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SEUSS, a member of a novel family of plant regulatory proteins, represses floral homeotic gene expression with *LEUNIG*

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SUMMARY

Proper regulation of homeotic gene expression is critical for pattern formation during both animal and plant development. A negative regulatory mechanism ensures that the floral homeotic gene *AGAMOUS* is only expressed in the center of an *Arabidopsis* floral meristem to specify stamen and carpel identity and to repress further proliferation of the floral meristem. We report the genetic identification and characterization of a novel gene, *SEUSS*, that is required in the negative regulation of *AGAMOUS*. Mutations in *SEUSS* cause ectopic and precocious expression of *AGAMOUS* mRNA, leading to partial homeotic transformation of floral organs in the outer two whorls. The effects of *seuss* mutations are most striking when combined with mutations in *LEUNIG*, a previously identified repressor of *AGAMOUS*. More complete homeotic transformation of floral organs and a greater extent of organ loss in all floral whorls were observed in the *seuss leunig* double mutants. By in situ hybridization and double and triple mutant analyses, we showed that this enhanced defect was caused by an enhanced ectopic and precocious expression of *AGAMOUS*. Using a map-based approach, we isolated the *SEUSS* gene and showed that it encodes a novel protein with at least two glutamine-rich domains and a highly conserved domain that shares sequence identity with the dimerization domain of the LIM-domain-binding transcription co-regulators in animals. Based on these molecular and genetic analyses, we propose that *SEUSS* encodes a regulator of *AGAMOUS* and functions together with *LEUNIG*.

Key words: *LEUNIG*, *AGAMOUS*, *APETALA2*, Co-repressor, Flower development, LIM domain binding protein, *Arabidopsis thaliana*

INTRODUCTION

Pattern formation and organ morphogenesis represents one of the most challenging and important questions in developmental biology. The ABC model of flower development elegantly explains how the identity of the four types of floral organs is specificied (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994). An *Arabidopsis* flower consists of four types of floral organs arranged in four concentric whorls. Four sepals develop in the outermost whorl (whorl 1), four petals develop in whorl 2, six stamens arise in whorl 3, and two carpels fuse with each other to form a gynoecium in whorl 4. The A, B and C classes of floral homeotic genes (also termed 'organ identity genes') function in specific and adjacent whorls to specify floral organ type. The mRNAs of most A, B and C genes are expressed only in the floral whorls where their activities are required (Drews et al., 1991; Jack et al., 1992; Mandel et al., 1992; Goto and Meyerowitz, 1994). Hence, proper transcriptional regulation of the A, B and C genes is crucial to the proper specification of floral organ type. Revealing the regulatory mechanism underlying floral homeotic gene expression, thus, poses the next major challenge in the field.

The regulation of the C class floral homeotic gene *AGAMOUS* (*AG*) is the most extensively studied. In *ag* loss-of-function mutants, stamens are replaced by petals, and carpels are replaced by a new flower. The generation of flowers within a flower reveals a second role of *AG* in maintaining the determinancy of the floral meristem (Bowman et al., 1989; Bowman et al., 1991; Mizukami and Ma, 1997). *AG* encodes a DNA-binding transcription factor of the MADS box family (Yanofsky et al., 1990; Huang et al., 1993). In wild-type, *AG* mRNA is only turned on at stage 3, when the sepal primordia just arise from the floral meristem (Smyth et al., 1990), and is only detected in the inner two whorls of a flower (Yanofsky et al., 1990; Drews et al., 1991). Its precise regulation requires the activity of both positive regulators *LEAFY* (*LFY*), *APETALA1* (*AP1*) and *WUSCHEL* (*WUS*) and negative regulators such as *LEUNIG* (*LUG*) and *APETALA2* (*AP2*) (Bowman et al., 1991; Drews et al., 1991; Weigel et al., 1992; Weigel and Meyerowitz, 1993; Liu and Meyerowitz, 1995; Lenhard et al., 2001; Lohmann et al., 2001). *LFY* and *WUS* were shown to bind directly to the second intron of *AG* and activate *AG* expression (Busch et al., 1999; Lohmann et al., 2001). However, the mechanism of negative regulation is less well understood.

