Dual role for *fimbriata* in regulating floral homeotic genes and cell division in *Antirrhinum*

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**Introduction**

Floral meristems produce four types of organs in concentric whorls in the order sepals, petals, stamens and carpels. The identity of the organs is controlled by the combinatorial action of genes which are expressed in specific regions of the floral meristem (Schwarz-Sommer *et al.*, 1990; Coen and Carpenter, 1993; Weigel and Meyerowitz, 1994). Mutations in these organ identity genes can be divided into three classes: in class *A* mutants, carpels replace sepals and stamens replace petals; in class *B* mutants, sepals replace petals and carpels replace stamens; in class *C* mutants, petals replace stamens and carpels are replaced by reiterative growth of sepals and petals. Based on these mutants, three genetic functions, *a*, *b* and *c*, have been proposed to specify organ identity in the combination *a*, *ab* and *bc*, and *c*, in whorls 1–4 respectively (Coen and Meyerowitz, 1991). In most cases, the genes required for these functions are expressed in domains that are precisely aligned with the morphological boundaries between whorls of organs, ensuring discrete changes in organ type from whorl to whorl (Jack *et al.*, 1992; Schwarz-Sommer *et al.*, 1992; Bradley *et al.*, 1993). However, the mechanisms responsible for this alignment between morphology and gene expression boundaries are unclear. A candidate gene involved in this process is the *fimbriata* (*fim*) gene of *Antirrhinum*, and its *Arabidopsis* orthologue, *UFO*, which affect both the identity and arrangement of organs within the flower (Simon *et al.*, 1994; Ingram *et al.*, 1995; Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995).

The *fim* protein contains a novel structural motif, the F-box, which is present in a range of proteins, including cyclin A, cyclin F and *CDC4*, that are involved in cell cycle control in yeast (Yochem and Byers, 1987; Zhang *et al.*, 1993, 1995; Bai *et al.*, 1994, 1996). Some F-box proteins, including Met30p and *GRR1*, act as transcriptional inhibitors and may also play a role in cell cycle control (Flick and Johnston, 1991; Thomas *et al.*, 1995). The precise role of *fim* in flower development is, however, unclear. We have used a combination of molecular and genetic approaches to address this problem in *Antirrhinum*.

Flowers of *fim* mutants described so far have organs consisting of petal/sepal tissue in the second whorl and petal/sepal/carpel tissue in the third whorl. In addition, mutant flowers sometimes fail to produce carpels and instead produce large numbers of mosaic organs (apical indeterminacy). These phenotypic effects can be accounted for by reduced expression of the *b* function gene *deficiens* (*def*) and *c* function gene *plena* (*ple*), suggesting that one role of *fim* is to promote transcription of organ identity genes (Simon *et al.*, 1994). Consistent with this, expression of *fim* is detected in meristems before any organ primordia are visible, shortly before the onset of *b* and *c* organ identity gene expression. Early *fim* expression encompasses the presumptive *b* and *c* domains: it is first seen in a ventral (abaxial) region of the floral meristem and then in the central region, after which it resolves into a broad ring internal to the sepal primordia, consistent with its role in regulating organ identity. At later stages, however, *fim* expression becomes localized to the boundary regions around the base of developing petal primordia and does not overlap with most of the domain of *b* or *c* activity. This raises the question of how *fim* interacts with the organ identity genes at different stages of development. In addition to affecting organ identity, *fim* mutants also show altered organ arrangement, united growth of organs and the occasional production of meristems in the axils.
of floral organs (lateral indeterminacy). Thus, fim also seems to play a role in establishing organ and whorl boundaries during floral development.

Here we describe two complementary approaches that have been used to study the action of fim. One involves isolating proteins that interact with FIM during flower development using the yeast two-hybrid system. The second approach involves generating a series of alleles with altered levels of fim activity. This is particularly important because previously analysed fim alleles are not nulls, obscuring the extent to which fim is required for the proper arrangement and identity of organs (Simon et al., 1994). To isolate a range of novel mutations at the fim locus, including null alleles, we exploited a weak fim allele (fim-619), which carries an insertion of the Tam3 transposon in the fim promoter. Tam3 is known to generate deletions and rearrangements in genes and can therefore be used as a localized mutagen (Martin et al., 1988; Almeida et al., 1989; Coen et al., 1989; Martin and Lister 1989; Hudson et al., 1990). Such transposon-induced derivatives can be screened for by PCR, as described in Caenorhabditis and maize (Rushforth et al., 1993; Das and Martienssen, 1995).

We show that FIM associates with a family of proteins, termed FAPs (FIM-associated proteins), closely related to Skp1 proteins. In yeast and humans, Skp1 proteins interact with F-box-containing proteins, directly or indirectly, to form a complex needed for protein degradation and cell cycle progression (Zhang et al., 1995; Bai et al., 1996; Connelly and Hieter, 1996). Thus, the similarity between FIM and F-box proteins is further substantiated by their selective degradation of regulatory proteins. This may occur by promoting expression and cell division, perhaps by promoting selective degradation of regulatory proteins. This may provide a mechanism by which morphological and gene expression boundaries can be aligned with each other during floral development.

Results

Molecular characterization of novel fim alleles

The role of fim in the control of flower development was investigated by site-selected mutagenesis, using fim-619 as the starting material. The fim-619 allele carries a Tam3 insertion 976 bp upstream of the start of the longest fim cDNA (start of the cDNA is denoted position 0). Ten events were detected from a PCR-based screen of 4020 progeny of fim-619 (M1 generation) and the corresponding seed was sown to give 10 M2 families. Screening the plants in these families by PCR, showed that seven of the 10 events were transmitted to the M2. Three untransmitted events could either have been somatic, or present in M1 plants which did not set seed. The seven transmitted alleles, named fim-676 to fim-682, were further characterized by PCR amplification and by cloning and sequencing the PCR products. M2 plants homozygous for the new alleles were then analysed by DNA blot hybridization.

