting with other MADS-box proteins (Egea-Cortines et al., 1999). The genes are independently induced early during flower development, but their expression later becomes mutually interdependent. The major *DEF* and *GLO* expression domains overlap in whorl 2 (the position of petals) and whorl 3 (the position of stamens), where the genes are expressed during the entire period of organogenesis and differentiation (Zachgo et al., 1995). Most of the findings made with *DEF* and *GLO* have been confirmed for their *Arabidopsis* orthologues *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), although their expression patterns differ in detail (Riechmann and Meyerowitz, 1997).

The contribution of *DEF* and *GLO* to the control of cell and

organ identity and cell-cell communication between layers that differ in genotype has been studied in somatically stable *def* and *glo* periclinal chimeras, obtained using genetically unstable transposon-induced alleles (Carpenter and Coen, 1990; Perbal et al., 1996). In L1 periclinal chimeras the wild-type *DEF* allele is present in L1 whereas L2/L3 carry the *def* mutant allele. The genetic constitution of the layers in L2/L3 chimeras is the opposite of that in L1 chimeras: L2/L3 carry the wild-type *DEF* (or *GLO*) allele and L1 the *def* (or *glo*) mutant allele. Complete or partial rescue of wild-type cell types and cell division patterns in the layer carrying the wild-type allele in such chimeras reveals cell-autonomous (or autonomous) control of differentiation. The wild-type layer may also influence the number and identity of cells in the adjacent, genetically mutant layer. This is the hallmark of non-autonomous control and implies cell communication. The absence of autonomous control is also indicative of interdermal communication during wild-type development. Petal size and morphology in L1 and L2/L3 periclinal chimeras were found to be intermediate between wild type and mutant, revealing a mutual non-autonomous influence of layers on cell type and cell divisions. The wild-type genes autonomously controlled the differentiation of petal cell types in the layer in which they were expressed, and influenced the types of cells formed in the adjacent mutant layer. The moderate non-autonomous influence of *DEF* and *GLO* in L2/L3 on epidermal features of petals and stamens and on organ shape could be, in part, attributed to trafficking of the DEF and GLO proteins from L2 to L1, but no DEF protein movement from L1 into inner layer cells could be detected (Perbal et al., 1996). The absence of *DEF*-mediated lateral communication between cells within a layer is evident from the sharp boundary between wild-type and mutant cells in sectorial chimeras (Carpenter and Coen, 1990; Perbal et al., 1996). *DEF* and *GLO* behaved similarly in petals of sectorial chimeras and in L2/L3 periclinal chimeras; however, no *glo* L1 chimeras could be obtained to compare the influence of *GLO* and *DEF* directly.

AP3 and *PI* differ in the extent of their autonomous and non-autonomous contributions to *Arabidopsis* organ identity. *PI* can control petal and stamen development from the epidermis, as revealed by the wild-type phenotype of X-ray-induced chimeras (Bouhidel and Irish, 1996). In contrast, *AP3* in L1 has little non-autonomous influence on sub-epidermal development in whorl 2, and no effect on the developmental fate of organs in whorl 3, according to more recent observations with transgenic *Arabidopsis* plants genetically engineered to mimic somatically stable transposon-induced alleles (Jenik and Irish, 2001). Whether the difference in the behaviour of class B genes in *Antirrhinum* and *Arabidopsis* and between the two class B proteins in *Arabidopsis* is real or is due to differences in the mode of production of chimeras is not known, and this question has been addressed in this report.

One disadvantage of somatic chimeras for the study of cell communication is that the genotypic differences between the cell layers are not heritable. This precludes genetic studies on the mechanisms underlying non-autonomous processes. They are also unsuitable for comparative studies of different genes in one species or orthologous genes in different species, because the genetic tools available for making chimeras (such as transposon-induced alleles for somatically stable chimeras, or genetic markers for X-ray-induced chimeras, or techniques for constructing graft chimeras and for vegetative maintenance of useful material) cannot always be applied to different genes and species. To overcome these problems we generated transgenic *Antirrhinum* and *Arabidopsis* plants that express *DEF* or *GLO* under the control of the epidermis-specific promoter of the *ANTIRRHINUM FIDDLEHEAD* (*AFI*) gene (A. Y. and N. Y., unpublished data). The *AFI* promoter is active over a long period of development, like the *AtML1* promoter used in comparable experiments (Sessions et al., 2000). Its function depends on cell position and not on cell lineage: the *AFI* promoter is active in the L1 meristematic layer and in L1-derived and differentiated epidermal cells, but not in L1-derived sub-epidermal cells (A. Y., N. Y. and M.-C. P., unpublished data). In this report we first show that the phenotypes of somatically stable *Antirrhinum* chimeras that express *DEF* in L1 under the control of its own promoter are reconstituted in transgenic plants expressing *DEF* under the control of the *AFI* promoter. By expressing *DEF* or *GLO* in the epidermis of transgenic plants we then demonstrate that epidermal B function is sufficient to ensure near normal sub-epidermal differentiation in *Arabidopsis*, but is less effective in *Antirrhinum*.

MATERIALS AND METHODS

Plant material

Plants were either grown in the greenhouse at a daytime temperature of 18-25°C and with additional light during the winter, or in a climate chamber under standard conditions (16 hours light, 18-20°C).

The *Arabidopsis thaliana* mutant *apetala3-3* (referred to as the *ap3* mutant in the text) was obtained from the Arabidopsis Biological Resource Centre (Ohio State University, Columbus) and the wild-type *Antirrhinum* line Sippe 50 from the Gatersleben collection. *niv*-98 (the progenitor of line 165E) and *pal*-rec2 were kindly provided by Rosemary Carpenter (John Innes Centre, Norwich, UK). The stable *deficiens* mutant *def*-B177 (referred to as the *def* mutant in the text) was obtained by transposon tagging (Schwarz-Sommer et al., 1992) and carried a *Tam2*-related insert (Z. Sch.-S., unpublished).

Binary vectors

Construction of the pHAF-XS and pBPF-XS vectors carrying a polylinker sequence (XS) and the *AFI* and *FDH* promoters, respectively, will be described in detail elsewhere (A. Y. et al., unpublished data). The full-length cDNAs for *DEF* or *AP3* and *GLO* or *PI*, extending from the ATG translation start codon and specifying the 12-amino acid c-myc epitope as a C-terminal translational fusion, were inserted between the *Xho*I and *Xba*I sites in the polylinker of the vectors. The resulting AFI::DEF, AFI::GLO, FDH::AP3 and FDH::PI constructs were used for plant transformation.

Transgenic lines

Arabidopsis was transformed using the standard in planta vacuum infiltration protocol (modified after Bechtold et al., 1993) and *Antirrhinum* line 165E was transformed according to the method of Heidmann et al. (Heidmann et al., 1998). Transgenic plants and their progeny were selected on plates of solid MS medium containing hygromycin (15 μg/ml for *Arabidopsis* and 10 μg/ml for *Antirrhinum*). Subsequent generations were grown without selection and, if necessary, the presence of the transgene(s) was confirmed by PCR or by RT-PCR. For simplicity, transgenic plants are termed epidermal chimeras (or transgenic chimeras) in this report to indicate epidermal expression of genes controlled by the *AFI* promoter.

