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The *FRUITFULL* MADS-box gene mediates cell differentiation during *Arabidopsis* fruit development

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SUMMARY

Fruit morphogenesis is a process unique to flowering plants, and yet little is known about its developmental control. Following fertilization, fruits typically undergo a dramatic enlargement that is accompanied by differentiation of numerous distinct cell types. We have identified a mutation in *Arabidopsis* called *fruitfull* (*ful-1*), which abolishes elongation of the silique after fertilization. The *ful-1* mutation is caused by the insertion of a DsE transposable enhancer trap element into the 5′ untranslated leader of the *AGL8* MADS-box gene. β-glucuronidase (GUS) reporter gene expression in the enhancer trap line is observed specifically in all cell layers of the valve tissue, but not in the replum, the septum or the seeds, and faithfully mimics RNA *in situ* hybridization data reported previously. The lack of coordinated growth of the fruit tissues leads to crowded seeds, a failure of dehiscence and, frequently, the premature rupture of the carpel valves. The primary defect of *ful-1* fruits is within the valves, whose cells fail to elongate and differentiate. Stomata, which are frequent along the epidermis of wild-type valves, are completely eliminated in the *ful* mutant valves. In addition to the effect on fruit development, *ful* cauline leaves are broader than those of wild type and show a reduction in the number of internal cell layers. These data suggest that *AGL8*/*FUL* regulates the transcription of genes required for cellular differentiation during fruit and leaf development.

Key words: *ful*, Fruit development, MADS-box gene, Cell division and expansion, Transposable enhancer trap, *Arabidopsis thaliana*

INTRODUCTION

The fruit, a plant organ mediating maturation and dispersal of the seed, is the end product of a successful fertilization process. Diverse forms of fruits are found in angiosperms, but in most plants the fruit consists of pericarp, which is derived from the ovary wall, and seeds, derived from the fertilized ovules. The fruits of over three thousand species of *Brassicaceae,* including *Arabidopsis thaliana,* are known as siliques and develop from a gynoecium consisting of two carpels that share a fused tissue called the septum (Rollins, 1993). On each side of the fruit the peripheral walls of the carpels, known as the valves, are joined to the replum, which is the outer margin of the septum (Fahn, 1967). The epidermis of the replum is visible as a suture that divides the two carpels, and it encases a vascular bundle that branches out into the placenta. After fertilization, the *Arabidopsis* gynoecium expands drastically to form an elongated silique in response to a hypothetical signal that emanates from the fertilized egg (Hensel et al., 1994). Upon maturation, a zone of dehiscence develops along the replum-valve junction such that the valves detach from the replum to release the seeds. Growth of the embryo, the ovules and the peripheral gynoecium valves involves cell differentiation and expansion and must be carefully coordinated during fruit development to generate the final form.

The cellular organization of the developing *Arabidopsis* gynoecium has been described previously (Hill and Lord, 1988; Okada et al., 1989; Sessions and Zambryski, 1995). The gynoecium arises from the center of the floral meristem (flower stage 6, as defined by Smyth et al., 1990) and forms a cylinder-like structure. At stage 9, four distinct tissue types can be recognized on the surface of the gynoecium, namely the apical stigma, the style, the carpel valves and the narrow stretch of epidermis covering the replum. In elongated siliques, such as those of *Arabidopsis*, the ovules are attached by their funiculi to the replum. Four rows of ovules are positioned alongside two vascular strands in the *Arabidopsis* silique. Within the valve, six layers of cells are organized into a well-defined pattern, with outer and inner epidermis sandwiching four internal cell layers. Immediately after fertilization, profuse cell division and expansion initiate in the valves as well as in the septum, in order to accommodate the rapid growth of the developing seeds, and elongation of the fruit is most noticeable (Müller, 1961). Anticlinal cell divisions are predominant throughout the developing valve tissue resulting in a thin and elongated valve. A number of specialized cell types differentiate post-

fertilization, such as stomatal cells in the valve epidermis (Bowman, 1994).

Many mutations affecting the gynoecium have been isolated in *Arabidopsis*. These mutations affect gynoecium specification (*agamous*: Bowman et al., 1989), patterning (*clavata*: Clark et al., 1993) and morphogenesis (*ettin*: Sessions and Zambryski, 1995; *crabsclaw*: Alvarez and Smyth, 1997; and *tousled*: Roe et al., 1997). Recently several mutants have been isolated that are capable of fruit development without fertilization processes, namely the *fie* (Ohad et al., 1996) and the *fis* (Chaudhury et al., 1997) mutants. However, few mutations affecting fruit development after fertilization have been studied (Okada et al., 1989).

Using transposon-mediated enhancer trap mutagenesis (Springer et al., 1995; Sundaresan et al., 1995), we have isolated a mutation that blocks elongation of the silique after fertilization. This restricted expansion results in a crowded silique full of seeds, and so the mutant was named *fruitfull* (*ful-1*). Both cell expansion and differentiation are affected in the carpel valves, creating a zigzag pattern in the replum epidermis. Molecular characterization of the mutant revealed that the enhancer trap had caused a loss-of-function mutation in the previously reported *AGL8* gene (Mandel and Yanofsky, 1995). Here we show that the *AGL8* MADS-box gene is required for the normal pattern of cell division, expansion and differentiation during morphogenesis of the *Arabidopsis* silique.

MATERIALS AND METHODS

Genetic screen

Parental lines (all in Landsberg *erecta* (Ler) background) carrying an Ac transposase gene were crossed to lines carrying the Ds-GUS enhancer trap DsE and gene trap DsG (Sundaresan et al., 1995; Springer et al., 1995), to generate F_1 plants in which the Ds element transposed to new sites. The F_2 seeds were screened for germinal transposants in MS medium (Carolina Biological Inc.) containing kanamycin (50 μg/ml) and α-naphthalene-acetamide (3.5 μM) (described in detail by Martienssen and Springer, 1997). The *ful-1* mutant was identified in the enhancer trap line ET3214. Subsequently *ful-1* was backcrossed to the wild-type Ler, and the following F_2 population was checked for segregation. Normal and *ful-1* plants segregated in a 3:1 ratio among 400 F_2 progeny of the heterozygous backcrossed plants, indicating that the mutation was recessive. In all subsequent experiments the backcrossed progeny was used.