The seven alleles carried deletions extending to the right of the Tam3 by various amounts. Two of the alleles had retained the original Tam3 insertion, whereas the five other alleles had lost Tam3 (Figure 1A). These types of deletion have previously been observed as the result of plant transposon activity and have been attributed to aberrant transposition events (McClimock, 1953, 1954; Martin et al., 1988; Coen et al., 1989; Federoff, 1989; Martin and Lister 1989; Hudson et al., 1990; Robbins et al., 1990). There were no striking similarities between the sequences around the right breakpoints of the seven deletions, although the breakpoints found in the fim-676 and fim-678 alleles were separated by only 2 bp. Four of the five deletion events in which Tam3 was lost had the same left breakpoint, 11 bp away from the transposon (bases −976 to −987, Figure 1B), indicating that they may have arisen from the same type of event.

Deletion of the fim open reading frame gives rise to a novel phenotype

Plants homozygous for the deletions in fim-677, fim-679, fim-681 or fim-682 all gave a similar phenotype (Figure 2D). This was more extreme than had previously been observed for any fim allele, confirming that previously described alleles were not null. At least half of the fim open reading frame (ORF) had been deleted in these new alleles, and DNA blots confirmed that no duplications of fim were present in the genome of these plants. Thus, the phenotype shown in these plants represented the null mutant phenotype of fim. Detailed analysis of the phenotype revealed the extent to which fim was involved in: (i) organ identity; (ii) whorl integrity; and (iii) determinacy.

Changes in floral organ identity. All null fim mutants produced flowers that showed changes in organ identity that were consistent with reduced b and c activity (Figure 2D and H). Flowers usually had a first whorl of sepal, and most organs internal to these were also sepal-like. Petal tissue was rare and was only present in small localized patches on the edges of internal sepals; stamenoid tissue was never observed, even under greenhouse conditions where the phenotype was less extreme than in plants grown in the field. Carpel tissue and ovoids were produced towards the centre of many flowers, especially flowers produced higher on the inflorescence spike (see later). The carpels were commonly united with adjacent organs and split or distorted.

Disruption of whorl integrity. Dissection of mature flowers from fim nulls suggested that organs internal to the first whorl were not arranged in regular whorls. To determine the developmental origin of this change in phyllotaxy, early floral meristems were examined by scanning electron microscopy. Flower meristems arose in the axils of bract primordia on the periphery of the inflorescence apex. The meristems were numbered sequentially, starting with the
Fig. 1. (A) Novel deletion events detected in the fim cluster. The fim ORF is shown as a black box, and the position of Tam3 in fim-619 indicated by a large black triangle. Null alleles are indicated by asterisks. The deletions in fim-676–fim-682 are shown as open boxes. Retained Tam3 elements are indicated as black triangles. (B) Sequence analysis of novel deletion events. The Tam3 insertion in fim-619 (position –976) is indicated by a triangle (position 0 corresponds to the end of the longest fim cDNA and the fim ATG is at position +97). The 10 bp imperfect duplication to the right of Tam3 in fim-619 is double underlined and a potential MADS box protein-binding site is enclosed in a dotted box. Sequences at the break points of each deletion allele are shown, with open boxes indicating deleted nucleotides to the left of the Tam3 insertion. Deletions to the right of Tam3 are indicated by dotted lines, with the number of bp deleted marked. Null alleles are indicated by asterisks. Triangles indicate retained Tam3 elements. The fim-678 and fim-679 alleles have rearranged bases at the right breakpoint (single underlined). Where deletions extend into the fim open reading frame, amino acid sequence is shown below the nucleotide sequence. The start of the putative fim-680 protein product is shown in bold.

youngest bract primordium at the top of the inflorescence apex (node 0). The development of flowers was divided into several stages as described by Carpenter et al. (1995): stage 0 (nodes 0–4, bract tongue stage); stage 1 (nodes 4–8, eye stage); stage 2 (nodes 8–10, loaf stage); stage 3 (nodes 10–12, pentagon stage); stage 4 (nodes 12–14, floritypic stage); stage 5 (nodes 15–18, petal mound stage).

In null fim mutants, floral development appeared normal until about stage 4. In wild-type meristems of this stage, a whorl of five sepal primordia was visible on the periphery of the floral meristem (Figure 3A). In fim null mutants, an extra primordium was frequently produced between the two ventral (abaxial) primordia (Figure 3B, arrow). This extra primordium appeared to be initiated later than the other sepals because prior to stage 4, the shape of the floral meristem in the null mutants was pentagonal, similar to that of wild-type meristems. The ventral primordia of the first whorl developed either into sepals (as in wild-type) or, more rarely, cylindrical filamentous organs (an example is labelled with an asterisk in Figure 3H). By early stage 5 in wild-type, a whorl of petal primordia was visible as five small mounds developing alternate and internal to the sepals (Figure 3C). However, in fim null flowers at this stage, it was usually not possible to allocate internal primordia to individual whorls. In addition, ventral primordia in the first whorl were occasionally united with more internal organ primordia of the flower (Figure 3D, arrow). In some flowers, organ primordia were arranged in a symmetrical fashion on either side of the dorsoventral axis of the meristem (Figure 3F), whereas in other flowers no coherent pattern for organ initiation was apparent. Organ primordia were often shaped abnormally and united with each other, the boundaries between developing organs often being poorly defined, making identification and counting of individual organs difficult. In wild-type, the production of petal and stamen primordia was closely followed by the initiation of two central carpel primordia (stage 6, Figure 3E and G). In fim null mutants there was a delay in the appearance of further primordia after the first 10–12 had emerged (Figure 3F and H). The later primordia which eventually formed appeared to be arranged in an increasingly abnormal way, and sometimes formed a spiral towards the centre of the flower (not shown).

Reduced determinacy. Various degrees of indeterminacy
were observed in fim nulls. Wild-type flowers are apically determinate, producing only four whorls of organs, and laterally determinate because no meristems arise in the axes of floral organs. In fim nulls, a gradient in the degree of indeterminacy was apparent along the inflorescence spike from lower to upper regions (Figure 4). Flowers produced low down on the inflorescence spikes (flower 0–10), commonly showed both lateral and apical indeterminacy (Figure 4). The internodes of these flowers became elongated after the production of 10–24 sepals, and there was a concurrent switch to spiral phyllotaxy. Such flowers produced no carpel-like structures on the main floral axis. In many cases, the internal organs of the flower were united with each other and, very occasionally, flowers terminated with united carpels similar to those found in the third whorl of def or glo mutants.