Table 1. CAPS and dCAPS markers used in this study

Marker name	Oligonucleotide sequence	Restriction enzyme	Restriction fragments for L*er* (bp)	Restriction fragments for Col (bp)
F2J6i (CAPS)	Forward: 5′CACTTGGGATGACTGCAAGA3′ Reverse: 5′TGATCTCCTTCTTGTGCCTATCCT3′	*Alu*I	239 117 33	272 117
F28H19i (CAPS)	Forward: 5′TTCGTCGAGAAGAAGTGTTTGT3′ Reverse: 5′AACCTCCATTGAGCCAAAGA3′	*Mse*I	395	320 75
F9L16Sp6 (CAPS)	Forward: 5′CTGATGGTGATGACCTTGGA3′ Reverse: 5′GGCTGCAAAAGCTGTCATTT3′	*Dra*I	784 382	692 382
F27F5ii (CAPS)	Forward: 5′TTGGAACTGCATGAACACAAA3′ Reverse: 5′TCCTTGGTCCAACCAAATTC3′	*Mse*I	405 200 143 75	553 143 75 54
Marker name	Oligonucleotide sequence	Restriction enzyme	Restriction fragments for wild type	Restriction fragments for mutant allele
seu-1 (dCAPS)	Forward: 5′ACAACAGATTCTGCTCTTCCGGAGGTA3′ Reverse: 5′TTACCTGCAAACACCGAACA3′	*Rsa*I	214	237
seu-2 (dCAPS)	Forward: 5′TCAGCCTATGGCTTTTCTTCA3′ Reverse: 5′CCATACATAGAGACGCACCACCT3′	*Mln*I	108	132
lug-1 (CAPS)	Forward: 5′CACTGGCTTATTTGGGTTTAGG3′ Reverse: 5′GAAAGCAGCAGTCAATAGAAAC3′	*Bsl*I	207 132	339
ap2-1 (dCAPS)	Forward:5′GAATCTTATAAAATAGGTATGTTTATCTG3′ Reverse: 5′GCGTCTTTGCCGTTACATTT3′	*Dde*I	197	172

LUG and *AP2* are two main negative regulators of *AG*. In *lug* and *ap2* mutants, *AG* mRNA is ectopically expressed in the outer two whorls of a flower, resulting in the homeotic transformation from sepals toward carpels, petals toward stamens, and a reduction in the number of floral organs in whorls 2 and 3 (Bowman et al., 1991; Drews et al., 1991; Liu and Meyerowitz, 1995). In addition, precocious expression of *AG* has been reported in *ap2* and *lug* mutants (Drews et al., 1991; Liu and Meyerowitz, 1995). Using *GUS* reporter genes fused to the *cis*-regulatory sequences of *AG*, the expression of the *AG::GUS* reporter genes was examined in *lug* and *ap2* mutants. This analysis indicated that *LUG* and *AP2* regulate *AG* expression at the level of transcription through the second intron of *AG* (Sieburth and Meyerowitz, 1997; Bomblies et al., 1999; Deyholos and Sieburth, 2000). *AP2* encodes a protein with two 68 amino acid repeats, dubbed the AP2 domain, that is predicted to perform functions of protein-protein dimerization and DNA binding (Jofuku et al., 1994; Riechmann and Meyerowitz, 1998; Nole-Wilson and Krizek, 2000). *LUG* encodes a glutamine-rich protein with seven WD repeats and was predicted to act as a transcriptional co-repressor (Conner and Liu, 2000). *lug* and *ap2* mutations enhance each others' effects with respect to floral organ identity transformation and floral organ loss (Liu and Meyerowitz, 1995). It has been proposed that *LUG*, the putative co-repressor, may be recruited by *AP2*, a DNA-binding transcription factor, to repress *AG* expression in the outer two whorls of a flower. Nevertheless, a lack of evidence indicating a direct physical interaction between *LUG* and *AP2* suggests that *AP2* and *LUG* might need other co-regulators to bridge their interactions. Alternatively, *AP2* and *LUG* may regulate each other indirectly via other transcription factors. In either scenario, the identification of additional regulators of *AG* is necessary.

We report the isolation and analyses of a new gene *SEUSS* (*SEU*). We showed that *SEU* functions as a repressor of *AG* and is a candidate co-regulator of *LUG*. *seu* mutants exhibit a phenotype similar to *lug*. Additionally, *seu* genetically enhances both *ap2* and *lug* in floral organ identity transformation and organ loss, and this effect of *seu* is mediated by an enhanced ectopic *AG* expression. *SEU* encodes a Q-rich protein with a putative dimerization domain, which was found in the LIM-domain-binding (Ldb) family of transcription co-regulators (Jurata and Gill, 1997). We propose that *SEU* may be required to mediate the interaction between *LUG* and *AP2* so as to repress *AG* expression in the outer two whorls of a flower. The detection of other genes with sequence similarity to *SEU* in a wide variety of plant species points to a crucial role of *SEU* and *SEUSS-LIKE* genes in higher plant development.

MATERIALS AND METHODS

Genetic analysis

Both *seu-1* and *seu-2* were induced by EMS in the Landsberg *erecta* (L*er*) ecotype. *seu-1* was isolated in a screen for genetic enhancers of *unusual floral organs* (Levin et al., 1998). *seu-2* was isolated in a screen for enhancers of *crabs claw* (Eshed et al., 1999). Both *seu-1* and *seu-2* were back-crossed to wild type (L*er*) three times before further genetic and phenotypic analyses.