Scanning electron microscopy

Tissues from wild-type Ler and *ful-1* plants were fixed overnight at 4°C in FAA (50% ethanol, 5% glacial acetic acid, 3.7% formaldehyde), dehydrated through an ethanol series and critical-point dried. Specimens were sputter-coated with gold and palladium (4:1) and examined in a Cambridge S360 scanning electron microscope using an accelerating voltage of 10 kV.

Thin sectioning

Young cauline leaves and floral organs of different stages from both *ful-1* and Ler plants were fixed in 2% glutaradehyde, and dehydrated through an ethanol series. The tissue was infiltrated with propylene oxide and embedded in Spurr's resin. 1 μm sections were cut and stained in Toluidine Blue. In addition, some of the floral organs were first stained for GUS expression before embedding and sectioning. The term 'cross section' refers to those sections that are perpendicular to the apical-basal axis of the fruit.

Characterization of the Ds insertion site

The genomic DNA flanking the *ful-1*::*Ds* insertion was amplified using Tail-PCR (thermal asymmetric interlaced PCR). The procedure was followed exactly as described in Liu et al. (1995) and Tsugeki et al. (1996). The PCR products were purified using Qiagen columns (Cat. 81041) and sequenced on an ABI automatic DNA sequencer. After obtaining sequence information of the flanking DNA at the Ds 5′ site, the insertion site was analyzed by sequencing using primers AGL8-1 (5′ ccacaaatatcatcgtc 3′), AGL8-2 (5′ cagatagatatgatcgatgtg 3′) and the Ds primers (Tsugeki et al., 1996). A total of 570 base pairs of flanking DNA were sequenced. The coding sequence matched completely with the previously reported *AGL8* cDNA sequence (Mandel and Yanofsky, 1995). 135 bp of the 5′ upstream promoter region and 150 bp of the first intron also matched the *AGL8* genomic sequence (C.F. and M.F.Y., data not shown).

RNA blot analysis

Poly(A)$^+$ RNA was isolated from Ler and *ful-1* plants using the Dynabeads Oligo(dT)$_{25}$ system (Dynal). RNA-blot analysis was done by established methods (Mandel and Yanofsky, 1995). The *AGL8*/*FUL* probe contained the full-length cDNA.

Genetic complementation

ful-1 carpels were pollinated with pollen from plants homozygous for a 35S::*AGL8* transgene (M. A. Mandel and M. F. Yanofsky, unpublished results). F_1 plants were allowed to self-pollinate, and GUS assays of 90 F_2 plants were performed on cauline leaves to select plants carrying the *ful-1* allele. The expected segregation ratio of 3:1 GUS+:GUS– plants was observed. Among the 70 GUS+ plants, 43 had normal, elongated siliques, 9 showed typical *ful-1* fruit morphology and 18 displayed a range of intermediate phenotypes. These numbers fit an 8:3:1 ratio and were therefore consistent with a single rescuing T-DNA locus. Five plants with intermediate phenotypes were allowed to self and were found to be *ful-1* homozygotes. The presence of the 35S::*AGL8* transgene in these five plants was confirmed by DNA-blot analysis (data not shown). The data were consistent with the conclusion that these intermediate phenotypes represented *ful-1* homozygotes whose phenotype was partially rescued by the 35S::*AGL8* transgene.

Staining method

Seedlings grown in MS medium and floral tissues collected from soil-grown plants were analyzed for their GUS expression patterns. The GUS solution contained 50 mM sodium phosphate buffer, pH 7.0, 1 mM EDTA, 0.5 mg/ml 5-bromo-4-chloro-3-indolyl β-D glucuronic acid (X-Gluc, Rose Scientific Inc.), 0.4% Triton X-100, 100 μg/ml chloramphenicol and 5 mM each of potassium ferri/ferrocyanide. Samples were vacuum infiltrated, incubated at 37°C for 24-36 hours, and depleted of chlorophyll in 70% ethanol (Springer et al., 1995; Sundaresan et al., 1995). Some flower samples were embedded in paraffin after GUS staining. 8 μm cross sections were made and viewed under dark-field microscopy.

RESULTS

Isolation and mutant phenotype of *fruitfull* (*ful-1*)

The *ful-1* mutation was identified during a large-scale insertional mutagenesis using enhancer and gene trap Ds transposable elements (Sundaresan et al., 1995; Springer et al., 1995). This system utilizes the maize Ac/Ds transposable elements and the reporter gene GUS. Transposition events were selected and screened for reporter gene expression patterns and mutant phenotypes. *ful-1* was identified in the F_3 progeny of an enhancer trap line (see Materials and methods).

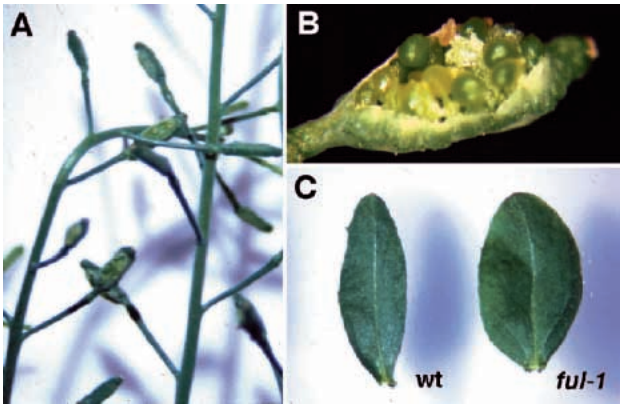

Fig. 1. Mutant phenotype of *ful-1* plants. (A) The developing siliques on a 6-week-old *ful-1* plant. (B) Example of the premature bursting of a *ful-1* silique (about 3 mm in length). (C) A comparison of the cauline leaves of *ful-1* and wild type (wt).