Phenotypes conferred by partial deletions of fim

Although the fim-680 deletion extended into the ORF, its mutant phenotype was less extreme than that of the null alleles: it showed greater determinacy, more petal and carpel tissue, and less internode elongation (data not shown). This weaker phenotype might be explained by the presence of an in-frame ATG, situated 38 amino acids (aa) into the ORF; the deletion in fim-680 extended only 108 bp (36 aa) into the ORF so the mutant could have produced a protein with 38 aa missing from the N-terminus (Figure 1B). The missing N-terminus is the region of FIM that is least conserved with its homologue from Arabidopsis, UFO (Ingram et al., 1995). It is therefore probable that this part of the protein is not absolutely required for FIM activity.

Two deletions gave very different phenotypes but had almost identical breakpoints in the 5′ promoter region of fim. The main difference in the structure of these alleles was whether or not they retained Tam3. In fim-678, which had lost Tam3 and 770 bp of promoter DNA, the deletion breakpoint was at –206, and in fim-676, which retained Tam3, the breakpoint was at –204. Homozygous fim-678

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**Fig. 2.** Phenotypes conferred by deletions at the fim locus. Flowering spikes from wild-type (A), fim-619 (B), fim-676 (C) and the fim-677 null (D). A typical flower is shown below each spike (E, F, G and H). Each spike shows a region of abortion (arrow), probably caused by environmental fluctuation. Decreasing amounts of petal and stamen tissue with increasing allele severity are apparent from left to right, as are increases in sepal tissue production. A high degree of apical and lateral indeterminacy is visible in the basal region of the null fim spike (D, indeterminate flower indicated by an arrowhead).
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**Fig. 3.** The development of **fim** null mutant flowers. Floral meristems from wild-type and null **fim-681** mutant plants are shown at nodes 13, 15, 17 and 19. At node 13 (stage 4), the dorsal region of the mutant flower appears normal but an extra primordium is visible between the ventral two organs (marked with an arrow in B). By node 15 (early stage 5) defects in the mutant are more apparent, illustrated by the extra ventral sepal which is united with a more internal organ (arrow in D, the two dorsal sepals are absent). At node 17 in wild-type, the second whorl petal primordia are small and stamen primordia are clearly visible (E, all but the most dorsal sepal removed). By contrast, at a comparable node in the **fim** mutant, organ primordia (probably sepals) internal to the first whorl are relatively large and those further in are hardly visible (F, all but the most dorsal first whorl organs removed). Whorl structure has also started to deteriorate. By node 19, retarded development of internal primordia is even more noticeable in the mutant (H, first whorl sepals have been left in place but have been partially dissected to reveal internal primordia) as compared with wild-type (G, all sepal primordia removed). United ventral sepals (H, arrow) and a filament structure (labelled with an asterisk in H) are also apparent in the mutant.

Plants gave a wild-type phenotype, suggesting that the 770 bp 5′ region deleted in these plants was not necessary for **fim** transcription. However, plants homozygous for **fim-676**, which retained Tam3, had a mutant phenotype with sepal/petal mosaic organs in the second whorl and a third whorl comprising one to two deformed stamens together with petal/sepal/carpel mosaic organs. The phenotype of plants carrying **fim-676** was more extreme than that of the **fim-619** progenitor which only showed slight abnormalities in the second whorl (Figure 2). Therefore, the presence of Tam3 in the **fim** promoter, in combination with the loss of promoter sequence to the right of Tam3, caused considerable disruption of **fim** function. This effect of Tam3 could be due to transcription factors or transposase...
proteins binding to its ends and interfering with fim expression. Alternatively, the effects of Tam3 could be due to physical distancing of 5' regulatory elements from the start of fim (Bradley et al., 1993; Chatterjee et al., 1996).

Expression of the fim alleles
The effects of the various deletions on fim expression were analysed by RNA in situ hybridizations on inflorescence apices. In wild-type, fim is first expressed in the ventral region of floral meristems and then in their centre before any floral organ primordia are visible (stage 2). The expression of fim then appears to spread outwards and, by the floritypic stage (stage 4), becomes localized as a ring around the centre of the floral meristem, adjacent to the sepal primordia (Simon et al., 1994). By stage 5, when petal primordia become visible, fim transcripts are present in domains around the base of each developing petal (Figure 5A). Throughout the rest of floral development fim remains localized at the junctions between sepals and petals, and between petals and stamens (Simon et al., 1994).

The deletion mutants showed reductions in fim expression which correlated with the severity of their phenotypes. The fim-678 allele, which had a 768 bp 5' deletion and a wild-type phenotype, showed wild-type fim expression (data not shown). In contrast, fim-676, which had a similar deletion but which retained Tam3 and showed a mutant phenotype, had a reduced level of transcript, although the distribution appeared to be temporally and spatially similar to wild-type (Figure 5D). Plants homozygous for fim-680, which gave an almost null phenotype, showed a very low level of fim transcript, although the spatial domain of expression seemed normal (not shown). Detection of fim transcripts in fim-680 was delayed compared with wild-type: transcripts first became visible at around node 12 compared with node 9 in wild-type. Plants homozygous for fim-679 or fim-681, which showed a null phenotype, had very low levels of transcript with the same spatial and temporal distribution as in fim-680 (not shown). The probe used for in situ analysis extended 3' of the deletion breakpoints in all of these alleles and could therefore detect transcripts truncated at the 5' end. No fim transcripts were detected in plants homozygous for the null alleles fim-682 and fim-677, which had lost the entire region covered by the probe (Figure 5G).

The results indicated that even when the entire 5' region between fim and Tam3 was deleted, information conferring spatially specific expression of fim was still present. Sequences 5' of the Tam3 excision site and/or in the 3' of the gene therefore play an important role in fim regulation. A candidate regulatory element is a putative MADS box protein-binding site 39 bp 5' of the Tam3 insertion site, which was not lost in any of the deletions studied (Figure 1B) (Schwarz-Sommer et al., 1990).