Transgenic *Antirrhinum* lines carried one to six copies of the transgene and transgenic *Arabidopsis* lines carried two to four copies,

Table 1. Overview of genotypes and phenotypes of transgenic plants

Genotype		Phenotype			
Endogenous genes	Epidermal transgenes	First whorl	Second whorl	Third whorl	Fourth whorl
Antirrhinum					
DEF; GLO		sepal	petal	stamen	carpel
def; GLO		sepal	sepal	carpel	–
DEF; glo		sepal	sepal	carpel	–
def; GLO	AFI::DEF	sepal	petaloid	carpeloid	carpel or –
DEF; glo	AFI::GLO	sepal	petaloid	carpeloid	carpel or –
DEF; GLO	AFI::DEF	sepal	petal	stamen	carpel
DEF; GLO	AFI::GLO	sepal	petal	stamen	carpel
DEF; GLO	AFI::DEF/GLO	petaloid	petal	stamen	short style split stigma
Arabidopsis					
AP3; PI		sepal	petal	stamen	carpel
ap3; PI		sepal	sepal	carpel	carpel
ap3; PI	AFI::DEF	sepal	petal*	stamen*	stamenoid‡
ap3; PI	FDH::AP3	sepal	petal*	stamen*	stamenoid‡
AP3; PI	AFI::DEF	sepal	petal	stamen	stamenoid‡
AP3; PI	AFI::GLO	sepal	petal	stamen	carpel
AP3; PI	FDH::AP3	sepal	petal	stamen	stamenoid‡
AP3; PI	FDH::PI	petaloid (weak)	petal	stamen	carpel
AP3; PI	AFI::DEF/GLO	petal*	petal	stamen	stamenoid‡
AP3; PI	FDH::AP3/PI	petal*	petal	stamen	stamenoid‡
AP3; PI	AFI::DEF/FDH::PI	petal*	petal	stamen	stamenoid‡
AP3; PI	FDH::AP3/AFI::GLO	sepal	petal	stamen	stamenoid‡

*Immature organs; ‡organs can proliferate inside whorl 3.

as determined by Southern hybridisation in the T0 and T1 generations, respectively. Copy number did not influence the phenotype of *Arabidopsis* lines. In *Antirrhinum* only plants carrying one copy of the transgene, obtained in the primary transformants or after outcrossing the transgene copies, showed phenotypic effects. The findings described in this report derive from observations on two independent transgenic *Antirrhinum* lines that carried a single copy of the transgene in question.

The presence of the c-myc epitope did not interfere with transgene expression as determined by observing the phenotype of transgenic plants transformed with an epitope-less variant of the transgenes. Unfortunately, attempts to detect the tagged proteins in situ, using antibodies directed against the epitope, were unsuccessful.

Microscopy and in situ hybridisation

Tissue preparation and in situ hybridisation with digoxigenin-labelled antisense RNA were carried out as previously described (Davies et al., 1996; Perbal et al., 1996). Cell walls of *Antirrhinum* sections were stained with Calcofluor or, for histological observations, with Toluidine Blue. Sections were viewed under fluorescent light or under bright-field illumination with a Zeiss Axiophot microscope.

For scanning electron microscopy (SEM) freshly harvested plant material was shock-frozen in liquid nitrogen, transferred to a cryo-chamber (Oxford Instruments), gold coated and examined in a Zeiss DSM 940 electron microscope at 5 kV.

All images were processed and assembled with the Adobe Photoshop and Canvas programmes.

RESULTS

Epidermal expression of *DEF* or *GLO*, separately or in combination did not affect the vegetative development of transgenic *Antirrhinum* or *Arabidopsis* plants. Floral phenotypes of transgenic plants in both species were variable within selfed and outcrossed populations, as well as within single plants. This was influenced slightly by the genetic background (stronger phenotypes were observed in Line 165E and weaker ones in the Pal-rec2 and Sippe 50 backgrounds in *Antirrhinum* and stronger phenotypes were found in the L*er* compared to the Col background in *Arabidopsis*) and, more markedly, by growth temperature. Variability could not be correlated with the age of the plant or the position of the flower within the inflorescence. Most of the data presented refer to the commonly observed intermediate to strong phenotypes. Table 1 gives an overview of the phenotypic alterations associated with epidermal expression of the transgene(s), together with the genotypes of plants examined.

Expression of the B function in the epidermis of *def* and *glo* mutant *Antirrhinum* plants reveals cell-cell communication during petal development

The AFI::DEF transgene was introduced into the *def* mutant background to test whether the *AFI* promoter is able to regulate *DEF* expression in the same manner as the endogenous *DEF* promoter and to reproduce the phenotype of somatically stable L1 chimeras (Perbal et al., 1996). In somatic chimeras L1-derived wild-type cells can invade the underlying L2 layer owing to rare periclinal cell divisions in L1; as a consequence, sub-epidermal cells derived this way express the *DEF* gene. This is not possible in *def*; AFI::DEF transgenic plants, because the epidermis-specific *AFI* promoter (in contrast to the endogenous *DEF* promoter) is inactive in sub-epidermal cells (see Introduction). *def*; AFI::DEF epidermal chimeras therefore provide a means of following more precisely the contribution of epidermal *DEF* to the control of organ identity. In addition, *glo*; AFI::GLO epidermal chimeras were obtained to determine whether epidermally expressed *DEF* and *GLO* differ in their developmental effects. However, in contrast to the differences observed between the phenotypes of *PI* and *AP3* L1 chimeras in *Arabidopsis* (see Introduction), the

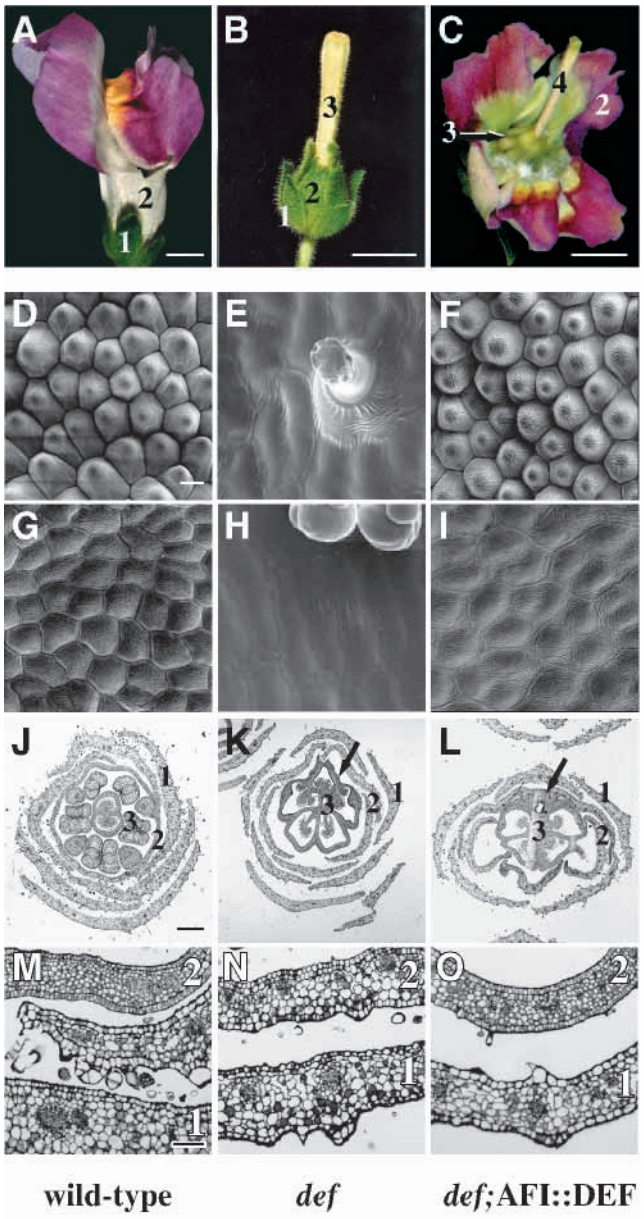

Fig. 1. Morphological features of *Antirrhinum* flowers: (left panels) wild-type; (middle panels) *def*; (right panels) *def*; AFI::DEF. (A-C) Photographs of whole mature flowers. The arrow in C indicates the shortened style of carpeloid third whorl organs (for comparison see the third whorl carpel in B). Bars, 5 mm. D-I: SEM micrographs of the inner (adaxial) epidermal surface of the upper (D-F) and the lower (G-I) regions of the second whorl organs. Bar, 20 μm. (J-L) Cross sections taken approximately in the middle of immature buds. Arrows in K and L point to the organ in the dorsal position in whorl 3, that is retarded in growth and is not visible in this section in wild-type (J) and transgenic flowers (L), but develops fully as a carpeloid organ in *def* mutant flowers (K). The flower in L, in contrast to the flower in C, did not develop a central carpel. Bar, 1 mm. (M-O) Cellular morphology of first- and second whorl organs in cross sections shown at a higher magnification. The shape and size of sub-epidermal cells in the transgenic second whorl organs in O is intermediate between the large and irregularly shaped sub-epidermal cells in whorl 2 of *def* mutants (N) and the small and more isometric sub-epidermal cells in wild-type petals (M). Bar, 100 μm. Numbers indicate whorls.

morphologies of *glo*; AFI::GLO and *def*; AFI::DEF chimeras were similar. In the following sections we therefore restrict our attention to the *def*; AFI::DEF chimeras.