LUG, *AP2* and *AG* all reside on chromosome 4 in the following order: *AP2* (16 cM) *LUG* (10 cM) *AG*. *SEU* resides on chromosome 1 (see below). To generate *seu lug* and *seu ap2* double mutants, *seu-1* homozygous flowers were fertilized with pollen from *lug-1*, *lug-2*, *lug*-3, *lug-8*, *ap2-1* or *ap2-2*, respectively. Seeds were collected from F_2 *seu* individuals (selected based on plant height and floral defects) and the respective double mutants were observed in 1/4 of the F_3 plants. To generate the *seu-1 lug-1 ag-1* triple mutants, *ag-1*/+ plants were fertilized with *lug-1* pollen. F_2 *lug-1* plants were crossed to *seu-1/seu-1*; *ap2-1/ap2-1* double mutants to generate *seu-1/+; + lug-1 ag-1/ap2-1 + +*. Approximately 10% of the F_2 families from this second cross segregated *lug-1 ag-1* double mutants in 3/16 of the progeny and the *seu-1 lug-1 ag-1* triple mutant in 1/16 of the progeny. The genotype of the *ag-1 lug-1 seu-1* triple mutant was confirmed by Co-Dominant Amplified Polymorphic Sequences (CAPS)- or dCAPS-based markers (Konieczny and Ausubel, 1993; Neff et al., 1998) developed for *lug-1* and *seu-1* (Table 1). To generate *seu-1/seu-1*; *lug-1 +/+ ap2-1* plants, *lug-1* individuals were crossed to *seu-1 ap2-1* double mutants. F_2 families segregated *seu-1/seu-1*; *lug-1 +/+ ap2-1* individuals with an enhanced *seu* phenotype in 1/8 of the progeny. The genotypes of these plants were confirmed by CAPS or dCAPS markers developed for *seu-1*, *ap2-1* and *lug-1* (Table 1).

Microscopic analyses

Scanning electron microscopy (SEM) samples were collected, fixed

and coated as previously described (Bowman et al., 1989; Bowman et al., 1991). Samples were examined on an AMRAY 1000A scanning electron microscope. Images were captured on a Polaroid camera. Whole-mount floral photographs were taken through a Zeiss Stemi SV6 dissecting microscope. Slides of longitudinal sections of inflorescences from in situ hybridization experiments were examined and photographed under a Zeiss AxioPlan2 microscope with Nomarski optics.

Positional cloning of *SEU*

A mapping population was generated by crossing *seu-1/seu-1* of the L*er* ecotype with wild-type Columbia (Col) ecotype. Genomic DNA was isolated from 305 of the F_2 *seu-1* plants and assayed by PCR-based markers. Linkage of *SEU* to the chromosome I markers GAPB and F16N3 led to the physical map (Fig. 5A). Finer mapping subsequently placed *SEU* 0.16 cM north of the marker F9L16Sp6 (Fig. 5A; Table 1). This placed *SEU* on the BAC clone F28H19 (AC006423). Sequencing and annotation by the Arabidopsis Genome Initiative predicts 13 open reading frames (ORFs) on F28H19. Among these ORFs is a glutamine-rich protein (F28H19.10). Fragments spanning the F28H19.10 ORF were amplified by PCR from *seu-1* and *seu-2* plants, respectively, and then directly sequenced. The mutational changes in the *seu-1* and *seu-2* alleles were confirmed by repeating the amplification and sequencing analysis.

PCR analyses using CAPS and dCAPS markers

dCAPS markers (Neff et al., 1998) were designed for both *seu-1* and *seu-2* alleles based on the mutations in *seu-1* and *seu-2* respectively (Fig. 5A). dCAPS marker for *ap2-1* was designed based on sequences published by Jofuku et al. (Jofuku et al., 1994). The CAPS marker for *lug-1* was based on Conner and Liu (Conner and Liu, 2000). These dCAPS or CAPS markers correctly distinguish wild-type from the mutant plants. The primer sequence for each marker is listed in Table 1. PCR amplification was performed under standard conditions.

Molecular analyses of *SEU*

A 5′ rapid amplification of cDNA ends (5′RACE) was carried out using the 5′ RACE kit-version 2.0 (Gibco/BRL). Three nested primers from the 5′ gene-specific region of *SEU* were used: oligo 293, 5′AAACCACTAAACCCGACGTT3′; oligo 265, 5′ GGGTCAGAC-TCAGCACCACT3′; oligo 178, 5′TATTTGGAGCATTCCCAAGC3′. 5′RACE products were cloned into pCRII-TOPO vector (Invitrogen) and sequenced. Blast searches identified six *SEU* EST clones. Five clones (AV521646, AV522370, AV531945, AV546257, AV553478) were provided by the Kazusa DNA Research Institute and one clone (AI997332) was purchased from Genome Systems Inc./Incyte Pharmaceuticals Inc. AV546257 is a full-length cDNA clone.