Expression of glo and def in fim mutants
The observed decrease in petal and stamen tissue in fim mutants suggested that b activity was reduced. In wild-type, the b function gene def is first expressed in the central region of floral meristems at node 10 just before the floritypic stage (stage 4). As second and third whorl primordia arise, def expression is lost from the centre of the floral meristem and becomes localized in the developing petal and stamen primordia (Schwarz-Sommer et al., 1992) (Figure 5B). Previous analysis of the fim-620 allele had shown that reduced fim activity led to a decrease and delay in the expression of def (Simon et al., 1994). A further reduction in def expression was observed in the fim nulls we generated in this study. About half of all floral meristems analysed showed no detectable def expression and in those that did show expression, it was delayed and restricted to small patches of cells in the central regions of young floral meristems. At later stages, small patches of def expression were occasionally detected in organs internal to the sepal whorl but these patches were very rare (Figure 5H).

In fim null mutants, expression of another b function gene, globosa (glo), was also found to be severely diminished. The glo expression pattern in fim nulls was very similar to that of def: it was absent from many floral meristems, and very much reduced and delayed in meristems where it was detected (data not shown). The temporal and spatial expression pattern of glo is known to be closely correlated to that of def in wild-type plants. However, probing adjacent sections of fim null buds at the late floritypic stage (nodes 13–14) indicated that both def and glo expression was found in small patches of cells, but that these patches only occasionally overlapped. This indicated that these genes were being independently activated in a stochastic manner in small areas within the b function domain at an early stage of meristem development. In later buds, probing of adjacent sections showed that the rare patches of glo expression observed did correlate with patches of def expression. This is consistent with studies which show that it is only after stage 6 of wild-type that mutual activation by DEF/GLO...
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Fig. 5. Expression of fim def and ple in fim mutants. Expression pattern of fim, def and ple in floral meristems at around node 16–17 of wild-type (A–C), fim-676 (D–F) and the fim-677 null (G–I). Expression of fim in fim-676 (D) is considerably reduced compared with wild-type (A), although the expression domain remains essentially unchanged. No fim transcripts were detected in fim-677 (G). The domain of def expression in fim-676 (E) is similar to wild-type (B), although large areas lacking def expression are observed within some second and third whorl organs. Two such organs are visible in transverse section (arrowed in E), one showing def expression at both lateral margins and the other showing a small amount of expression at one lateral margin. A very low level of def expression can be seen near the centre of the fim-677 null floral meristem (H), although many fim null floral buds show no def expression. Expression of ple in fim-676 (F) was slightly delayed and restricted as compared with wild-type (C). Expression of ple in fim-677 (I), when present, was delayed and restricted to a small region in the centre of the floral meristem. Less than 50% of fim-677 flowers showed any ple expression at node 16. The positions of sepal (s), petal (p), stamen (st) and carpel (c) primordia in wild-type meristems are shown in (A). Scale bar is 100 μm.

heterodimers becomes necessary to maintain expression of def and glo (Zachgo et al., 1995).

In fim-680, which showed an almost null phenotype, the amounts of def and glo transcript detected, although still very low, were higher than in the null mutants, confirming that there was some fim activity in these plants (data not shown).

In fim-676 mutants, mosaic organs composed of sepal and petal tissue were produced in the second and third whorls. Similar types of mosaic organs were also reported in the fim-620 mutant, where it was shown that regions of sepal and petal tissue separated by lines of only one or two cells of intermediate identity (Simon et al., 1994). Since both fim-620 and fim-676 carry transposon insertions in the fim promoter, it is possible that patches of b function expression were being caused by transposon excision during early flower development. However, the mosaic organs appeared at a reproducible frequency, independent of temperature, whereas Tam3 excision is known to be temperature-sensitive, suggesting that transposition events are an unlikely explanation for most patches.

A more likely explanation for the mosaic organs was that they reflected an early role of fim in allocating cells in the floral meristem to express b function genes. In weak fim alleles, such as fim-676 and fim-620, only a subset of cells might be allocated, giving rise to discrete sectors with b activity. To investigate this possibility, we studied the expression of def and glo in fim-676 plants. Expression was very variable in early floral meristems (nodes 12–14, stage 4), with some floral meristems showing almost wild-type def and glo transcript levels and others showing very reduced levels. Expression always occurred within the b domain and was not appreciably delayed compared with wild-type. Early patches of glo expression were always completely overlapped by def expression, although the domain of glo appeared to be
slightly more restricted than that of def (Figure 6B). The coordinated expression of both genes early in development indicated that their activation was most likely not stochastic. In older buds (stages 5 and onwards), expression of the two genes was coincident and maintained a patchy pattern. Some areas of second and third whorl organs showed a wild-type level of def and glo, while adjacent areas of the same organs showed no expression. When organs were sectioned transversely, patches of def and glo expression were found to be most common on the lateral edges of organs rather than in the midrib region (Figure 5E). In wild-type meristems at later stages, developing sepal tissue showed a wild-type level of expression whereas thicker regions (assumed to be developing sepal tissue) showed no expression.

**Activation of ple and flo varies between early and late flowers of fim mutants**

The presence of carpel tissue in flowers higher up on the inflorescence of fim nulls suggested that the c function was active in these flowers. However, flowers lower down showed considerable apical indeterminacy, similar to flowers lacking c function. To clarify these effects on c function, expression of ple was investigated in the fim mutants.

Decreased and delayed ple expression was observed in fim-676, fim-680 and all fim null flowers. In wild-type flowers, expression of ple is first observed at late stage 3 in a central region of the floral meristem and then in the presumptive stamen and carpel primordia as they form (Figure 5C) (Bradley et al., 1993). In fim-676 plants, which have a reduced level of fim expression, ple transcripts were present at a reduced level, and the domain of ple expression was restricted to a slightly more central area of the meristem than normal, similar to the pattern reported in fim-620 plants (Figure 5F) (Simon et al., 1994). In fim null mutants, ple expression was never observed until primordia internal to the first whorl were clearly visible (stage 5), a delay of five nodes compared with wild-type (Figure 7A) (Bradley et al., 1993). In some floral buds, ple expression was entirely absent, even at late stages (Figure 7B). The variable timing and levels of ple expression correlated with the variation in phenotype observed along the inflorescence of fim nulls. Sections of floral meristems lacking ple expression at late stages tended to show inflorescence-like characters typical of flowers low down on the inflorescence.