Second whorl petaloid organs of *def*; AFI::DEF flowers are morphologically aberrant, forming a short tube at the base and a pigmented, partly unfolded lobe at the top (Fig. 1C). The three ventral organs display the typical curvature of wild-type ventral petals and include several cell types that are characteristic of wild-type ventral petals. The inner epidermal surface within the lobe region is made up of distinctive conical cells and, within the tube region, isometric flat cells are found, similar to wild-type petals and different from the second whorl sepals seen in *def* mutants (Fig. 1D-I).

The size of *def*; AFI::DEF petals is intermediate between that of wild-type petals and the second whorl sepals of the *def* mutant (Fig. 1A-C). Cross sections reveal that the sub-epidermal cells (as well as cells within the inner and outer epidermis) are smaller and more regular in shape than those of sepals or *def* second whorl organs (Fig. 1M-O). This shift towards wild-type morphology in the sub-epidermis suggests a limited degree of control of sub-epidermal cell division and cell shape by the epidermis, since the number of smaller cells has to increase in order to produce organs that are larger than in the *def* mutant. The intensity of the green colour in sub-epidermal cells in *def*; AFI::DEF petals is intermediate between mutant and wild type, indicating incomplete suppression of chlorophyll synthesis by a non-autonomous mechanism. Thus, as in somatic L1 chimeras (see Introduction), petal epidermal cell types and pigment synthesis are autonomously controlled by epidermal *DEF*, whereas epidermal *DEF* influences but cannot fully control petal cell identity in the sub-epidermal layers. As a consequence, epidermal B function is only partly sufficient to influence petal shape and size. These relationships are depicted schematically in Fig. 2.

To determine if epidermal signalling is achieved by trafficking of class B transcripts, the expression of *DEF* and *GLO* was monitored in *def*; AFI::DEF and *glo*; AFI::GLO flowers, respectively. As shown in Fig. 3, expression of the transgenes is confined to the outermost cell layer. The absence of detectable amount of sub-epidermal RNA in the transgenic chimeras corresponds to the absence of protein movement from L1 to L2 in somatic L1 chimeras (Perbal et al., 1996).

Epidermal B function cannot direct stamen development

The potential of AFI::DEF to restore stamen development in the *def* mutant is far less pronounced compared to petal restoration. The upper portion of the severely carpeloid *def*; AFI::DEF third whorl organs is often short and occasionally split (Fig. 1C). Their basal parts are frequently fused, in contrast to the absence of fusions between third whorl stamens in the wild type. This indicates defects in stamen initiation and early development. It is not possible to determine whether epidermal cell types correspond to that of a stamen or a carpel, because the style of a carpel and the filament of a stamen are superficially indistinguishable in terms of the type and arrangement of cells in the two organs. Development of stigmatic papillae at the tip of the carpeloid organs is frequently incomplete or abolished, revealing that this event is autonomously controlled. Epidermal expression of *DEF* is sufficient to suppress organ formation at

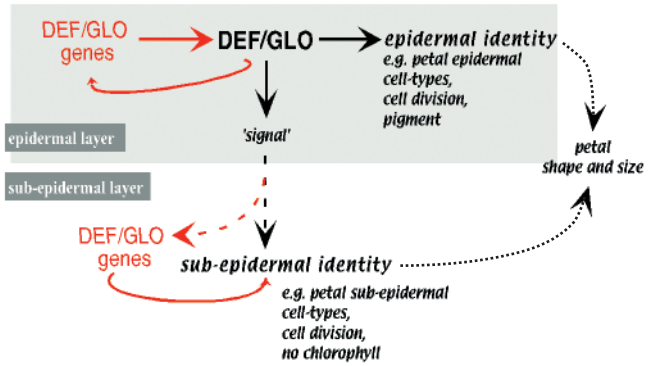

Fig. 2. Schematic summary of experimental observations on the influence of epidermal B function on petal development in *Antirrhinum*. Autonomous control is indicated by the solid arrows and non-autonomous control is shown by the broken arrows. The contribution of the endogenous *DEF*/*GLO* genes in wild-type sepals is indicated in red. All other control events are common to AFI::DEF/GLO wild-type sepals and *def* (or *glo*) mutant petals expressing AFI::DEF (or AFI::GLO). The dotted arrows on the right indicate an epidermal influence on petal shape and size, which are also affected by sub-epidermal events (Perbal et al., 1996), not discussed in this report.

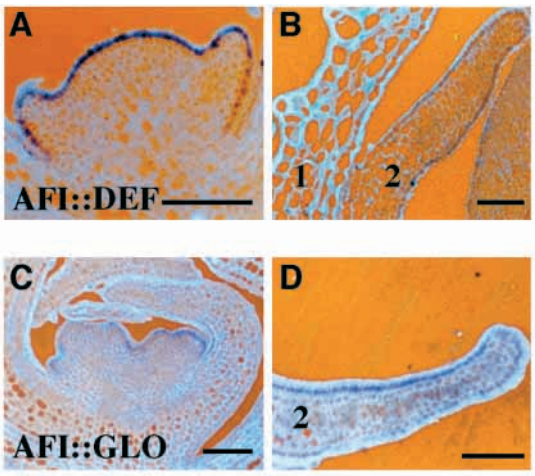

Fig. 3. In situ analysis of *DEF* (A,B) and *GLO* (C,D) expression in longitudinal sections of *def*; AFI::DEF (A,B) and *glo*; AFI::GLO (C,D) flowers. The hybridisation signal is confined to the outermost cell layer in young flowers (A,C) and in older petals (B,D). The sections were stained with calcofluor and the hybridisation signal appears as a purple stain in fluorescent light. Bars,100 μm. Numbers indicate whorls.

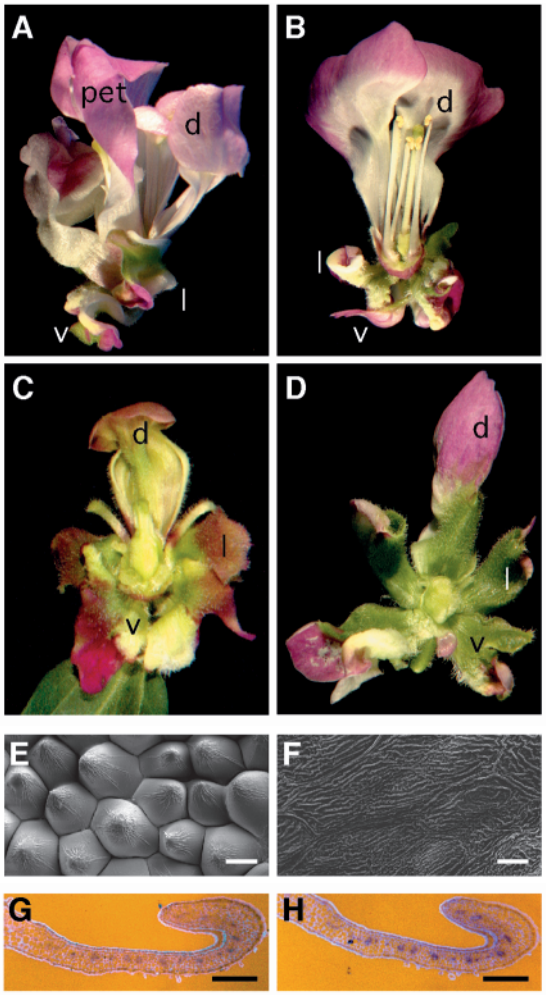

Fig. 4. Morphological features of *Antirrhinum* flowers that express the AFI::DEF/GLO transgenes in a wild-type background. (A-D) Mature flowers displaying various first whorl organ transformations. (A) Lateral view of flower with slightly aberrant petals (pet). (B-D) Frontal view of flowers, with some organs (all petals in B, all petals and stamens in C and all inner organs in D) removed. An anomalous carpel is shown in C. For comparison, B shows a wild-type carpel developing in a flower with a severe first whorl transformation. The position of the dorsal (d), lateral (l) and ventral (v) sepals are indicated. (E,F) SEM of inner epidermal cells within the lobe (E) and tube (F) regions of a petaloid sepal. Bars, 20 μm. (G,H) Expression pattern of *DEF* (G) and *GLO* (H) in sequential cross sections from a first whorl organ. The weak sub-epidermal hybridisation signal within the marginal region is most obvious around vascular bundles. Epidermal expression is present in this, and in the adjacent, region. Bars, 200 μm.