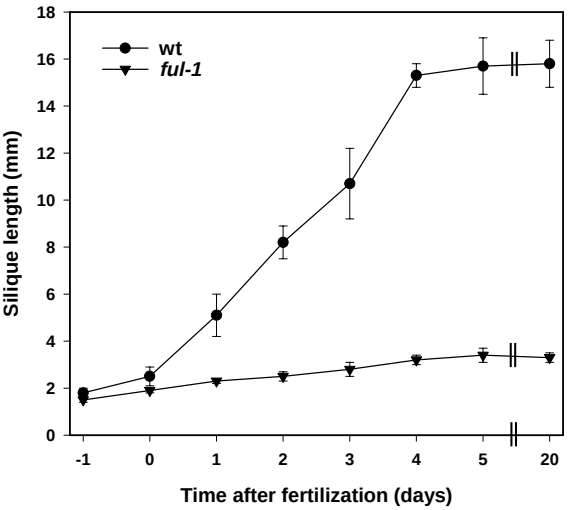

Fig. 2. Elongation rate of the silique following fertilization. Averages for 3-5 siliques are given for each time point for both wild type (wt, Ler) and mutant (*ful-1*). Standard errors are indicated by bars. Day −1, the day before fertilization, (flower stage 12, according to Smyth et al., 1990). Day 0, the day of fertilization (flower stage 13). Days 1-20, number of days after fertilization (stages 14-20).

The most drastic effect of the *ful-1* mutation was observed in the silique that failed to elongate, with seeds tightly compressed inside, as shown in Fig. 1. Backcrossing to 'wild type' Landsberg *erecta* (Ler) confirmed that *ful-1* is a recessive mutation (Materials and methods). To address whether the *ful-1* mutation was caused by the insertion of the Ds-GUS enhancer trap element (DsE), we analyzed co-segregation between the mutant phenotype and expression of the GUS reporter gene. Among a total of 200 mutant plants all were GUS positive, and one-third of the wild-type plants were GUS negative, as expected in the case of complete linkage between the GUS reporter and the mutation. Genomic DNA analysis showed that the mutant plant carried a single transposed Ds element, as expected (data not shown).

Seeds in the mutant plants looked normal except for a smaller size, and they remained arranged in four rows as in the wild type. However they were highly compacted inside the silique. Compared with Ler siblings, the number of seeds in each silique of *ful-1* mutants was reduced by 25%, and the dry weight of each *ful-1* seed was reduced by 30% (data not shown), while the length of the silique was reduced by more than 80% (Fig. 2). Frequently we observed a dramatic phenotype of the seeds bursting out of the siliques a few days after fertilization (Fig. 1B), whereas in normal siblings dehiscence occurred much later, by an orderly separation at the junction between the replum and the valves once the silique was mature. Additionally, the wild-type siliques elongated at a steady rate after pollination, and by day 5 the silique had elongated nearly eightfold compared to the size before pollination (Fig. 2). In contrast the *ful-1* siliques had expanded merely twofold by 20 days after pollination (Fig. 2). Another phenotype observed in the *ful-1* plants is that the cauline leaves are wider than those of wild-type siblings (Fig. 1C).

Differentiation of the valve epidermis

Since the *ful* mutation most dramatically affected the shape of the silique, we investigated *ful-1* silique development in detail. Floral organs in *ful-1* mutants developed normally during early stages (data not shown). At stage 12, still prior to fertilization, the surface of the gynoecium had already differentiated into stigma, style and valves. In Ler, which will be referred to as 'wild type', the carpel valve epidermal cells are small and stomatal precursors can already be identified by their shape. The demarcation between the valves becomes readily distinguishable. However, in the valve epidermis of *ful-1* mutants, cells were smaller and uniformly arranged, and there were no stomatal precursor cells. The demarcation between the valves and the replum epidermis was not visible. In contrast to the situation in the valves, the *ful-1* mutant epidermal tissues in stigma and style were indistinguishable from wild type and displayed normal cuticular thickening and interspersed stomata.

Immediately after fertilization (stage 13), the epidermal cells in the wild-type valves become irregular in shape and larger than the replum epidermal cells. Stomata begin to form in the carpel valve epidermis. The differences between the wild-type and the *ful-1* carpel epidermal cell morphology became more dramatic from this stage onwards and were particularly evident following stage 15, as revealed by scanning electron microscopy (Fig. 3). Whereas the stigmatic tissue and the style resembled those in the wild type (Fig. 3D), cell differentiation in the replum and valves in the *ful-1* mutants did not proceed

Table 1. Epidermal cell numbers in valve cross sections

	Stage*	Inner epidermis†	Outer epidermis‡
ful-1	12	54±4	150±2
Wild type	12	23±3	145±3
ful-1	16	63±4	160±6
Wild type	16	27±4	152±2

*Valves of *ful-1* and wt (Ler) at the flower stages 12 (prior to fertilization) and 16 (after fertilization) were compared.

†Inner epidermal cells of one valve excluding the replum.

‡Outer epidermis covering both valves and replums. Average number ± s.d. from three thin sections each of two silique samples are given.

normally (Fig. 3E). The epidermal cells covering the valves failed to expand and stomatal cells failed to develop (Fig. 3F), while in the wild type, functional stomata were present in high numbers (Fig. 3C). Interestingly, in the *ful-1* mutants the epidermal cells of the replum continued to expand but not in the direction of the silique main axis; instead they adopted irregular and more rounded shapes (Fig. 3K). The differences between wild-type and *ful-1* siliques became more evident as fruit development proceeded. Whereas the wild-type valve cells continued to grow rapidly, *ful-1* valve cells apparently stopped growing altogether (Fig. 3K and below). Outer epidermal cells of the replum continued to expand and grew to approximately the same length as in the wild type, but instead of forming parallel rows of cells, they adopted a wavy zigzag arrangement, almost perpendicular to the main axis of the fruit (Fig. 3K). This mediated a modest lateral growth of the silique and accommodation of the growing seeds.