The inflorescence-like characteristics of some flowers on fim nulls was further analysed by studying expression of the meristem identity gene floricaula (flo) (Coen et al., 1990). In wild-type meristems, fim and flo have a complementary expression pattern (Simon et al., 1994). In fim nulls, the expression pattern of flo was indistinguishable from wild-type up to stage 5, showing that fim does not affect the early expression pattern of flo. At later stages, indeterminate flowers showed strong flo expression in the same domains as observed in the main inflorescence apex: flo was expressed in the youngest organ primordia (morphologically comparable with bracts) and in the secondary meristems, but was absent from the apical region (Figure 7C).

**Identification of FIM-associated proteins (FAPs)**

The analysis of the nulls indicates that fim plays a role both in regulating expression of organ identity genes and in the pattern of meristem growth. To gain further insights into how fim might affect these processes, we used the yeast two-hybrid system to identify proteins that interacted with FIM. A fusion protein, comprising FIM and the GAL4 DNA-binding domain, was made by cloning the entire FIM-coding region into the vector pGBT9, to give plasmid pBD/FIM. This plasmid did not itself activate transcription: when yeast transformants were grown on medium lacking histidine, growth was much reduced. The presence of carpell tissue in flowers higher up on the inflorescence of fim nulls suggested that the c function was active in these flowers. However, flowers lower down showed considerable apical indeterminacy, similar to flowers lacking c function. To clarify these effects on c function, expression of ple was investigated in the fim mutants.

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To identify proteins that interact with FIM, the HF7c(BD/FIM) strain was transformed with an expression
Fig. 7. Expression of ple and flo in flowers of fim null mutants. Flowers from a fim-677 null at around node 30–35 probed with ple (A and B) and with flo (C). The meristem in (A) shows lateral indeterminacy but also shows apical ple expression and would presumably have formed an apically determinate flower. In contrast, the meristem shown in (B) shows no ple expression and would probably have formed an indeterminate inflorescence-like structure. This is confirmed by probing the adjacent section with flo (C), revealing strong flo expression in young primordia and axillary meristems, typical of the flo expression pattern of wild-type inflorescence apices. Scale bar is 100 μm.

library (Davies et al., 1996) in which a plasmid vector carrying the GAL4 activation domain had been coupled to Antirrhinum floral cDNA. A total of $2.4 \times 10^6$ transformants were screened for enhanced growth in the absence of histidine. The 340 resulting candidates were then tested for activation of the LacZ gene. This gave 27 positive colonies, from which the plasmids were isolated and retransformed into another yeast strain (SFY526) carrying pBD/FIM to confirm that they could still activate LacZ.

Of the resulting transformants, only 16 could activate LacZ conditional on pBD/FIM also being present in the yeast host. All 16 plasmids were then partially sequenced. The predicted proteins encoded by the 16 activation domain-containing plasmids, termed Fim-associated proteins (FAPs), came from three closely related genes: fap1 (seven plasmids), fap2 (seven plasmids) and fap3 (two plasmids) (Figure 8). From the sequence available, the predicted amino acid sequence of FAP1 was 84% identical to FAP2, and FAP1 and FAP2 were both 70% identical to FAP3 (Devereaux et al., 1984). The fact that only proteins belonging to the same family came through the screen confirmed that they had been selected on the basis of association with FIM, rather than through any hypersensitivity in the yeast two-hybrid system. The sequences were most highly conserved at their C-terminal ends and contained a variable region in the centre. Database searching using the ‘Blast’ algorithm revealed that the FAP family showed strong similarity to the human p19Skp1 protein and SKP1 from yeast, which are required for protein degradation and cell cycle progression (Altschul et al., 1990; Zhang et al., 1995; Bai et al., 1996; Connelly and Hieter, 1996).

**Expression of FAPS in floral meristems**

If the FAP–FIM interaction is of functional significance in vivo, there should be an overlap between the expression domains of the fim and the fap genes. To test this, in situ hybridizations were carried out on wild-type Antirrhinum inflorescences using the inserts from the 16 fap clones. All fap RNA probes gave a very similar expression pattern. High expression was seen in inflorescence and floral meristems and provascular tissue (Figure 9A and B), all areas known to be in a state of active cell division (Fobert et al., 1994; V. Gaudin et al., submitted). The earliest overlap between fim and fap expression in the flower occurred at about stages 2–3, before the sepals had formed and when fim was expressed in the central dome of the floral meristem. At stage 4, fap expression was observed throughout the floral meristem (Figure 9B), whereas fim was restricted to a ring around the periphery of the central dome. At later stages, as the floral primordia emerged, fap expression was mainly within primordia and to a lesser extent between primordia (Figure 9C), whereas fim expression was restricted to the base of petal primordia (Figure 5A). Expression of the faps continued to be seen in later stages of organ development, particularly in actively dividing ovule and anther tissue (Figure 9D). To summarize, the fap expression domain was broader than, and incorporated, that of fim throughout floral development.

**Discussion**

We have shown that FIM associates specifically with a class of proteins implicated in protein degradation during the cell cycle of yeast and humans. The FIM-associated proteins (FAPs), isolated by the yeast two-hybrid system, fall into a family comprising three related proteins, FAPs 1–3. The FAP family shows strong similarity to Skp1 proteins from yeast and human. In yeast and humans, Skp1 proteins interact with F-box-containing proteins, to form a complex needed for protein degradation and cell cycle progression.
Fig. 8. Sequence of FAPs. Predicted amino acid sequences for FAP1, FAP2 and FAP3 compared with the human (HS) and yeast (SC) Skp1 proteins. Amino acid identities are boxed in black and conservative amino acid changes in grey. The black triangle represents an additional 30 amino acids present in the yeast protein. The genes are more highly conserved at their 3' ends and are more variable in the central region. The fap clones sequenced are not complete at their 5' ends.