the position of the stamenodium (Fig. 1L), whose development is retarded in the third whorl of the wild type, but which develops fully as a fifth carpeloid organ in the third whorl of *def* mutants (Fig. 1K). In all these respects the morphology of third whorl organs in transgenic epidermal chimeras resembles that of somatic L1 chimeras. Interestingly, flowers carrying the AFI::DEF transgene in the temperature sensitive *def*-101 mutant background develop fertile stamens when grown at a moderate temperature (not shown). Under these growth conditions third whorl organs of *def*-101 mutant flowers initiate as separate organs, but they remain carpeloid during further development (Zachgo et al., 1995). This suggests that epidermal *DEF* can control stamen identity when supplemented with a very low level of endogenous B function. Temperature-shift experiments are in progress to define the developmental stage until which endogenous B function is essential for stamen development and after which stamen differentiation can be controlled by epidermal DEF alone.

Fourth whorl carpels do not initiate in *def* mutants (Fig. 1K; Schwarz-Sommer et al., 1992; Tröbner et al., 1992). Expression of *DEF* in L1 of somatic chimeras restores carpel initiation, indicating that it is under non-autonomous control, since organ

initiation involves cell divisions in inner layers (Perbal et al., 1996). In transgenic chimeras the flowers in an inflorescence may (Fig. 1C), or may not (Fig. 1L), develop fourth whorl carpels. This variability perhaps relates to fluctuations in the activity of the *AFI* promoter, which provides the *DEF* function, during early stages of development when *DEF* is known to be critical for carpel initiation (Zachgo et al., 1995).

Epidermal signalling enhances first whorl petaloidy in flowers that simultaneously express *DEF* and *GLO* in the epidermis

Mechanisms that non-autonomously activate endogenous class B genes can reinforce *DEF*/*GLO* expression in cells carrying the wild-type *DEF* and *GLO* genes, but have no influence on *DEF* and *GLO* expression in *def* or *glo* mutant cells. One question relevant to events occurring in the wild type therefore is whether the limitations on cell communication observed in *def*; AFI::DEF chimeras are valid when all cells carry wild-type *DEF*/*GLO* genes. This can be answered by ectopically expressing *DEF*/*GLO* in the epidermis of wild-type sepals and carpels. Although wild-type first and fourth whorl organs are not completely devoid of class B gene transcription (Tröbner et al., 1992; Zachgo et al., 1995), sepals and carpels of wild-type flowers carrying either AFI::GLO or AFI::DEF alone are phenotypically wild type. This suggests that levels of endogenous *DEF* and *GLO* expression in whorls 1 and 4 are too low to permit the formation of functional heterodimers with the DEF or GLO proteins expressed by the individual epidermal transgenes. Plants that were doubly transgenic for AFI::DEF and AFI::GLO (designated as AFI::DEF/GLO plants) were therefore generated and their first and fourth whorl organs were examined for morphological changes relative to *def*; AFI::DEF second and third whorl organs, respectively, to answer the question posed above.

Fourth whorl carpels in all wild-type backgrounds remained almost completely normal, only occasionally displaying a short style or a split stigma in flowers of double transgenic plants (Fig. 4C). Reduction or absence of stigmatic papillae at the tip of the stigma was the only indication of autonomous control of epidermal cell type by AFI::DEF/GLO in whorl 4. Thus, simultaneous, high-level epidermal expression of *DEF* and *GLO* in a wild-type background is essentially unable to cause any substantial changes in carpel morphology in whorl 4, reminiscent of the limited influence of epidermal *DEF* function on third whorl development in a *def* mutant background in transgenic or somatic L1 chimeras.

AFI::DEF/GLO flowers frequently formed petaloid first whorl organs in all genetic backgrounds tested. In colour, surface structure (Fig. 4E,F) and morphology (Fig. 4C), these resembled second whorl organs of *def*; AFI::DEF flowers (Fig. 1C), although overall their phenotype was more variable and less uniform, even within a flower. Petaloid development was more pronounced in the dorsal first whorl sepal and was least obvious in the lateral organs (Fig. 4A-D). Petaloid dorsal sepals were often split into two halves and ventral sepals appeared as composite structures displaying two or more ‘lobes’. Some of these ‘lobes’ remained sepal-like and green, while the adjacent ‘lobe’ resembled wild-type petals. However, even sepaloid regions were enlarged and modified in shape compared to genuine sepals, thus revealing non-autonomous control of sub-epidermal cell number and organ shape in whorl 1.

Analysis of *DEF*/*GLO* transcription by RT-PCR (which can distinguish between endogenous transcripts and transcripts originating from the transgene) indicated that the level of endogenous gene expression is enhanced in petaloid parts of the organs, relative to that in wild-type sepals (not shown). In situ studies detected weak sub-epidermal *DEF*/*GLO* expression in older petaloid first whorl organs, and also epidermal expression in regions where sub-epidermal *DEF*/*GLO* expression is absent (Fig. 4G,H). Thus, transcription of *DEF*/*GLO* in the epidermis does not always facilitate sub-epidermal *DEF*/*GLO* expression.

As mentioned above, sub-epidermal *DEF*/*GLO* expression in epidermal chimeras can result from invasions of wild-type L1 cells (Perbal et al., 1996). Such invasions are characterised in petals by the presence of a chlorophyll-less, fully petaloid margin made up of wild-type L1-derived sub-epidermal cells that express *DEF*/*GLO*. L2-derived *def* mutant sub-epidermal cells inside the margins are revealed by their light green colour, due to non-autonomous partial suppression of chlorophyll synthesis. The morphology conferred by sub-epidermal cell invasion in petaloid sepals of *def*; AFI::DEF flowers slightly resembles the phenotype of petals of somatic L1 chimeras, as illustrated by the dorsal organ in Fig. 4C. Since the *AFI* promoter is inactive in L1-derived sub-epidermal cells (see Introduction), *DEF*/*GLO* expression there has to be independent of *AFI* promoter activity. It is possible that AFI::DEF/GLO expression enhances epidermal transcription of the endogenous *DEF*/*GLO* genes, which then can be maintained by the autoregulatory mechanism even if epidermal cells acquire a sub-epidermal identity.

In many transgenic first whorl organs, however, the structure of petaloid sectors cannot be explained by invasions of cells from the L1 layer. For instance, dorsal first whorl organs of severely affected flowers are usually completely petaloid, and show no residual sub-epidermal sepaloidy (Fig. 4A,B), as neither do some of the petaloid sectors of lateral or ventral organs (Fig. 4B-D). For L1 invasions to account for such complete transformations, all L2 cells within fully petaloid sectors would have to be ‘expelled’ by L1 cells. This seems very unlikely. Even less likely are processes that reverse L1 invasion, to allow the formation of a sepaloid sector on top of a fully petaloid one (lateral organ in Fig. 4A). Enhancement of endogenous *DEF*/*GLO* transcription in L2 induced by signalling from the petaloid epidermal cells (Fig. 2) would seem to be the most probable explanation for these observations. Whether enhancement by signalling is achieved by transcriptional activation of otherwise silent endogenous genes, or by reinforcement and maintenance of low-level transcription cannot be decided.