Cell division and expansion

We further analyzed the cellular organization of the carpel valves in transverse sections. It was noticed that there were more than twice as many cells in the inner valve epidermis in the mutant as in normal siblings (Fig. 4A,C and Table 1), indicating a failure in cell division control. This alteration had occurred even before anthesis, such that twice as many small cells were observed in the mutant inner epidermal layer (Fig. 4A and Table 1). The outer epidermis of the *ful-1* valve had a similar number of cells to the wild type (Fig. 4A,C and Table 1), but cell expansion did not occur in the mutant after fertilization (Fig. 4C). The replum epidermis consists of 10 files of narrow rectangular cells in the wild type, and a similar number of cell files was observed in the *ful-1* replum (data not shown). After fertilization, cell size increased rapidly in normal valves, in both the apical-basal and transverse dimensions. However, cell expansion was not observed in either axis in the *ful-1* mutant valve (Figs 3K, 4C). Interestingly, the septum of the *ful-1* mutant appeared to follow a separate pathway, and was seemingly capable of responding to signals for growth and differentiation after fertilization. The septum cells divided and expanded, progressively filling up all the space in the ovary together with the developing seeds (Fig. 4C and data not shown). Medial and lateral vascular bundles throughout the valves were reduced in size and poorly differentiated (Fig. 4E), while the vascular bundles in the replum between the two valves appeared normal (Fig. 4A,C). However the exact nature of the defect in the vascular tissue was unclear. The poor differentiation of the vascular bundles did not allow us to distinguish whether specific vascular cell types were missing.

Cauline leaf shape and midvein differentiation

In addition to the defective silique, *ful-1* plants also had more rounded cauline leaves, and this phenotype cosegregated with

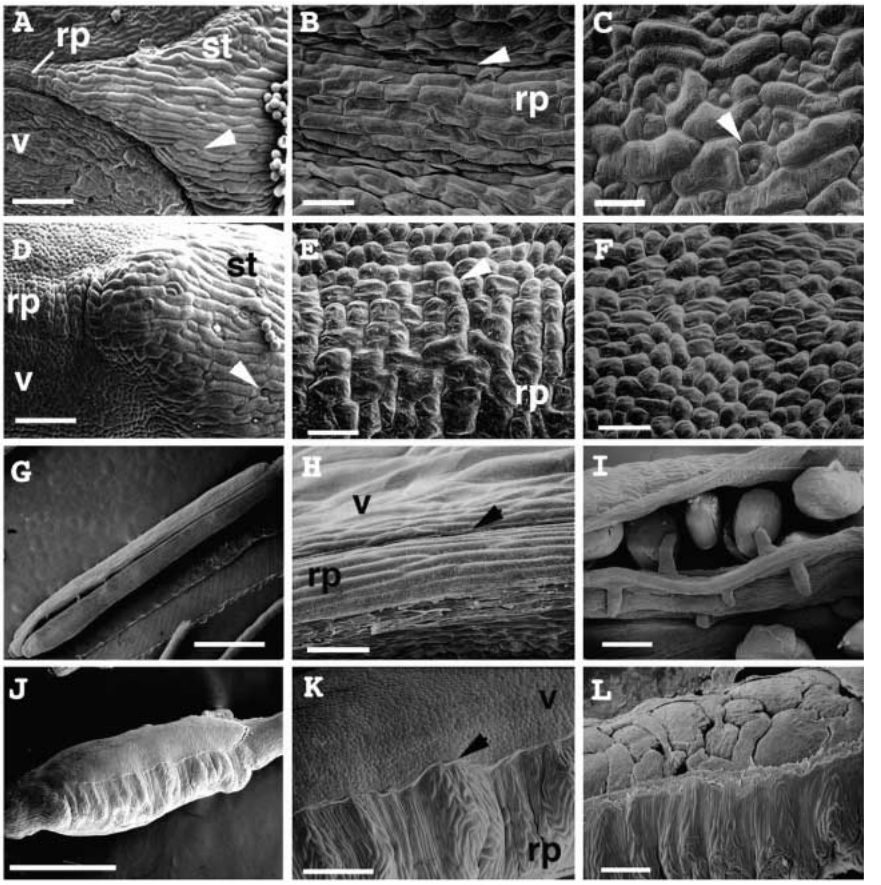

Fig. 3. Scanning electron micrograph of wild-type (A-C, G-I) and *ful-1* (D-F, J-L) fruits. (A) Stage 15 wild-type gynoecium at the junction between style (st), replum (rp) and valve (v) regions. Note stomata in style epidermis (arrowhead). (B) Close-up of the medial side of the carpel in A. The demarcations between the valves and replum are clearly visible (arrowhead). (C) Close-up of the lateral side of the carpel in A. Note stomatal cells (arrowhead). (D) Stage 15 *ful-1* gynoecium in a similar view to that in A. The style cells appear wild type and stomata are present (arrowhead). Note poorly developed valve epidermis. (E) Close-up of the medial side of the carpel in D. Note the irregularly shaped replum epidermal cells. The demarcation between this region and the valve (arrowhead) is not well defined. (F) Close-up of the lateral side of the carpel in D. Cells in the valve epidermis are small, unorganized and lack stomatal cells. (G) Stage 18 wild-type silique (9 mm in length). Dehiscence of the valves has begun. (H) Close-up of the silique in G. Cells in the valve (v) and in the replum (rp) have greatly elongated and are arranged along the silique main axis. Note the zone of dehiscence (arrowhead). (I) Medial view of a dehiscent wild-type silique. The replum is a flat layer and seeds are arranged in rows. (J) A *ful-1* silique (stage 18, about 3 mm in length). The replum region is broad and the cells adopt a wavy, zigzag pattern. (K) Close-up of the silique in J. Dehiscence fails to take place (arrowhead). The replum cells are similar to wild-type, although they are mainly arranged perpendicular to the main axis of the fruit. The epidermal cells of the valve remain small and undifferentiated. (L) The valve has been removed from a *ful-1* silique to show the dense packing of seeds inside the fruit. Bars, 100 μm (A,D); 20 μm (B,C,E,F); 2 mm (G); 50 μm (H); 200 μm (I,K,L); 1 mm (J).