Fig. 9. Expression of faps in wild-type inflorescences. Expression pattern of faps in the inflorescence apex (A); stage 4 floral meristem (B); stage 6 floral meristem (C) and later floral bud (D). All faps give a similar expression pattern; those shown are fap1 (A–C) and fap2 (D). Expression correlates with regions where cells are actively dividing: the inflorescence meristem (I), bracts (Br), early floral meristems (F), sepals (S), petals (P), stamens (St), carpels (Ca), ovary (O) and anthers (An). Some expression is also seen in provascular tissue. Scale bar is 100 μm.

cycle progression (Zhang et al., 1995; Bai et al., 1996; Connelly and Hieter, 1996). Thus, the similarity between FIM and F-box proteins is further substantiated by their ability to interact with similar partners. A role for the FIM–FAP complex in flower development is supported by the RNA expression pattern of the fap genes. These genes are expressed in inflorescence and floral meristems in a pattern that incorporates the domain of fim expression. Based on the phenotypic and expression analysis of the various fim alleles generated by site-selected mutagenesis, the FIM–FAP complexes might play two roles in flower development: (i) regulation of organ identity gene expression; and (ii) establishing morphological boundaries.

Regulation of organ identity gene expression
The b function is largely absent from the flowers of fim null plants: they contain almost no petal or stamen tissue and the b function genes, def and glo, are both expressed at greatly reduced levels compared with wild-type. In those cases where def and glo transcripts are detected, they first appear as small patches within the b function domain (stage 4). There is little overlap between early
patches of def and glo expression, indicating that both genes are activated in a stochastic manner in fim nulls. An early role of fim may therefore be to coordinate activation of def and glo expression, although the detectable level of b activity in the nulls indicates that fim is not the only means for promoting def and glo expression. At later stages, interactions between def and glo appear to be involved in maintaining their expression so that by about stage 6, the rare patches of def and glo expression in fim null mutant flowers are found to coincide. This is consistent with previous results which show that autoregulatory control of def and glo expression does not become important until around stage 6 (Tröbner et al., 1992; Zachgo et al., 1995).

The homology between FIM–FAP and proteins from other systems suggests a possible mechanism by which fim might influence b activity. F-box proteins and their partners in other systems have been shown to affect the activity of cell cycle proteins and transcription factors, most probably by promoting protein degradation (Flick and Johnston, 1991; Barral et al., 1995; Kumar and Paietta, 1995; Thomas et al., 1995; Bai et al., 1996). By analogy, the FIM–FAP complex might act by promoting degradation of a transcriptional inhibitor of def and glo. As expression of fim moves out from the centre of the meristem, the inhibitor could be removed in its wake, allowing the b genes to be activated. This is consistent with the pattern and timing of gene expression in the meristem: fim transcripts are first detected in the central dome, one to two nodes before expression of def and glo; expression of fim then spreads out to the periphery of the dome, with def and glo expression following behind.

A key requirement of any model for FIM function is to explain how the effects of fim on b activity persist until late stages of development, even though fim is only transiently expressed in the central dome of wild-type floral meristems. The persistent effects of fim might have two explanations: (i) early transient expression of fim might allocate cells to express b genes and this pattern is then maintained during later stages independently of fim; or (ii) late expression of fim at the boundaries of primordia might act non-autonomously to promote b expression throughout the primordia.

Analysis of the development of mosaic organs in fim-676 mutants supports a transient role for fim in activating b. Expression of def and glo initiates in distinct patches in second and third whorl organ primordia of fim-676 flowers. These patches may represent subsets of primordium cells that have been allocated to express b function genes early on. At later stages, expression of b function genes is still observed in discrete patches with sharp boundaries, correlating with areas of petal tissue observed in the mature flowers of fim-676 plants. These discrete regions of b activity presumably include the clonal descendants of patches seen earlier on in development. The pattern of b activity in fim-676 flowers can therefore be explained by allocation of cells early on in development which then persists through cell division as the flowers develop. According to this view, fim would act transiently, ensuring that cells within the b domain of wild-type meristems are allocated to express both def and glo at early stages.

Allocation might be explained if the FIM–FAP complex promotes degradation of a transcriptional inhibitor of b genes early on, such that an active transcriptional state is then maintained through subsequent development. The existence of this sort of de-repression mechanism is supported by the analysis of CURLY LEAF in Arabidopsis, a gene which inhibits organ identity gene expression and encodes a product with homology to the Polycomb group of proteins, thought to be involved in cell allocation during Drosophila development (Goodrich et al., 1997). However, CURLY LEAF mainly affects c activity in floral meristems at later stages of development, so other genes would presumably have to play a comparable role in repressing b function early on. Early allocation is also compatible with studies of cell lineages in Antirrhinum petal primordia which have shown that lineage restrictions occur between petals and adjacent organs at about the time that fim expression starts to diminish in organ primordia (Vincent et al., 1995).

In addition to affecting b, the fim nulls also show reduced and delayed c activity. There is a decrease in reproductive tissue and an increase in organ number, correlating with delayed expression of the c function gene ple. In the absence of fim, there appears to be a gradient of ple activation along the inflorescence, with upper flowers showing ple expression earlier in their development than lower flowers. This is similar to the graded increase in c function activity observed towards the top of Arabidopsis inflorescences carrying mutations in meristem identity genes (Irish and Sussex, 1990; Huala and Sussex, 1992; Weigel et al., 1992; Schultz and Haughn, 1993; Wilkinson and Haughn, 1995). It is unlikely that fim directly maintains ple expression since, by the time that ple expression is clearly detected, fim shows a complementary expression pattern. A more likely explanation is that early transient expression of fim in the central dome of the floral meristem is needed to activate ple at the right time, perhaps by a similar mechanism to that proposed for regulation of b genes.

The availability of null alleles also allowed the interaction of fim with the meristem identity gene flo to be investigated. These two genes show complementary expression patterns: expression of fim initiates in the central dome of the floral meristem at about the same time that flo declines in this region (stages 2–3), and at later stages flo is expressed within petal primordia whereas fim is expressed at their boundaries. This raises the possibility that fim and flo might inhibit each other’s expression. In fim nulls, however, early flo expression is not significantly altered, indicating that fim is not necessary for restricting flo. Nevertheless, it is still possible that flo inhibits fim expression: the restriction of fim to the central dome at stage 3 could, for example, reflect the decline of flo expression specifically in this region. This is not easy to test in Antirrhinum because flo is also needed earlier on to activate fim in the central dome of floral meristems. In Arabidopsis, where LFY (the flo orthologue) is not needed to activate UFO (the fim orthologue), expression of UFO has been shown to become more extensive in Ify mutants (Lee et al., 1997), consistent with LFY playing a role in restricting the domain of UFO.