Positional preferences for first whorl organ transformations in the double transgenic chimeras correspond to preferences observed in mutants in which spatial control of class B gene expression is impaired (Wilkinson et al., 2000). This coincidence suggests that the enhanced petaloidy of AFI::DEF/GLO first whorl organs is influenced by a corresponding distribution of endogenous factors that promote the manifestation of the B function. Apparently, these factors are crucial for wild-type petal development – in addition to class B genes – and are uniformly expressed in the second whorl, but less regularly distributed in the first whorl. Fluctuations in *AFI* promoter activity in the first whorl

may, in addition, cause variability in the size and phenotypic appearance of organs.

As summarised in Fig. 2, simultaneous epidermal expression of *DEF* and *GLO* in the first whorl autonomously controls cell shape and non-autonomously influences, to a limited extent, the petal cell identity and organ shape in the sub-epidermal layers. This is similar in overall effect to the influence of epidermal class B function on sub-epidermal cell fates in whorl 2. In addition, epidermal signalling can reinforce endogenous sub-epidermal *DEF/GLO* transcription and thereby enhance petaloid development. The variability of this event indicates that additional factors required for petal development are non-uniformly distributed.

Phenotypes of flowers expressing *DEF* in the epidermis of an *ap3* mutant indicate a great degree of non-autonomy for the *DEF* function in *Arabidopsis*

That *DEF* is functional in *Arabidopsis* was demonstrated previously (Irish and Yamamoto, 1995; Samach et al., 1997). To test its ability to control petal and stamen organ identity by autonomous and non-autonomous processes when expressed in the epidermis, the AFI::DEF transgene was introduced into an *ap3* mutant background. Restoration of wild-type petal and stamen features, as shown in Figs 5D,E and 6, indicates that sub-epidermal development can, in large measure, be controlled by *DEF*-dependent signalling from the epidermis, in contrast to the more limited influence of epidermal *DEF* in *Antirrhinum* discussed above.

Second whorl petals of transgenic flowers have an essentially wild-type shape and are larger than true sepals or *ap3* second whorl organs (Fig. 5C,D). In surface morphology, as well as in size and shape of their sub-epidermal cells they are indistinguishable from wild-type petals (Fig. 6). The shape of stamens in the third whorl is close to wild type, and pollen production occurs in the theca (Fig. 6L). Since sub-epidermal cells in petals and pollen in stamens originate from L2, these findings indicate non-autonomous control of development by epidermal class B function in the transgenic chimeras. Epidermal *DEF* also conditions a mild stamenoid transformation of carpels within the third whorl (Fig. 6C), because PI is expressed in the centre of the flower and can form functional heterodimers with DEF (Samach et al., 1997).

However, petals and stamens in *ap3*; AFI::DEF flowers remain immature, in that elongation of petals and stamen filaments after stage 12 of development (Smyth et al., 1990), suppression of chlorophyll formation, and pollen maturation are incomplete. Since the floral phenotype of *ap3*; FDH::AP3 plants (not shown) did not differ from that of *ap3*; AFI::DEF epidermal chimeras we can exclude the possibility that inability of *DEF* to direct late developmental events in *Arabidopsis* is responsible for late defects. Possibly, *AFI* promoter activity decreases during later stages, and hence *DEF* (or *AP3*) activity might not reach the threshold necessary for wild-type function (see below). Incomplete late development may then relate to properties of the *AFI* promoter, rather than to temporal changes in cell communication. However, in spite of this potential limitation, the full developmental potential of epidermal *PI* observed with X-ray-induced chimeras (Bouhidel and Irish, 1996) could be reproduced in *ap3*; AFI::DEF epidermal chimeras.

Expression of *DEF* and *GLO* in the epidermis of wild-type *Arabidopsis* plants results in changes in organ identity at ectopic positions

The effect of epidermal *DEF* and *GLO* expression in a wild-type background was studied to see whether the autonomous and non-autonomous influence of epidermal B function on petal and stamen identity is whorl-specific.

Epidermal expression of *DEF* causes mild to severe stamenoid transformations in fourth whorl carpels, and proliferation of additional (usually 2+4) immature stamenoid organs within whorl 3 (Fig. 7B), reminiscent of the phenotype of *superman* mutants (Sakai et al., 1995). The homeotic changes are more pronounced in double transgenic lines carrying the AFI::DEF/GLO transgenes (Fig. 7D). This enhancement could be due to an insufficient level of endogenous *PI* expression in the centre of the flower, as was also observed on comparing transgenic lines expressing *AP3* and *AP3*/*PI* under the control of the CaMV 35S promoter (Krizek and Meyerowitz, 1996). AFI::DEF /GLO flowers also display petaloid transformation of first whorl organs that resemble immature wild-type petals and whose shape, colour (Fig. 5B) and surface features (not shown) are very similar to those of second whorl organs of *ap3*; AFI::DEF flowers. Thus, unlike the case in *Antirrhinum*, endogenous class B genes do not seem to facilitate epidermal *DEF/GLO* non-autonomy in *Arabidopsis*. Furthermore, homeotic transformation of first whorl organs is more uniform in *Arabidopsis*, compared to the pronounced variability of *Antirrhinum* AFI::DEF/GLO first whorl organs (Figs 5B, 4A-D). This suggests that in *Arabidopsis* factors other than class B genes that promote the adoption of petal identity are similarly distributed in whorls 1 and 2. Experiments performed with FDH::AP3 and FDH::AP3/PI transgenes and not described in detail in this report resulted in phenotypes similar to those described for AFI::DEF and AFI::DEF/GLO (Table 1).

The APETALA3/GLOBOSA combination is non-functional in *Arabidopsis*

Surprisingly, epidermal expression of *GLO* alone has no phenotypic effect (Fig. 7E) although, as shown above, AFI::GLO is functional in *Arabidopsis* when combined with AFI::DEF. Endogenous *AP3* expression is sufficient for mild homeotic first whorl transformation when combined with epidermal *PI* expression (Fig. 7F), but even enhancement of epidermal *AP3* expression in FDH::AP3/AFI::GLO double transgenic plants does not result in phenotypic alterations in whorl 1 (Table 1). It seems then that the AP3/GLO heterodimer (in contrast to the DEF/PI heterodimer) either cannot form or is not functional in *Arabidopsis*. The reason why the AP3/GLO combination does not function in *Arabidopsis* was not pursued. It could either be due to impaired heterodimer formation and/or to inadequate interaction of the protein complex with ternary factors. Our inability to detect the role of *GLO* in *Arabidopsis* illustrates the limitations of using heterologous species as test systems to evaluate the function of proteins in their normal cellular environments.

In situ hybridisation studies with *DEF* and *PI* in *Arabidopsis* epidermal chimeras indicate the absence of mRNA trafficking

DEF expression was determined in AFI::DEF/GLO transgenic plants with an otherwise wild-type background to discover

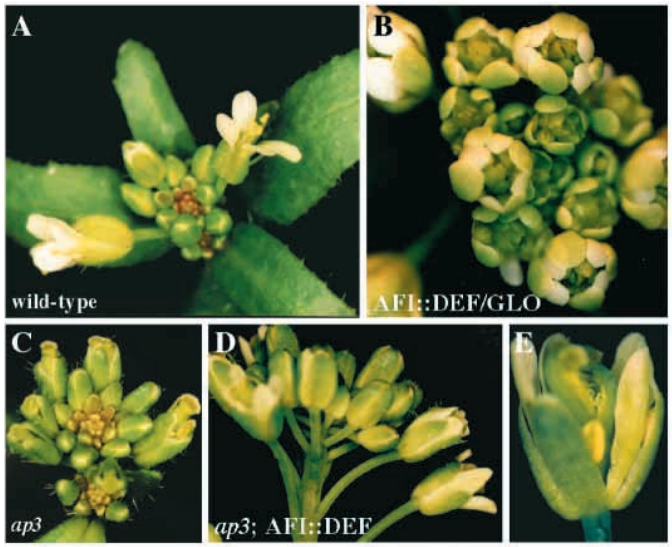

Fig. 5. Phenotypes of wild-type (A), AFI::DEF/GLO (B), *ap3* (C) and *ap3*; AFI::DEF (D,E) *Arabidopsis* flowers. In E a sepal has been removed to reveal immature petals and stamens. Incomplete suppression of sub-epidermal chlorophyll synthesis by the epidermal transgenes in the petaloid first whorl of wild-type flowers (B) and in the second whorl of *ap3* mutant flowers (D,E) is revealed by the slightly green colour of the organs compared to the white colour of mature petals (A,B). Note the immature stamens in the centre of AFI::DEF/GLO flowers in B (viewed by SEM in Fig. 7D) and immature petals and stamens in the *ap3*; AFI::DEF flowers in D and E (viewed by SEM in Fig. 6C).