the small silique phenotype, shown in Fig. 5. Cross sectioning of the leaf tissue showed that the cells in the *ful-1* cauline leaf appeared loosely packed and disorganized (Fig. 5D). The mutants had only 4-5 internal cell layers, instead of 5-6 cell layers in the wild type (Fig. 5C, D). Also, the midvein in the cauline leaves of *ful-1* was found to be less developed in that there were fewer vascular cells in cross section of the developing *ful-1* cauline leaves than in normal leaves (Fig. 5D).

Analysis of the *ful::Ds* insertion site and genetic complementation

Sequencing revealed that the Ds element had inserted into the untranslated leader of the *AGL8* gene (Fig. 6A), which had been previously cloned and sequenced by virtue of its homology to MADS-box transcription factors (Mandel and Yanofsky, 1995). Using a probe from the coding region, *AGL8* mRNA was not detectable in flowers from homozygous *ful-1* mutant plants on RNA gel blots (Fig. 6B). Thus, the DsE insertion had abolished *AGL8* gene expression, suggesting that *ful-1* leads to a complete loss of *AGL8* function. DNA sequence analysis further revealed that the DsE insertion had resulted in alterations of the nucleotides at both ends of the DsE element (Fig. 6A). For this reason, the DsE element could not be remobilized.

To further confirm that the observed phenotypes were caused by the DsE insertion into *AGL8*, we tested whether the *AGL8* cDNA was able to rescue the *ful-1* mutant phenotype. Transgenic plants carrying the *AGL8* cDNA under the control of the strong CaMV35S promoter, were crossed with *ful-1* mutants (see Materials and methods). Carpels of homozygous F_2 *ful-1* plants that contained the 35S::*AGL8* transgene displayed a significant elongation of the valves, although some aspects of the *ful-1* silique were still observed, such as a wavy arrangement of the replum epidermal cells. Typical examples of these phenotypes are shown in Fig. 7. A closer inspection of these siliques showed the initiation of stomata differentiation in the valve epidermis (data not shown), which was never observed in *ful-1* mutant plants. These results demonstrate that the 35S::*AGL8* transgene can partially rescue the *ful-1* mutant phenotype. The absence of a full rescue may reflect a difference in the level or the timing of *AGL8* and 35S promoters within the carpel valves. Finally, in the wild-type background 35S::*AGL8* expression resulted in very subtle changes in their appearance, including occasional early flowering (data not shown).

The gene expression pattern of *FUL*

Because the enhancer trap had landed in the 5′ untranslated region of the *AGL8*/*FUL* gene, it was possible to analyze the endogenous gene expression pattern by monitoring the GUS reporter gene expression. We compared expression of the *AGL8* gene in Ler (Mandel and Yanofsky, 1995) with expression of the GUS reporter gene driven by the enhancer trap element in homozygous *ful-1* mutant and in heterozygous plants. It has been previously shown by RNA blots that *AGL8* is expressed in the stem and cauline leaves, and strongly expressed in the flowers, but not in the roots (Mandel and Yanofsky, 1995). We found that the GUS expression pattern faithfully mimics the *AGL8* expression revealed previously by RNA gel blot and in situ hybridization (Mandel and Yanofsky, 1995). The most intense staining was seen in the inflorescence meristem (Fig. 8B,C) and in the carpel valves (Fig. 8B,D-G). GUS was localized to the vascular tissue of the stem and cauline leaves (Figs 8B, 5A,B), but not in the roots (Fig. 8A), or the vegetative shoot apical meristem or in the hypocotyl (data not shown).

In addition to the results matching those of prior RNA analysis, we showed that after fertilization, GUS accumulation