**Growth at morphological boundaries within the flower**

Two aspects of the null phenotype indicate that fim plays a role at morphological boundaries in the flower. First,
meristems are often produced at the boundaries between floral organs in null flowers, giving rise to axillary shoots (lateral indeterminacy). Secondly, the boundaries between organs are often displaced, resulting in united organs and a loss of whorled phyllotaxy. The expression of fim in wild-type correlates with these effects. For instance, an extra ventral organ is frequently observed in the first whorl of fim null mutants, correlating with early expression of fim in this region of wild-type. Thus, localized fim expression in early meristems may repress growth and/or prim-ordium initiation. Later expression of fim at the boundaries between primordia might also repress growth, ensuring lateral determinacy and whorled phyllotaxy. In affecting boundaries between primordia, fim shows similarities to the no apical meristem (nam) gene of petunia, which is also expressed between floral organs where it is thought to reduce growth (Sousa et al., 1996).

The similarities between fim and F-box genes from yeast suggest that fim may influence growth by modulating the cell cycle. This is also consistent with down-regulation of the cyclin D3b gene in boundary regions expressing fim (V.Gaudin et al., submitted). One possibility is that the FIM–FAP complex is involved in degrading a regulatory protein needed for cell cycle progression. Another possibility is that FIM competes for a FAP needed for cell cycle progression.

Although fim regulates boundary growth, it is not absolutely required for the formation of morphological boundaries because they still form in null mutants even though they are in abnormal positions. Furthermore, over-expression of the fim orthologue, UFO, in Arabidopsis does not result in any obvious reduction in growth, indicating that growth effects of fim may depend on other spatially restricted factors (Lee et al., 1997).

The significance of fim affecting both growth at boundary regions and organ identity gene expression may be that it provides a means of coupling the patterns of meristem growth to the regulation of gene activity. For example, the outer boundary of b gene expression appears to be centred within a domain of fim expression by stage 5. If the effects of fim on growth also ensure that morphological boundaries are centred within the fim domain, the anatomical and expression boundaries would be automatically aligned. This dual aspect of fim may be comparable with the role of GRR1 in yeast, which encodes an F-box protein thought to coordinate gene expression with cell growth and division (Barral et al., 1995). The superman gene of Arabidopsis also appears to have a dual role in b gene expression and cell proliferation (Schultz et al., 1991; Bowman et al., 1992; Sakai et al., 1995). It is therefore possible that coupling between transcriptional regulation and growth, as exemplified by the FIM–FAP complex, is a general mechanism for aligning expression domains in meristems.

Materials and methods

Strategy for site-selected mutagenesis of the fim gene

To generate deletions and rearrangements caused by Tam3, several plants homozygous for fim-619 (the M0 generation) were placed at 15°C, a temperature that favours Tam3 activity (Carpenter et al., 1995). The fim-619 allele carries a Tam3 insertion 976 bp upstream of the start of the longest fim cDNA (start of the cDNA is denoted position 0). This allele is unstable due to the excision of Tam3, giving germline revertants at a rate of ~1–2% under field conditions (Simon et al., 1994). The M0 plants were self-pollinated and 268 seed capsules individually harvested. Each capsule was sown to give a family of 15 M1 plants (total of 4020 plants). The M1 plants were also self-pollinated and seed pooled within each family.

Plants in the M1 generation were expected to be heterozygous for any germinal events which had occurred in the M0. To allow these events to be detected by PCR, leaves were harvested and pooled from each M1 family of 15 plants and genomic DNA was extracted from the pools. Three sets of oligonucleotide primers (oligos) were designed: oligos A and B were complementary to each end of Tam3, C and D were based on sequences near the Tam3 insertion site, and E–K corresponded to sequences within the 6.5 kb genomic region containing fim and its 5' and 3' regions (Figure 10). Screening with Tam3 oligos (A and B) in combination with fim-specific oligos (E–K) should detect events in which Tam3 had transposed to a new site within fim, or deletions adjacent to Tam3. In addition, fim-specific oligos in combination with oligos adjacent to the Tam3 insertion site (C and D) should detect deletions in which Tam3 had excised. The sensitivity of PCR for detecting rare events was tested by making serial dilutions of genomic DNA from homozygous fim-619 individuals in an excess of wild-type DNA. Dilutions were tested with oligos A and E or with B and F. In control reactions, both combinations gave the expected products of ~1 kb with undiluted fim-619 DNA, and no products with wild-type DNA. Amplification of a 1:1000 dilution of fim-619 wild-type DNA with either oligo combination gave 1 kb PCR products which were visible on an ethidium bromide-stained gel and which strongly hybridized to a fim probe on DNA blots (probe Y, Figure 10; data not shown). A dilution of 1:10 000 also gave bands detectable on DNA blots probed with fim, although they were not visible on ethidium bromide gels. Screening DNA pooled from 500 plants in one PCR reaction therefore allowed ready detection of heterozygous events by ethidium bromide gel staining.

To facilitate screening, DNA from M1 families was further pooled in a combinatorial fashion so that each family was represented in a unique combination of two large pools. This minimized the number of reactions needed to identify the family in which an event had occurred. The large pools contained DNA from a maximum of 11 families (165 plants) so that new events were well within the limit of detection (1 in 500 plants). Only events found in two large pools were considered to be genuine.

DNA preparation and PCR screening

For DNA extraction, leaf samples were ground in liquid nitrogen and added to extraction buffer [50 mM EDTA, pH 8; 0.1 mM NaCl; 0.1 M Tris–HCl, pH 8; 1% (w/v) sodium dodecyl sulfate (SDS)]. Phenol/chloroform extractions were performed and DNA was precipitated with 10% (v/v) sodium acetate and 70% (v/v) isopropanol, washed in 70% ethanol, air-dried and resuspended in TE buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA) containing 5 μg/ml RNase A. DNA concentrations were determined by optical density at 260 μm and gel analysis.