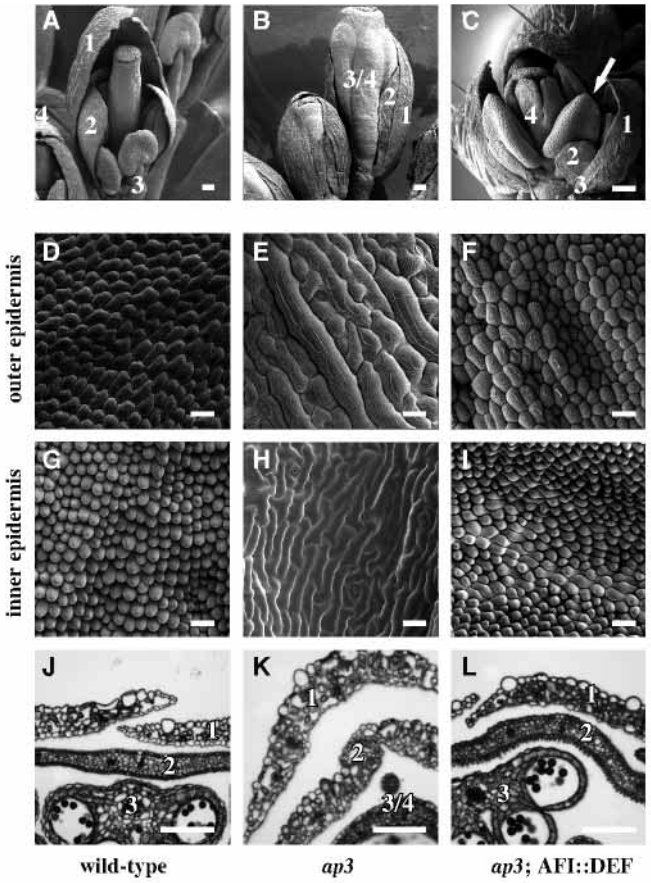

Fig. 6. Morphological features of *Arabidopsis* flowers: (left panels) wild type; (middle panels) *ap3*; (right panels) *ap3*; AFI::DEF viewed by SEM. (A-C) Overall morphology of young flowers. Some of the sepals and petals in the front have been removed. The arrow in C indicates a wild-type-like stamen in the transgenic flower. Bars,100 μm. (D-I) Outer (D-F) and inner (G-I) epidermal surfaces of second whorl organs. Bars, 20 μm. (J-L) Cross sections taken approximately in the middle of immature buds. Note the similarity of sub-epidermal petal cells (in whorl 2), and the presence of pollen in stamens (in whorl 3), in wild type (J) and in *ap3*; AFI::DEF (L). Bars, 100 μm. Numbers indicate the whorl.

whether RNA trafficking could account for the non-autonomous effects seen in *Arabidopsis*. As shown in Fig. 8A-C, this can be excluded, since *DEF* expression during early and late developmental stages is only detectable in the outermost cell layer. The in situ hybridisation pattern for *DEF* also shows that *AFI* promoter activity decreases during late development of floral organs, as indicated by the weak hybridisation signal in older sepals compared to (developmentally younger) petals or (stamenoid) carpels of the same flower (Fig. 8B). Furthermore, epidermal expression of *DEF* in stamens is reduced compared to petals or (stamenoid) carpels.

AP3 and *PI* are not expressed in AFI::DEF/GLO first whorl petaloid sepals (Fig. 8D,E) suggesting that epidermal expression of the endogenous class B genes cannot be influenced by *DEF*/*GLO*. In addition, hybridisation of sections from a FDH::AP3/PI flower with a PI probe (Fig. 8F) demonstrates the absence of a sub-epidermal hybridisation signal in whorl 1. Thus, in contrast to *Antirrhinum*, where the endogenous class B genes were shown to be inducible by the epidermally expressed functions (Fig. 4G,H), B function provided by the AFI::DEF/GLO or FDH::AP3/PI epidermal transgenes does not influence transcription of the endogenous *PI* or *AP3* genes in *Arabidopsis*.

DISCUSSION

Communication between cell layers during acquisition of identity by floral organs in *Antirrhinum*

The identity of floral organs becomes manifest as specific combinations of cell and tissue types derived from different layers. The number, shape and size of the different cells that arise during development must be co-ordinated, in order to produce complex mature structures with distinct functions. Our observations on transgenic *Antirrhinum* flowers expressing the *DEF* and *GLO* functions, which control organ identity, in the epidermis show that cell-cell communication indeed contributes to petal development, as revealed by alterations in cell shape and number as well as suppression of chlorophyll synthesis in the sub-epidermal tissue. In *Antirrhinum*, the developmental influences of epidermally expressed *DEF* and *GLO* are comparable, indicating that the two proteins do not differ in their ability to affect the differentiation of sub-epidermal cells.

However, non-autonomous control of the fate of L2 cells by L1 cells in *def* or *glo* mutant petals is incomplete, suggesting that the DEF/GLO proteins themselves must be present in sub-epidermal cells to ensure normal development. This finding implies that autonomous control of a subset of target genes in

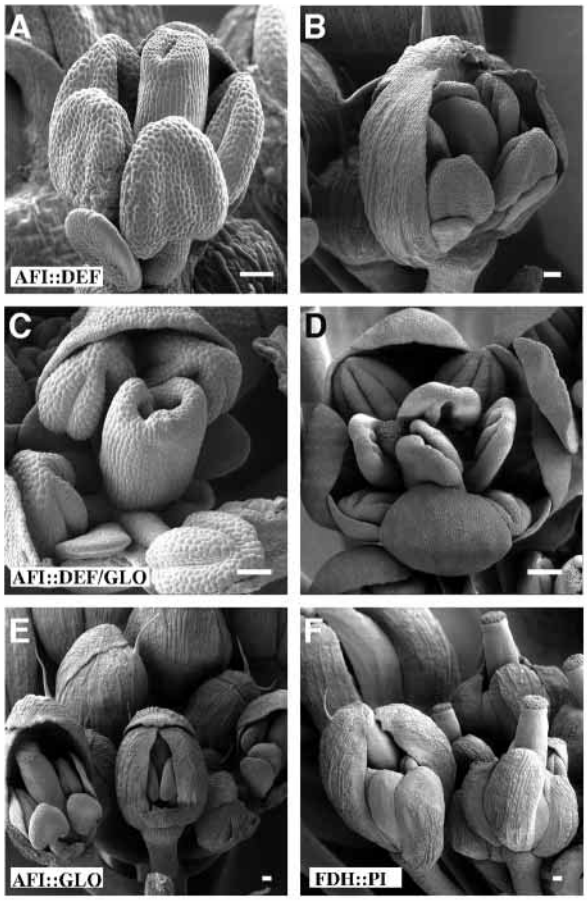

Fig. 7. SEM micrographs of *Arabidopsis* flowers expressing epidermal transgenes in a wild-type background. Strong (B,D) and weak (A,C) phenotypes are shown for the AFI::DEF (A,B) and AFI::DEF/GLO (C,D) transgenes. In A-D some or all first whorl organs were removed. Note the differences in shape and surface structure between first whorl organs in B and D. The flowers with wild-type sepals in E indicate that AFI::GLO has no effect when expressed alone; in contrast, expression of the FDH::PI transgene results in transformation of aberrant sepal/petal mosaic organs. Bars, 50 μm.