In situ experiments were performed as previously described (Liu et al., 2000). For northern analyses, total RNA was isolated using the Tri-reagent RNA isolation system (Sigma) from leaves and inflorescences containing flowers at all stages. mRNA was subsequently isolated from the total RNA using the polyATrack mRNA isolation system III (Promega). 2.5 μg mRNA was fractionated on 1% denaturing formaldehyde-agarose gels, blotted, hybridized and washed according to the method of Ausubel et al. (Ausubel et al., 1991). A 874 bp *Kpn*I fragment corresponding to the 3′ end of *SEU* (2685-3559 bp) was used as a probe.

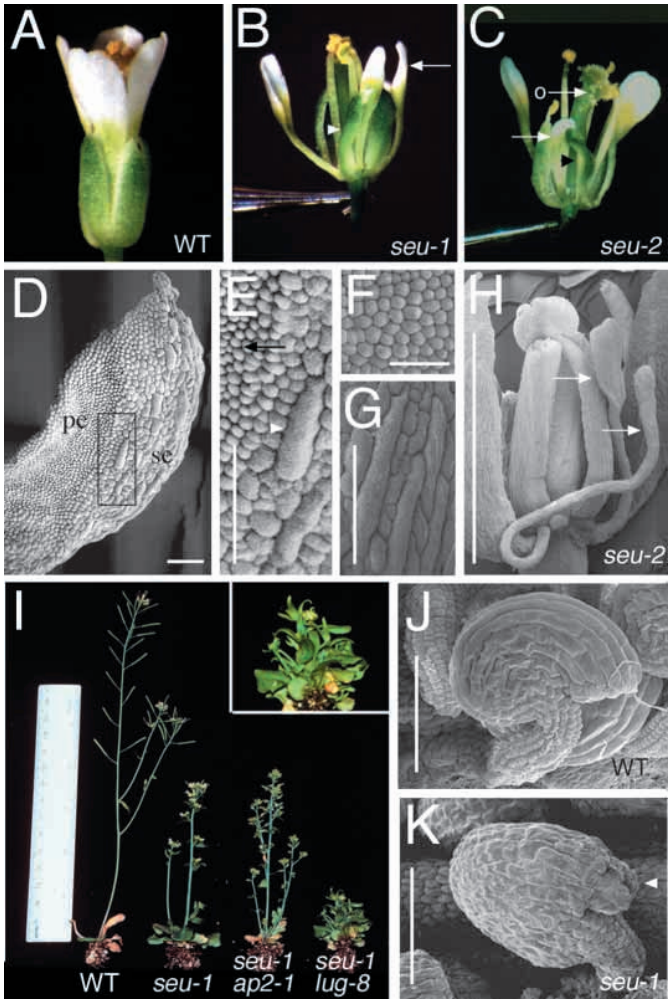

Fig. 1. Phenotypes of *seu* mutant plants. A-C,I are photographs; D-H and J-K are scanning electron micrographs. (A) A wild-type flower. (B) A *seu-1* mutant flower with narrow petals (arrow) and narrow sepals (arrowhead). (C) A *seu-2* mutant flower exhibiting a whorl 1 stamen/carpel mosaic organ (arrowhead) and a whorl 2 petal that is reduced in size and is staminoid (arrow). The tip of the gynoecium is unfused, exposing the ovules (o). (D) Abaxial surface of a *seu-1* whorl 1 organ. Both petal (pe) and sepal (se) cells are present. (E) An enlargement from the boxed area in D. Both round-shaped petal cells (arrow) and rectangular-shaped sepal cells (arrowhead) are present. (F) Wild-type petal cells. (G) Wild-type sepal cells. (H) A *seu-2* mutant flower. Note the long tubular organs (arrows) in whorl 2. These tubular organs are likely petal in identity because of the petal blade at the tip of the tube. (I) Wild-type and mutant plants. All plants are L*er* ecotype. The ruler is 15 cm long. The *seu-1* plant is about half the height of the wild-type one; *seu-1 ap2-1* is similar in height to *seu-1; seu-1 lug-8* double mutant is less than half the height of *seu-1* and is bushy. Inset box is an enlarged picture of a *seu-1 lug-8* plant. (J) A mature wild-type ovule. (K) A *seu-1* ovule with an abnormal protrusion at the micropylar end (arrowhead). Scale bars in D, E, G, J, K are 100 μm; bar in F is 50 μm; bar in H is 1 mm.