Oligonucleotides were synthesized and purified in a synthesizer according to the manufacturer’s instructions (Pharmacia Biotech) and were as follows: (A) 5'-CAC GGC CCA ATT CAC ATC TTT A-3'; (B) 5'-CTC GGC ACG TTT CAC ATC TTT A-3'; (C) 5'-GTT TGA TGT GTA CTC CTT TTT GCC-3'; (D) 5'-CTT GTC GTC CCT CAC

Fig. 10. The fim locus. The position covered by the fim cDNA is shown as a box, with the solid region indicating translated sequence and the direction of transcription shown by a large arrow. The Tam3 insertion in fim-619 is indicated as a black triangle. Positions of oligos A–K (used in PCR screening) are indicated by small arrows and probes X and Y are shown hatched.
the BD/FIM bait and tested for β domain plasmids that were only positive by filter assay in the presence in situ and Methods for tissue preparation, digoxigenin labelling of RNA probes In situ hybridization described in Simon et al. probe used to detect Elmer). The primers used for sequencing flanked the pGAD424 cloning (Feilotter et al. Winston, 1987). Candidate positives were re-transformed into HF7c strain HF7c carrying BD/FIM (Gietz domain. 240 yeast DNA preparation from the remaining positive colonies was resulting clone, pJAM2036, was fully sequenced and is referred to fusion of the open reading frame to the GAL4-binding domain. The reading frame into the vector pGBT9, which encodes the yeast GAL4 activation plasmids were selected by their ability to grow more quickly on strain HF7c carrying BD/FIM (Gietz domain. The primers used for sequencing flanked the pGAD424 cloning (Feilotter et al. Winston, 1987). Candidate positives were re-transformed into HF7c strain HF7c carrying BD/FIM (Gietz domain. The primers used for sequencing flanked the pGAD424 cloning (Feilotter et al. Winston, 1987). Candidate positives were re-transformed into HF7c strain HF7c carrying BD/FIM (Gietz domain. The primer included the T7 promoter. The purified PCR products were used as templates for T7 RNA polymerase.

Molecular and phenotypic analysis of new fim alleles PCR products were cloned into pGEM4z (Promega) by fusing overhangs using T4 DNA polymerase and then ligating into Smal-cut vector under blunt-end conditions. Plasmids were isolated using QiaQuick columns (Qiagen, Hybaid Ltd, UK) and sequenced by dideoxynucleic acid methods using the Sequenase II kit according to the manufacturer’s instructions (US Biochemicals). Methods for isolation of genomic DNA for gel blot analysis and preparation of DNA probes were as described previously by Bradley et al. (1993).

Plants for SEM analysis were grown in growth chambers at 20°C. SEs were prepared as described in Green and Linstead (1990). The resulting clone, pJAM2036, was fully sequenced and is referred to as BD/FIM.

The prey was kindly provided by Hans Sommer (Davies et al., 1996) and consisted of a cDNA library from floral tissue, directionally cloned into the vector pGAD424 which carries the yeast GAL4 activation domain. PCR amplification was used to verify SaII and BamHI restriction sites into the fim sequence, allowing an in-frame fusion of the open reading frame to the GAL4-binding domain. The resulting clone, pJAM2036, was fully sequenced and is referred to as BD/FIM.

The FIM bait was generated by subcloning the full-length fim open reading frame into the vector pGBT9, which encodes the yeast GAL4 DNA-binding domain. PCR amplification was used to verify SaII and BamHI restriction sites into the fim sequence, allowing an in-frame fusion of the open reading frame to the GAL4-binding domain. The resulting clone, pJAM2036, was fully sequenced and is referred to as BD/FIM.

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Plants for SEM analysis were grown in growth chambers at 20°C. SEs were prepared as described in Green and Linstead (1990). The mutant fim-619 (originally called sep-619) was derived from stock JI.98 (nivearecurrens-98) during a large-scale mutagenesis programme (Carpenter and Coen, 1990, Simon et al., 1994).

Yeast two-hybrid screening and further analysis The FIM bait was generated by subcloning the full-length fim open reading frame into the vector pGBT9, which encodes the yeast GAL4 DNA-binding domain. PCR amplification was used to verify SaII and BamHI restriction sites into the fim sequence, allowing an in-frame fusion of the open reading frame to the GAL4-binding domain. The resulting clone, pJAM2036, was fully sequenced and is referred to as BD/FIM.

The prey was kindly provided by Hans Sommer (Davies et al., 1996) and consisted of a cDNA library from floral tissue, directionally cloned into the vector pGAD424 which carries the yeast GAL4 activation domain. 240 μg of the library was then used to transform the yeast strain H7 carrying BD/FIM (Gietz et al., 1992). Colonies that contained activated plasmids were selected by their ability to grow more quickly on plates lacking histidine. Candidate positives were also tested for β-galactosidase activity using a filter assay (Bredon and Naysmith, 1985). A crude yeast DNA preparation from the remaining positive colonies was transformed into the Escherichia coli strain HB101 (Hoffman and Winston, 1987). Candidate positives were re-transformed into H7 carrying BD/FIM (Feilotter et al., 1994) or SPS526 (Bartel et al., 1993), with or without the BD/FIM bait and tested for β-galactosidase activity. Those activation domain plasmids that were only positive by filter assay in the presence of the BD/FIM bait were sequenced using the automated ABI PRISM® Dye Terminator Cycle sequencing Ready Reaction Kit system (Perkin Elmer). The primers used for sequencing flanked the pGAD424 cloning sites, as described previously (Davies et al., 1996).

In situ hybridization Methods for tissue preparation, digoxigenin labelling of RNA probes and in situ hybridization were as described in Bradley et al. (1993). The probe used to detect fim transcript was prepared from pCTW6 as described in Simon et al. (1994). Probes used to detect ple, def and flo transcripts were as described in Bradley et al. (1993). The probe used to detect glo transcript was prepared as described in Tröbner et al. (1992). The probes used to detect the gap genes were from PCR-amplified products, using primers to the pGAD424 vector, of which one primer included the T7 promoter. The purified PCR products were used as templates for T7 RNA polymerase.

Accession numbers The sequence information presented in this paper has been assigned the following EMBL database accession numbers: Antirrhinum majus mRNA for fimbriata-associated protein 2 (Y14856), Antirrhinum majus mRNA for fimbriata-associated protein 2 (Y14857) and Antirrhinum majus mRNA for fimbriata-associated protein 1 (Y14858).

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Carpenter,R., Copesy,L., Vincent,C. and Coen,E.S. (1996) Budding yeast SKP1 links the ubiquitin machinery through a novel motif, the F-box.


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