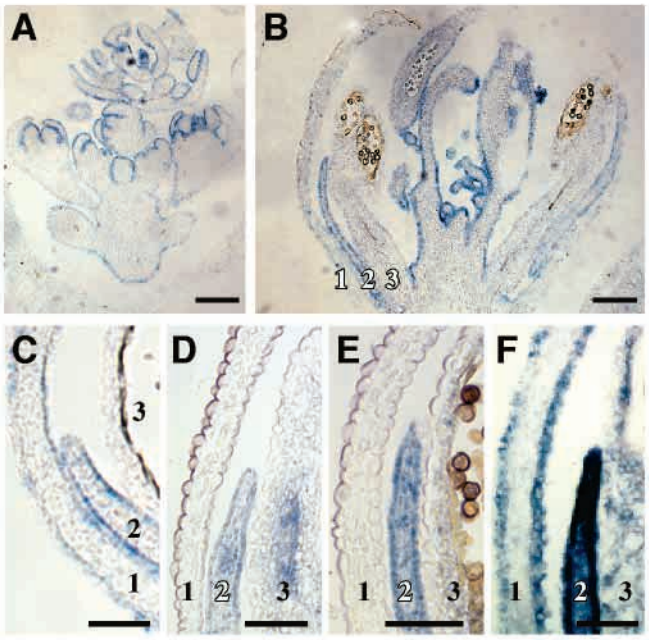

Fig. 8. Expression of *DEF* (A-C), *AP3* (D) and *PI* (E,F) mRNAs in *Arabidopsis* inflorescences and flowers doubly transgenic for the AFI::DEF/GLO (A-E) and FDH::AP3/PI (F) transgenes. (A-C) Epidermal expression of the AFI::DEF transgene in differentiating organs of very young (A) and older (B,C) flowers. Bars, 100 μm. (C-F) The region encompassing the first and second whorl organs of older AFI::DEF/GLO (C-E) and FDH::AP3/PI flowers (F) shown at higher magnification. Bars, 200 μm. Endogenous epidermal *AP3* (D) and *PI* (E) expression is not detectable in AFI::DEF/GLO first whorl organs and *PI* mRNA is not found in sub-epidermal cells in first whorl organs in FDH::AP3/PI flowers (F). Sections were viewed under bright-field illumination. Numbers indicate the whorl.

L2 is necessary for sub-epidermal development. Indeed, wild-type sepals that carry the wild-type *DEF*/*GLO* genes in all layers (but are devoid of B function) can develop fully wild-type petal sectors when *DEF*/*GLO* are functional in the epidermis. Endogenous *DEF*/*GLO* transcription is enhanced in sub-epidermal cells of such transgenic first whorl organs and this is most probably achieved by *DEF*/*GLO*-controlled signalling from the epidermis (Fig. 2). This non-autonomous mechanism would result in a balance of B function in adjacent layers, which is perhaps necessary for normal petal development.

The influence of L1 or epidermis on stamen development is very limited, and this limitation is valid in the third whorl of L1 periclinal chimeras (Perbal et al., 1996) and *def* mutant AFI::DEF transgenic flowers, as well as in the fourth whorl of the wild-type AFI::DEF/GLO double transgenic plants. This suggests that stamen development depends on the concerted action of the B function in all cell layers. However, it is difficult to determine the extent to which the mechanisms of cell communication in petals and stamens differ, because stamen development (but not petal development) is very sensitive to changes in *DEF*/*GLO* function during organ initiation and at early stages in the differentiation process (Zachgo et al., 1995). Such early defects then irreparably affect organ identity in the third whorl, precluding study of cell communication during later development. Ongoing experiments with plants expressing the AFI::DEF transgene in the temperature sensitive *def*-101 mutant background will allow us to study the epidermal contribution of the B function during later stages of stamen development.

Communication between layers during specification of identity in floral organs of *Arabidopsis*: conflicting observations in different experiments

In *Arabidopsis* epidermal B function provided by the AFI::DEF or FDH::AP3 transgenes is sufficient to control petal and stamen epidermal cell identity and all sub-epidermal developmental events. Epidermal control is equally efficient in the second and third whorls of *ap3* mutant flowers and in the first and fourth whorls of wild-type flowers. This agrees well with the developmental potential of epidermally expressed PI (Bouhidel and Irish, 1996), but is at variance with the whorl-specific differences in the response of layers to the B function and the absence of epidermal control of stamen development

observed with transgenic *AP3* epidermal chimeras (Jenik and Irish, 2001). To explain this contradiction we assume that the 927-bp *AP3* promoter fragment used to control expression of the *AP3* transgene in the latter experiments is insufficient to achieve wild-type *AP3* expression levels in individual layers in whorl 3, although it seems to be capable of directing wild-type stamen development when used to express *AP3* in all layers simultaneously. This assumption is supported by the non-autonomy of *PI* in directing stamen development, observed with X-ray induced sectorial chimeras – and hence under conditions where a class B gene is controlled by its endogenous promoter (Bouhidel and Irish, 1996). According to this interpretation wild-type organ identity in *Arabidopsis* can be governed by wild-type epidermal B function via signalling to sub-epidermal cells, or by a direct transcriptional control of class B target genes by sub-optimal levels of B function in all layers. Furthermore, as in *Antirrhinum*, the two class B genes in *Arabidopsis* do not differ with respect to their potential to control organ identity by cell communication.

We would like to note that signal intensity in the epidermal layer appears to be crucial for detection (or exclusion) of non-autonomy of the function controlling production of that signal. For instance, as recently reported, epidermal *APETALA1* (*AP1*) function in transgenic *Arabidopsis* flowers expressing *AP1* under the control of the L1-specific *AtML1* promoter in an *ap1* mutant background revealed two different classes of mutant phenotypes (Sessions et al., 2000). The majority of plants displayed second whorl organs (which fail to develop in the *ap1* mutant) with petal epidermal cell types, but without influence on sub-epidermal cell identity. The stronger class (representing a minority of transgenic lines) revealed a more pronounced rescue of petaloid identity of sub-epidermal cells, together with a 'gain of AP1 function' phenotype and ectopic activation of *AP3*. Whether the level of *AP1* expression provided by the ML1::AP1 transgene exceeds the wild-type level in the restored petals, or whether it is more wild-type-like in the weaker plants is not known. It is therefore difficult to decide whether the non-autonomous features of *AP1* are conferred by enhanced signal intensity due to over-expression, or, vice verse, low signal intensity due to insufficient *AP1* expression prohibits its detection of non-autonomous functions of *AP1*. To conclusively determine the role of signalling between layers in wild-type development for any given gene it will be important to compare transgenic chimeras (possibly representing enhanced or decreased transcript levels) with genetic chimeras (representing the endogenous level of transcription), as exemplified by the class B genes in this report.

Differences in the non-autonomous control of organ identity in *Arabidopsis* and *Antirrhinum*

The difference between the two species with regard to the extent of non-autonomy of the B function in *Arabidopsis* compared with *Antirrhinum* reported here is not due to intrinsic properties of the AP3/PI or DEF/GLO proteins themselves, because we used the same pair of genes in both species. It seems rather that the mechanisms involved in sending/receiving a B-function-dependent 'signal' between layers differ. Possibly, factors are present in *Arabidopsis* that reinforce the ability of the DEF/GLO (and AP3/PI) proteins to control epidermal production and transmission, or sub-epidermal reception of the signal. The nature of the epidermal 'signal' remains unknown, although *DEF*/*GLO* RNA trafficking most likely can be excluded. Similarly, protein movement from L1 to L2 was not observed in previous studies with DEF in *Antirrhinum* (Perbal et al., 1996) or AP3 in *Arabidopsis* (Jenik and Irish, 2001). At the site of reception it appears that a subset of the sub-epidermal target genes controlled by the B function in *Antirrhinum* requires the physical presence of class B proteins in these cells, while these targets can be activated indirectly, by signalling, in *Arabidopsis*. It is also possible that, at least in petals, epidermal signalling in both species can control most if not all sub-epidermal target genes, but in *Antirrhinum* the threshold for this control is high and cannot be fully satisfied by the epidermal signal. According to this scenario, the transcriptional control of *DEF*/*GLO* target genes and petal L2 identity is facilitated by, but does not completely depend on, the physical presence of the DEF/GLO proteins. This can now be tested by comparing gene expression in petals of wild type and transgenic epidermal chimeras; one would expect to find only quantitative differences if this idea is correct.