RESULTS

seu mutants exhibit defects in floral organ identity and organ number

EMS-induced *seu-1* and *seu-2* mutants (Materials and Methods) initially appeared similar in the severity of their phenotypes. Further characterization showed that *seu-2* was slightly stronger than *seu-1*. For both alleles, late-arising flowers exhibited more severe phenotypes than early-arising flowers. In the late-arising flowers, the organ number in whorls 2 and 3 is reduced (Fig. 1B,C; Table 2). On average, only 3 organs are found in whorl 2, and 5 organs in whorl 3. 7% of

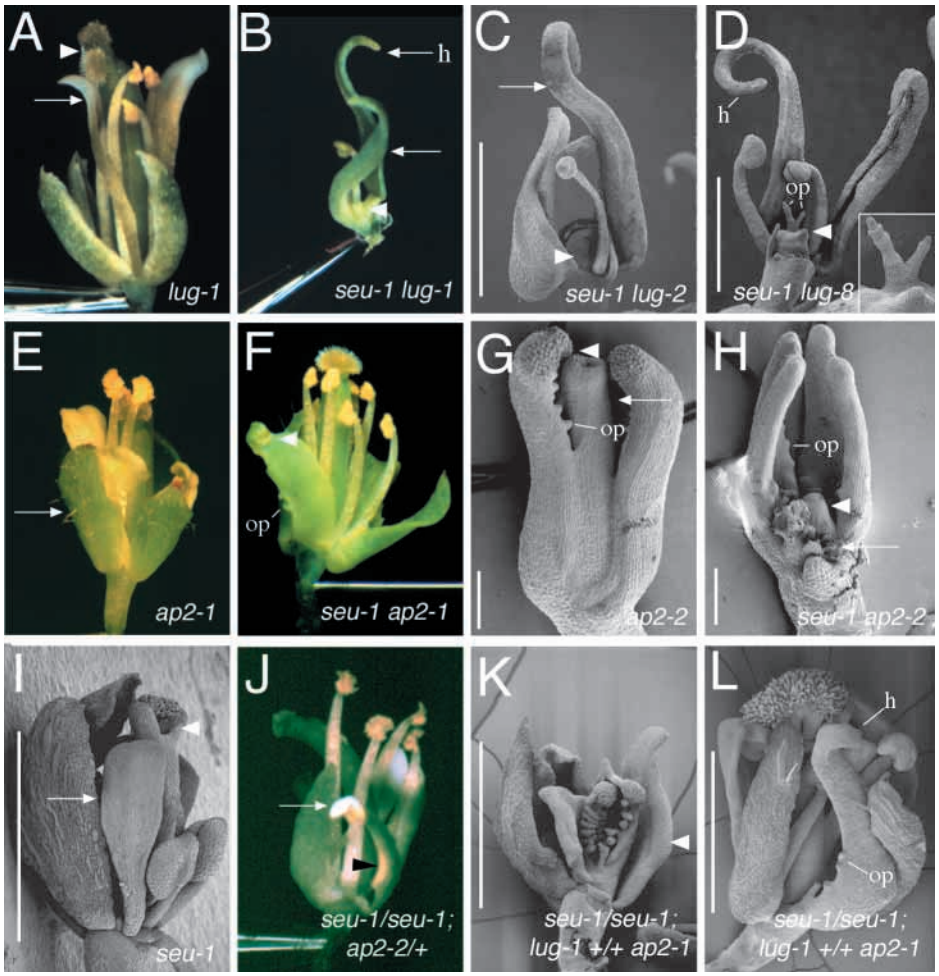

Fig. 2. *seu-1* enhances the floral defects of *lug* and *ap2*. A-B,E-F,J are photographs; C-D,G-I,K-L are SEMs. (A) A *lug-1* mutant flower with narrow petals (arrow) and split gynoecium (arrowhead). (B) A *seu-1 lug-1* double mutant flower at twice the magnification of the flower in A. The *seu-1 lug-1* flower is roughly 25% size of a wild-type flower. Only two carpelloid organs with horns (h) are formed in whorl 1. A single stamen forms in whorl 3. Whorl 4 is reduced to a small mound of tissue (arrowhead). (C) A *seu-1 lug-2* flower. Whorl 1 organs are carpelloid (arrow) but lack stigmatic tissue and ovules. Whorl 4 is just a small mound of tissue (arrowhead). (D) A *seu-1 lug-8* mutant flower. The small mound of tissue in whorl 4 (arrowhead) forms two ovule primordia (op). Inset box is an enlargement of the ovule primordia. (E) An *ap2-1* mutant flower. Whorl 1 organs are leaf-like with trichomes characteristic of leaves (arrow). (F) A *seu-1 ap2-1* double mutant flower. Whorl 1 organs are carpelloid with stigmatic tissues (arrowhead) and ovule primordia (op). (G) An *ap2-2* flower. The relatively normal whorl 4 gynoecium is indicated (arrow). Whorl 1 organs are carpelloid with stigmatic tissus (arrowhead) and ovule primordia (op). (H) A *seu-1 ap2-2* double mutant flower. Whorl 1 organs are carpelloid with ovule primordia (op) along their margin but lack stigmatic tissues. A stamen/sepal mosaic organ was removed (arrow) to reveal whorl 4 that is reduced to a mound of tissue (arrowhead). (I) A *seu-1* flower. Two whorl 1 sepals have been removed to reveal a narrow petal (arrow) and an unfused gynoecium (arrowhead). (J) A *seu-1/seu-1; ap2-2/+* mutant flower. The presence of *ap2-2*/+ enhances *seu-1*/*seu-1* as shown by a stamenoid/carpelloid organ (arrowhead) in whorl 1 and a stamenoid petal (arrow) in whorl 2. (K) A *seu-1/seu-1; lug-1 + /+ ap2-1* flower. The presence of *lug-1 + /+ ap2-1* further enhances *seu-1*/*seu-1*. A whorl 1 organ with both stamen and carpel characteristics is indicated (arrowhead). (L) A *seu-1/seu-1; lug-1 +/ + ap2-1* flower. Note the carpelloid whorl 1 organ with ovule primordia (op) on the organ margin and a horn (h) on the top. Scale bars in C, D, I, K, and L, 1 mm; in G and H, 100 μm.