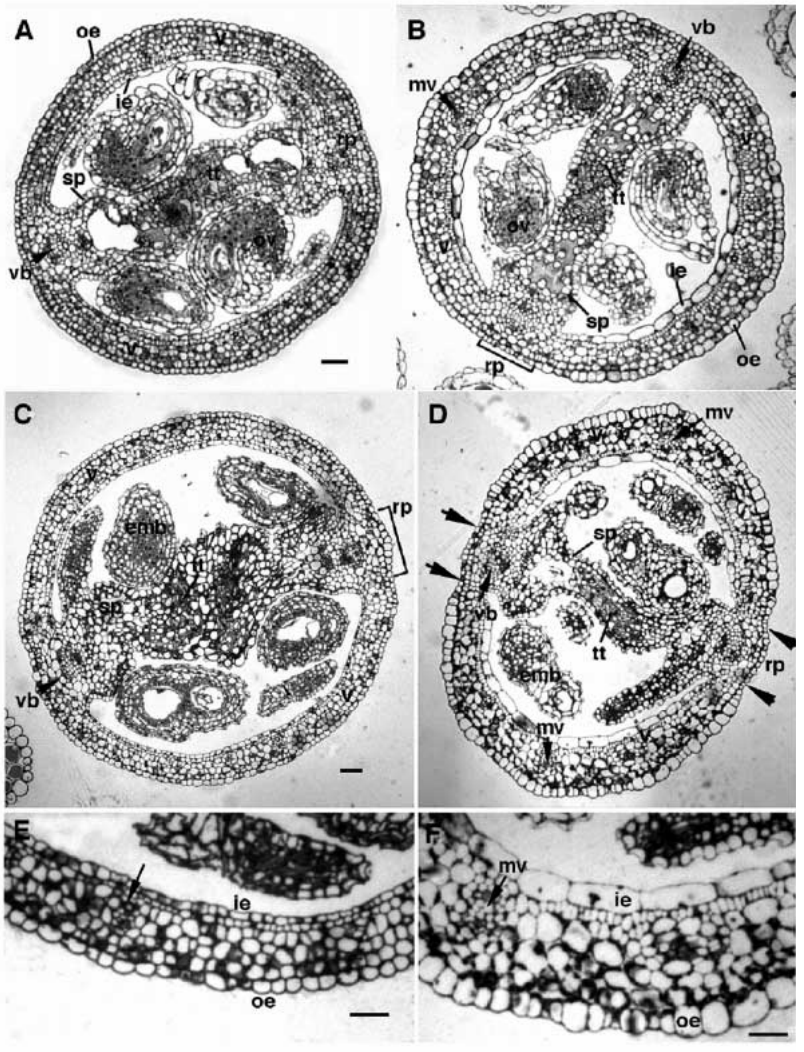

Fig. 4. Medial transverse cross sections of fruits before (stage 12) and after fertilization (stage 16). (A) *ful-1*, stage 12. (B) Ler, stage 12. (C) *ful-1*, stage 16. (D) Ler, stage 16. (E) and (F) are enlargments of parts of C and D, respectively. The dehiscence zones are indicated by arrowheads. The arrow in E points to a poorly differentiated vascular bundle in the *ful-1* valve. ie, inner epidermal cells; oe, outer epidermal cells; ov, ovule; rp, replum; sp, septum; tt, transmitting tract; vb, replum vascular bundle; v, valve; mv, valve medial vascular bundle; emb, developing embryo. Bars, 50 μm.

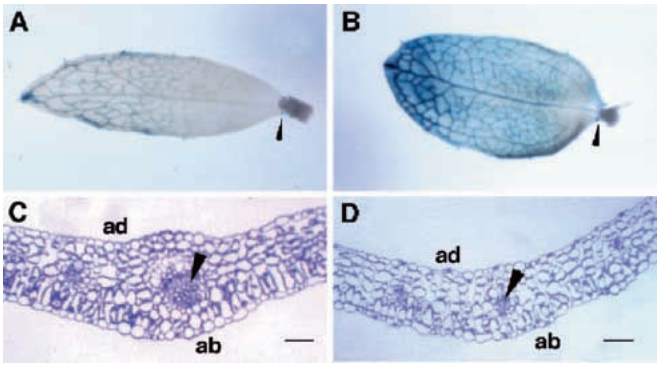

Fig. 5. Cauline leaf phenotype and GUS localization pattern. (A) Heterozygous (*ful-1*/+) and (B) homozygous (*ful-1* mutant) cauline leaves, at about 1 cm in length. Both were stained for GUS expression. Arrowheads point to the stipules at the base of the leaf with GUS staining. (C, D) Medial transverse thin sections of wild-type and *ful-1* young cauline leaves respectively. Arrowheads point to the mid vascular bundles. ad, adaxial side of the leaf; ab, abaxial side of the leaf. Bars, 50 μm.

in the carpel appeared to become confined to the apical and basal parts of the valves, but GUS activity was undetectable in mature brown fruit (after stage 17, data not shown). In transverse sections of the flower, GUS staining was confined to all cell layers of the valves, but was absent from the replum, the septum and the transmitting tract (Fig. 8E-G). Strong reporter gene expression was also observed in cauline leaves (Fig. 5A,B). Our analysis further revealed that GUS expression in homozygous mutant plants was first detected in the most distal vascular tissue of the cotyledons and the first pair of emerging leaves (Fig. 8A). GUS expression was also evident in the style and the vascular tissue of the sepals and inflorescence stems (Fig. 8B,D). GUS was also found in stipules (Fig. 5A,B) and in the nectaries of the flower (data not shown). The pattern of GUS accumulation in the *ful-1*/+ heterozygous siblings was weaker but qualitatively similar to that found in *ful-1* homozygous mutant plants (data not shown).

DISCUSSION

MADS-box proteins are a class of DNA binding regulatory proteins that are evolutionarily conserved among eukaryotes (Theißen and Saedler, 1995; Purugganan et al., 1995). A large number of key regulators involved in flower development are MADS-box genes that specify floral meristem identity or floral organ identity, including *AGAMOUS*, *APETALA1*, *APETALA3*, *CAULIFLOWER* and *PISTILLATA* in *Arabidopsis* (Yanofsky et al., 1990; Mandel et al., 1992; Jack et al., 1992; Kempin et al., 1995; Goto and Meyerowitz, 1994), and their orthologs in *Antirrhinum* and other species (Schwarz-Sommer et al., 1990). These proteins share a conserved DNA-binding MADS-domain at their amino termini. In addition to the five MADS-box genes defined by mutations, at least 20 additional members of the family have been isolated in *Arabidopsis*. The expression patterns of these genes suggest that they play diverse roles in plant development (Rounsley et al., 1995). We have identified a loss of function allele for the *AGL8* gene, and determined that it has an important role in fruit morphogenesis.