The epidermal contributions to stamen development in *Arabidopsis* and *Antirrhinum* differ dramatically. This appears to be due to differences in early mechanisms that operate during organ initiation in whorl 3, which can be regulated by signalling from the epidermis in *Arabidopsis*, but depend on a concerted and B-dependent control in all layers in *Antirrhinum*. In fact, stamen-specific cell division patterns in whorl 3 are independent of the presence of class B gene expression before stage 6 of development in *Arabidopsis* (Hill and Lord, 1989), but stamen development is irreversibly affected in *Antirrhinum* by reducing the level of B function long before flowers reach a comparable developmental stage (Zachgo et al., 1995).

Interestingly, a less prominent role for cell-cell communication between layers in *Antirrhinum* compared to *Arabidopsis* was also observed for *FLORICAULA* (*FLO*; Coen et al., 1990) and *LEAFY* (*LFY*; Weigel et al., 1992), a pair of orthologues that control floral meristem identity in *Antirrhinum* and *Arabidopsis*, respectively. While *LFY* is fully non-autonomous in each layer (Sessions et al., 2000), this is true for *FLO* only when it is sub-epidermally expressed (Hantke et al., 1995). The difference in the relative contribution of cell communication to cell identity in *Antirrhinum* and *Arabidopsis*, observed in the case of class B genes and also for genes that control meristem identity, is intriguing, because it demonstrates that plants can use different strategies to realise their wild-type morphologies. The availability of genetically stable transgenic plants will allow us now to search for the genes responsible for conferring the observed restrictions on cell communication in *Antirrhinum*.

We are grateful to Drs Sabine Zachgo, Peter Huijser and Brendan Davies for discussions during preparation of this manuscript, Dr Paul Hardy for proof-reading the text and Sybille Richter and Manfred Pohe for the maintenance of plants. This work was in part supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 243) to Z. Sch.-S.

REFERENCES

Bechtold, N., Ellis, J. and Pelletier, G. (1993). In-planta Agrobacterium-

mediated gene-transfer by infiltration of adult *Arabidopsis thaliana* plants. *Compt. Rend. Acad. Sci. Ser. III-Life Sci.* **316**, 1194-1199.

Bouhidel, K. and Irish, V. F. (1996). Cellular interactions mediated by the homeotic *PISTILLATA* gene determine cell fate in the *Arabidopsis* flower. *Dev. Biol.* **174**, 22-31.

Carpenter, R. and Coen, E. S. (1990). Floral homeotic mutations produced by transposon-mutagenesis in *Antirrhinum majus*. *Genes Dev.* **4**, 1483-1493.

Coen, E. S., Romero, J. M., Doyle, S., Elliott, R., Murphy, G. and Carpenter, R. (1990). *floricaula*: a homeotic gene required for flower development in *Antirrhinum majus*. *Cell* **63**, 1311-1322.

Davies, B., Egea-Cortines, M., de Andrade Silva, E., Saedler, H. and Sommer, H. (1996). Multiple interactions amongst floral homeotic MADS box proteins. *EMBO J.* **15**, 4330-4343.

Davies, B., Motte, P., Keck, E., Saedler, H., Sommer, H. and Schwarz-Sommer, Z. (1999). *PLENA* and *FARINELLI*: redundancy and regulatory interactions between two *Antirrhinum* MADS-box factors controlling flower development. *EMBO J.* **18**, 4023-4034.

Egea-Cortines, M., Saedler, H. and Sommer, H. (1999). Ternary complex formation between the MADS-box proteins SQUAMOSA, DEFICIENS and GLOBOSA is involved in the control of floral architecture in *Antirrhinum majus*. *EMBO J.* **18**, 5370-5379.

Hantke, S. S., Carpenter, R. and Coen, E. S. (1995). Expression of *floricaula* in single cell layers of periclinal chimeras activates downstream homeotic genes in all layers of floral meristems. *Development* **121**, 27-35.

Heidmann, I., Efremova, N., Saedler, H. and Schwarz-Sommer, Z. (1998). A protocol for transformation and regeneration of *Antirrhinum majus*. *Plant J.* **13**, 723-728.

Hill, J. P. and Lord, E. M. (1989). Floral development in *Arabidopsis thaliana*: a comparison of the wild type and the homeotic *pistillata* mutant. *Can J. Bot.* **67**, 2922-2936.

Irish, V. F. and Yamamoto, Y. T. (1995). Conservation of floral homeotic gene function between *Arabidopsis* and *Antirrhinum*. *Plant Cell* **7**, 1635-1644.

Jenik, P. D. and Irish, V. F. (2001). The Arabidopsis floral homeotic gene *APETALA3* differentially regulates intercellular signaling required for petal and stamen development. *Development* **128**, 13-23.

Krizek, B. A. and Meyerowitz, E. M. (1996). The *Arabidopsis* homeotic genes *APETALA3* and *PISTILLATA* are sufficient to provide the B class organ identity function. *Development* **122**, 11-22.

Perbal, M.-C., Haughn, G., Saedler, H. and Schwarz-Sommer, Z. (1996). Non-cell-autonomous function of the *Antirrhinum* floral homeotic proteins DEFICIENS and GLOBOSA is exerted by their polar cell-to-cell trafficking. *Development* **122**, 3433-3441.

Riechmann, J. L. and Meyerowitz, E. M. (1997). MADS domain proteins in plant development. *Biol. Chem.* **378**, 1079-1101.

Sakai, H., Medrano, L. J. and Meyerowitz, E. M. (1995). Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. *Nature* **378**, 199-203.

Samach, A., Köhalmi, S. E., Motte, P., Datla, R. and Haughn, G. W. (1997). Divergence of function and regulation of class-B floral organ identity genes. *Plant Cell* **9(N4)**, 559-570.

Satina, S. and Blakeslee, A. F. (1941). Periclinal chimeras in *Datura stramonium* in relation to development of leaf and flower. *Am. J. Bot.* **28**, 862-871.

Schwarz-Sommer, Z., Hue, I., Huijser, P., Flor, P. J., Hansen, R., Tetens, F., Lönnig, W.-E., Saedler, H. and Sommer, H. (1992). Characterization of the *Antirrhinum* floral homeotic MADS-box gene *deficiens*: evidence for DNA binding and autoregulation of its persistent expression throughout flower development. *EMBO J.* **11**, 251-263.

Schwarz-Sommer, Z., Huijser, P., Nacken, W., Saedler, H. and Sommer, H. (1990). Genetic control of flower development by homeotic genes in *Antirrhinum majus*. *Science* **250**, 931-936.

Sessions, A., Yanofsky, M. F. and Weigel, D. (2000). Cell-cell signaling and movement by the floral transcription factors LEAFY and APETALA1. *Science* **289**, 779-781.

Smyth, D. R., Bowman, J. L. and Meyerowitz, E., M. (1990). Early flower development in Arabidopsis. *Plant Cell* **2**, 755-767.

Szymkowiak, E. J. and Sussex, I. M. (1996). What chimeras can tell us about plant development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 351-376.

Tröbner, W., Ramirez, L., Motte, P., Hue, I., Huijser, P., Lönnig, W.-E., Saedler, H., Sommer, H. and Schwarz-Sommer, Z. (1992). *GLOBOSA*: a homeotic gene which interacts with *DEFICIENS* in the control of *Antirrhinum* floral organogenesis. *EMBO J.* **11**, 4693-4704.

Weigel, D., Alvarez, J., Smyth, D. R., Yanofsky, M. F. and Meyerowitz, E. M. (1992). *LEAFY* controls floral meristem identity in *Arabidopsis*. *Cell* **69**, 843-859.

Wilkinson, M., de Andrade Silva, E., Zachgo, S., Saedler, H. and Schwarz-Sommer, Z. (2000). *CHORIPETALA* and *DESPENTEADO*: general regulators during plant development and potential targets of FIMBRIATA-mediated degradation. *Development* **127**, 3725-3734.

Zachgo, S., de Andrade Silva, E., Motte, P., Tröbner, W., Saedler, H. and Schwarz-Sommer, Z. (1995). Functional analysis of the *Antirrhinum* floral homeotic *DEFICIENS* gene in vivo and in vitro by using a temperature-sensitive mutant. *Development* **121**, 2861-2875.