whorl 1 organs display partial homeotic transformation and possess sepal/petal or sepal/carpel mosaics (Fig. 1C-G; Table 2). Whorl 2 organs are most often narrow petals, but stamenoid petals were occasionally observed (Fig. 1C). Alternatively, petals can be replaced by filamentous or tubular structures (Fig. 1H). Whorl 3 stamens are typically reduced slightly in size. The whorl 4 gynoecium is often slightly split at the top (Fig. 1C, Fig. 2I). Sometimes, horn-like protrusions are seen at the gynoecium apex (data not shown).

In addition to defects in floral organ identity and organ number, *seu* plants exhibit other defects including narrow floral organs (Fig. 1B-C, Fig. 2I), narrow leaves (data not shown), reduced plant height (Fig. 1I) and increased lateral branching (Fig. 1I). Furthermore, the number of seeds per silique is reduced. On average, *seu-1* produces 18.1±6.2 (n=15) seeds per silique while wild-type (L*er*) produces 62±7.4 (n=11) seeds per silique. Occasionally, *seu-1* ovules develop abnormally with a protrusion from the micropylar end (Fig. 1J,K). The *seu* phenotype indicates that *SEU* plays diverse roles during plant development.

seu genetically enhances *lug*

With the exception of the reduced plant height, the floral, ovule, and vegetative defects of *seu* mutants are similar to, but weaker than, those of *lug*. To understand the relationship between *seu* and *lug*, we generated and characterized *seu lug* double mutants. *seu lug* double mutant flowers display a dramatically enhanced phenotype characterized by a reduction in flower size and floral organ number and an enhanced carpelloidy of whorl 1 organs (Fig. 2B-D; Table 2). Most often, only two whorl 1 organs are formed. These whorl 1 organs are often carpelloid as evidenced by their epidermal cell morphology, the formation of horns (a character of *lug* carpels), and the expression of *AG* (see later). Whorl 2 organs are completely absent. In whorl 3, one stamen is occasionally formed, averaging 0.4 per flower. Whorl 4 carpels develop into a small stub or mound of tissues. Interestingly, structures derived from carpel marginal meristems (i.e. ovule, stigma, style, and septum) are not observed on whorl 1 carpelloid organs, nor on whorl 4 mounds (Fig. 2B,C). An exception to this is the *seu-1 lug-8* double mutant (*lug-8* is a weak allele)

Table 2. Effects of *seu, lug, ap2* and *ag* mutants on organ number and organ identity

Genotype	Number of organs in whorl 1	Number of organs in whorl 2	Number of organs in whorl 3	Number of organs in whorl 4	% of whorl 1 organs displaying homeotic transformation	Number of flowers examined
Wild type	4.0±0.0	4.0±0.0	5.8±0.39	2.0±0.0	0%	17
seu-1/seu-1	3.8±0.4	3.0±1.0	4.8±0.90	2.0±0.18	7.4%*	86
seu-1/seu-1; ap2-2/+	4.0±0.0	2.2±0.77	4.4±0.83	1.5±0.52	25%†	15
seu-1/seu-1; lug-1 +/+ ap2-1	3.7±0.7	1.6±1.1	3.2±1.0	1.9±0.47	38%†	14
lug-1/lug-1	4.0±0.0	0.7±0.72	2.7±0.72	1.7±0.49	40%‡	15
seu-1/seu-1; lug-1/lug-1	3.2±0.68	0.0±0.0	0.4±0.91	0.0±0.0	88%§	15
seu-1/seu-1; lug-8/ lug-8	2.9±0.47	0.4±0.94	1.2±0.97	0.4±0.65	ND	14
ag-1/ag-1	4.0±0.0	4.0±0.0	6.0±0.0	43±3.5**	0%	8
lug-1 ag-1/lug-1 ag-1	4.0±0.0	3.9±0.33	5.6±0.88	41±4.2**	0%	9
seu-1/seu-1; ag-1/ag-1	3.6±0.79	4.1±0.38	5.9±0.38	ND	0%	7
seu-1/seu-1; lug-1 ag-1/lug-1 ag-1	3.9±0.27	3.8±0.58	4.4±1.0	16.9±5.7**	9%¶	14