FUL/AGL8 is widely expressed during plant development

Analyses of the *FUL*::*GUS* reporter fusion revealed that the *FUL* gene is weakly expressed in rosette leaves during vegetative development and is subsequently strongly upregulated in the shoot apex upon the transition to flowering. Throughout inflorescence development GUS activity remains strong in the floral shoot apex and in the vascular tissues of the cauline leaves. High levels of GUS activity are also apparent in the central dome of young flower primordia, beginning at around stage 3. Subsequently, GUS activity is observed in developing carpels, where strong expression becomes localized to the valves of the carpels and resulting fruits. In addition, GUS expression was detected in the style, as well as in nectaries and stipules. The observed *FUL*::*GUS* expression pattern is in close agreement with previous RNA gel blot and *in situ* hybridization analyses (Mandel and Yanofsky, 1995). Furthermore, we have localized *FUL* expression in vascular tissues during vegetative development, which had not been analyzed previously. In addition it is clear from our cross sections that *FUL*/*AGL8* is not expressed in the replum and other organs within the gynoecium (Fig. 8E-G).

In the gynoecium, *FUL* is expressed at a high level in the valves, and it is the carpel valves that show the most striking mutant phenotype in homozygous *ful-1* mutant plants. Although *FUL* is expressed at high levels in inflorescence apices, scanning electron microscopy failed to uncover any alterations in inflorescence or floral meristems in the mutant plants (data not shown). Thus, our data suggest that *FUL* is only required for normal fruit and cauline leaf development despite its widespread expression pattern. One possibility is that, if *FUL* plays a role in vegetative and inflorescence development, it may be redundant with one of the other MADS-box genes whose expression pattern is known to overlap with that of *FUL*/*AGL8* (Rounsley et al., 1995).

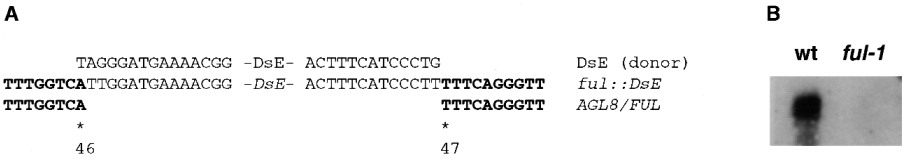

Fig. 6. Diagram of the DsE enhancer trap position (A) and RNA blot analysis of *FUL* expression (B). (A) The DsE element is inserted into the 5′ untranslated region of the *FUL* gene, at the position between 46 and 47 of the transcribed nucleotide sequence of the *AGL8* gene (Mandel and Yanofsky, 1995). The ends of the DsE element are drawn, and the bold letters denote the sequence of the *AGL8* gene. (B) 2 μg of poly(A)$^+$ RNA from aerial tissues of 20-day-old wild-type Ler (wt) and *ful-1* plants were loaded, blotted and probed with an *AGL8*/*FUL* cDNA probe.

Fig. 7. Genetic complementation. Wild-type Ler (top), *ful-1*; 35S::*AGL8* (middle) and *ful-1* (bottom) mature siliques at stage 17 (about 3 days after fertilization). Bar, 1 mm.

FUL inhibits cell division and promotes cell expansion in the valve

The Ler silique elongates by a factor of 8 after fertilization, while the *ful-1* silique has essentially lost the ability to extend, resulting in a short silique with overcrowded seeds. The primary defect responsible for this phenotype is a failure of cell differentiation and cell growth in the carpel valves. After fertilization, all cell types in mutant valves ceased to expand, the vascular tissue developed poorly and stomatal cells did not form in the valve epidermis. Strikingly, only the valves were affected and not the replum, which continued to grow and was forced into a zigzag pattern as a result. Although the most obvious defects occurred after fertilization, cell shapes were already distorted in mutant valves even before pollination. This was most obvious in the inner epidermis of the valves, which consisted of numerous tiny cells in the mutant as compared to fewer enlarged cells in the wild type. Thus, *FUL* has multiple functions during cell differentiation, being able to promote cell expansion as well as inhibit cell division in specific cell types.

FUL and the role of cell-cell interactions in fruit development

Fruit development in *Arabidopsis* is induced by the successful fertilization of the female gametophyte. Although *Arabidopsis* plants are able to undergo limited seed development without fertilization in certain mutant backgrounds (Ohad et al., 1996; Chaudhury et al., 1997), under normal conditions signals must communicate between the fertilized gametophyte and the valve tissues (Hensel et al., 1994), possibly via the replum. The *ful-1* carpel lacks most aspects of the complex response of the wild type to the fertilization signal. This, together with the fact that *FUL* is expressed in all cell layers of the carpel valves before and after fertilization, suggests a role for *FUL* in establishing competence of these cells to respond to a signal provided by the fertilization process.

The nature of this signal is not known but could involve plant hormones. It is known from work in other species, such as tomato, that ethylene, auxins, cytokinin and gibberellins follow elaborate changes during early fruit development (reviewed by Gillaspy et al., 1993). However, spraying *ful-1* mutant flowers