*Mostly petal/sepal mosaics.
†Mostly stamen/sepal or carpel/sepal mosaics.
‡Similar to †; also see Liu and Meyerowitz (1995).
§Mostly sepal to carpel transformations.
¶Only petal/sepal mosaics.
**Number of organs formed interior to whorl 3.
ND, not determined; values are mean ± s.d.

where some ovule primordia were occasionally observed in either whorl 1 organs or whorl 4 mounds (Fig. 2D). Vegetative defects were also enhanced in the *seu lug* double mutants (Fig. 1I). Although *lug* single mutations have no effect on plant height, *seu-1 lug-1* double mutants are only 12% of wild-type height (2.7±0.9 cm; *n*=20), much shorter than *seu-1* (11.4±1.6 cm; *n*=10). In summary, the *seu lug* double mutant flowers exhibit increased carpelloidy in whorl 1, enhanced organ loss in whorls 1-3, a reduction of whorl 4 gynoecium, and a loss of carpel marginal tissues. An overall reduction of flower size and plant height was also observed.

seu genetically enhances *ap2*

Since *AP2* plays a major role in *AG* repression and *ap2* interacts with *lug* synergistically (Bowman et al., 1991; Liu and Meyerowitz, 1995), we sought to determine the relationship between *ap2* and *seu*. Both weak *ap2-1* and strong *ap-2-2* alleles were used for the analysis. The weak *ap2-1* flower develops leaf-like whorl 1 organs and staminoid whorl 2 petals (Fig. 2E) (Bowman et al., 1989; Bowman et al., 1991). In *seu-1 ap2-1* double mutants, first whorl organs are converted to carpelloid structures as evidenced by the presence of stigmatic tissue and ovule primordia and the absence of leaf-like trichomes (Fig. 2F). In the strong *ap2-2* flowers, whorl 1 organs are carpelloid, whorl 2 and 3 organs are absent, and in whorl 4 a relatively normal gynoecium is formed (Fig. 2G) (Bowman et al., 1989; Bowman et al., 1991). Similar to *ap2-2*, *seu-1 ap2-2* double mutant whorl 1 organs are carpelloid (Fig. 2H). However, the *seu-1 ap2-2* whorl 1 carpels have less stigmatic tissue and fewer ovules than *ap2-2*. The most obvious difference between *ap2-2* single mutant and *seu-1 ap2-2* double mutant is in whorl 4 where only a small mound of tissue develops (Fig. 2H). Hence, *seu* enhances the defects of weak *ap2-1* in homeotic transformation and organ loss and enhances

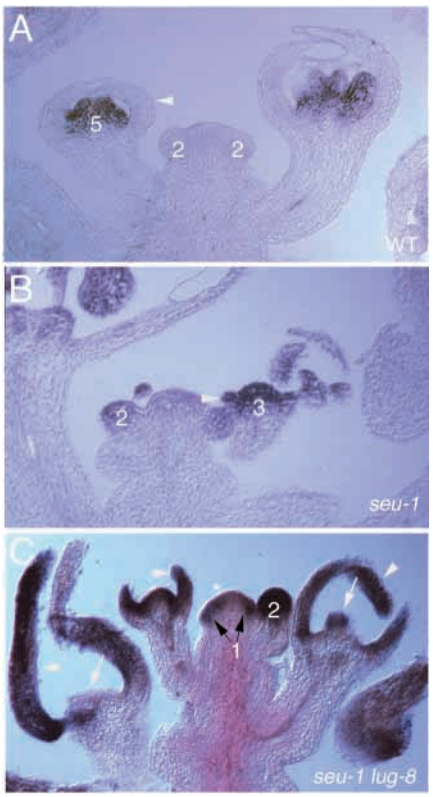

Fig. 3. Ectopic and precocious expression of *AG* in *seu* single and *seu lug* double mutants. 8 μm longitudinal sections of *Arabidopsis* inflorescences were hybridized with an *AG* antisense probe. Numbers indicate stages of floral development [based on Smyth et al. (Smyth et al., 1990)]. (A) A wild-type inflorescence. *AG* mRNA is detected in the center of the stage 5 flower and is not detected in sepal primordium (arrowhead). *AG* mRNA is not detected in stage 2 floral meristems. (B) A *seu-1* inflorescence. *AG* mRNA is detected in the stage 2 floral meristem and in the sepal primordia of stage 3 floral meristem (arrowhead). (C) A *seu-1 lug-8* double mutant inflorescence. *AG* mRNA is detected as early as the stage 1 and stage 2 floral meristems. Expression of *AG* mRNA is also detected in groups of cells (marked with a *) in the inflorescence meristem that might represent pre-stage 1 cells. *AG* mRNA is detected in the sepals of all flowers (arrowheads). Note the severely reduced whorl 4 (white arrows). The pink color in the stem is residual Eosin stain and does not reflect hybridization signal.