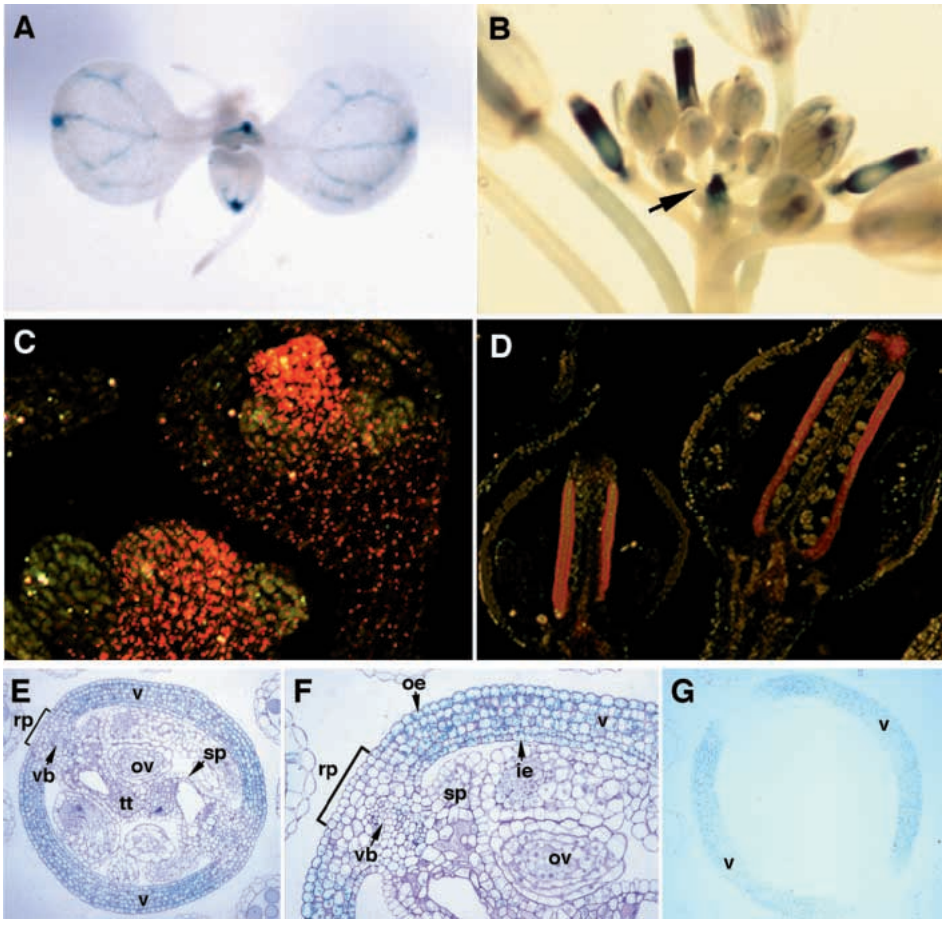

Fig. 8. GUS histochemical localization patterns in *ful-1* plants. (A) An 11-day-old *ful-1* seedling. (B) An inflorescence. The blue color represents the GUS localization. In addition, a few flower buds were dissected away to reveal GUS accumulation in the *ful-1* floral meristem, indicated by the arrow. (C, D). Dark-field microscopy showing the GUS localization in the inflorescence apical meristem (left in C), stage 6 gynoecium (right in C), and stage 10-12 carpel valves (D). (E-G) GUS localization in thin cross sections (1 μm) of *ful-1* mutant fruits. ie, inner epidermal cells; oe, outer epidermal cells; rp, replum; ov, ovule; sp, septum; tt, transmitting tract; v, valve; vb, replum vascular bundle. (E) Medial transverse cross section of a GUS-stained carpel was sectioned and counter-stained in Toluidine Blue. (F) Enlargement of the upper left area of A. GUS activity is reflected by the blue speckles that accumulate in all cell layers of the valve. (G) A similar thin section to A, but without Toluidine Blue staining.

with gibberellic acid, cytokinin or auxin did not rescue the *ful-1* phenotype (data not shown), suggesting that the defect in *ful-1* mutants is not simply a deficiency in these hormone levels, although it might be a lack of sensitivity to the hormones.

In wild-type carpels, *FUL* gene expression could not be detected in the replum or in the septum. Further, the lack of *FUL* expression in mutant valves often causes a failure of replum tissues to separate normally during dehiscence. This suggests that *FUL* may regulate the transcription of a cell-cell interaction factor required for dehiscence in the neighboring replum. Alternatively, cell-to-cell movement of the FUL protein itself may be directly responsible for cooperation between growth of the valves and of the replum. Cell-cell trafficking of MADS-box transcription factors has been implicated in coordination between cell layers in floral meristems (reviewed by Hake and Char, 1997), and failure to coordinate growth may result in subtle alterations in replum architecture leading to a failure of dehiscence. Dehiscence (pod shattering) in *Brassica* species is agronomically important because of seed loss at harvest (Petersen et al., 1996), suggesting that *FUL* may be of some value in manipulating this trait.

Vascular development in the cauline leaf

Outside the floral organs, *FUL* is expressed widely in the vasculature of vegetative tissues, although at a lower level. Detection of *AGL8*/*FUL* mRNA by RNA gel blot in vegetative tissues (Mandel and Yanofsky, 1995) argues against an artifact of the promoter GUS fusion system. Our GUS fusion data extend previous findings because we have localized *FUL* gene expression to the vascular tissues. A mutant phenotype was observed only in *ful-1* cauline leaves, which were broader and more rounded than in wild type, with the internal cell layers more loosely packed. It has recently been shown that the cell number in leaves of the *angustifolia* (*an*) and *rotundifolia* (*rot*) leaf shape mutants is the same as in wild type, indicating that leaf shape in these mutants is regulated by expansion polarity genes (Tsuge et al., 1996). The overall number of cells in the *ful-1* cauline leaf may also be the same as in wild type, so that the fewer layers of more loosely packed cells in the mutant may contribute to the broader shape of the leaf.

Alternatively, *FUL* may control cauline leaf shape through an effect on midvein differentiation. The diameter of the midvein is considerably reduced in *ful-1* cauline leaves, and the vein and surrounding tissues appear to be less developed than the tissue of wild type (Fig. 5C,D). *midribless (mbl)* mutants in *Panicum* (Fladung et al., 1991) and in pearl millet (Rao et al., 1988) have poorly differentiated midveins, and have broader leaves than normal. Interestingly, these mutants also have reduced carpels. Similarly, *ovaryless* (*ovl*) mutants of barley lack both ovaries in the flower and fully differentiated midveins in the vegetative leaves (Seip and Tsuchiya, 1979). Although carpel morphology is much more severely affected in these grasses than in *ful-1 Arabidopsis*, the phenotypes may be related. For example, *midribless* and *ovaryless* mutants in grasses result in partially indeterminate floral meristems (Fladung et al., 1991). The *AGL8*/*FUL* gene is strongly expressed in inflorescence meristems and may contribute to determinancy along with related MADS-box genes *APETALA1* and *CAULIFLOWER* in *Arabidopsis* (C. F., Q. G., R. M. and M. F. Y., unpublished results).

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