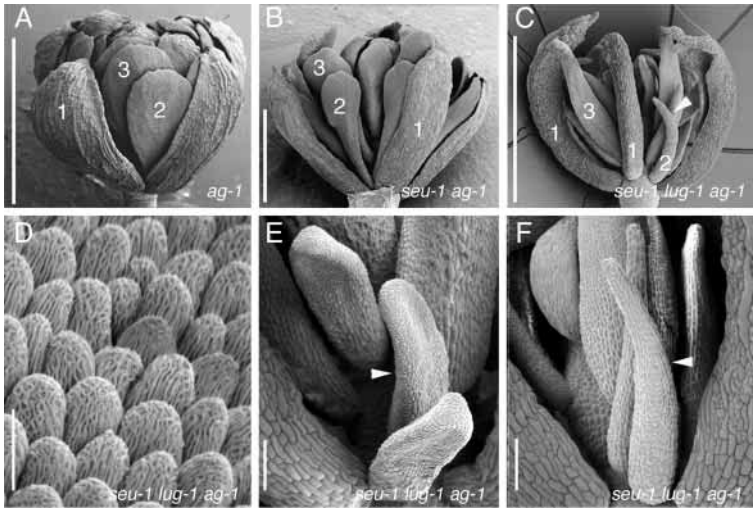

Fig. 4. SEMs of *seu-1 ag-1* and *seu-1 lug-1 ag-1* flowers. Number indicates whorl number. (A) An *ag-1* flower. Whorl 1 organs are sepals, and whorls 2 and 3 organs are petals. Whorl 4 is another flower repeating the $(\text{sepal-petal-petal})_n$ pattern. (B) A *seu-1 ag-1* double mutant flower. Whorl 1 organs are narrow sepals. Whorls 2 and 3 are narrow petals. Whorl 4 is another flower. (C) A *seu-1 lug-1 ag-1* triple mutant flower. The whorl 1 sepals are very narrow and canoe-shaped. Whorls 2 and 3 organs are either blade-like or club-like (arrowhead). (D) A close-up image of the epidermal cells in the blade-like organ. These epidermal cells exhibit characteristics of petal cells. (E) A close-up image showing several developing blade-like organs (arrowhead) in whorls 2 and 3 of a *seu-1 lug-1 ag-1* triple mutant flower. (F) A close-up image showing several club-like organs (arrowhead) in whorls 2 and 3 of a *seu-1 lug-1 ag-1* triple mutant flower. Scale bars in A, B, and C are 1 mm; scale bar in D is 10 μm; scale bars in E and F are 100 μm.

the defects of strong *ap2-2* primarily in the whorl 4 gynoecial development.

In the homozygous *seu* mutant background, the strong *ap2-2* allele behaves as a dominant enhancer of *seu*. While *seu-1/seu-1* plants display homeotic transformations in only 7.4% of whorl 1 organs, *seu-1/seu-1*; *ap2-2/+* plants display homeotic transformations in 25% of whorl 1 organs (Table 2) with a greater degree of homeotic transformation (Fig. 2I,J). Furthermore, the *lug-1* allele behaves as a dominant enhancer in the *seu-1/seu-1; ap2-1/+* background. Carpelloid and staminoid transformations are observed in 38% of whorl 1 organs in *seu-1/seu-1*; *lug-1 +/+ ap2-1* flowers (Table 2; Fig. 2, compare 2K,L with J). In summary, the degree of mutant severity with respect to homeotic transformation can be ordered as follows: *seu-1/seu-1* < *seu-1/seu-1; ap2-2/+* < *seu-1/seu-1*; *lug-1 +/+ ap2-1* < *ap2-2/ap2-2* (<: less severe than). Therefore, *seu*, *lug* and *ap2* exhibit both synergistic and dominant genetic interactions.

AG is ectopically expressed in *seu* single and *seu lug* double mutants

To test if the carpelloid and stamenoid homeotic transformation of whorl 1 organs and the reduction of organ number observed in the *seu* single and *seu lug* double mutant flowers are primarily caused by the ectopic expression of *AG*, we examined *AG* mRNA expression by in situ hybridization. In wild-type flowers, *AG* mRNA is first detected at stage 3 in the center of a floral meristem (Fig. 3A) (Drews et al., 1991). In contrast, *AG* mRNA was sometimes detected in all four whorls in stage 3 *seu* flowers (Fig. 3B). Additionally, *AG* mRNA was sometimes detected in stage 2 *seu-1* floral primordia (Fig. 3B). Thus, *seu* causes both ectopic and precocious *AG* mRNA expression. In *seu-1 lug-8* double mutant flowers, the ectopic *AG* expression was enhanced as shown both by a greater extent of ectopic *AG* expression in whorl 1 organs and by a higher percentage of whorl 1 organs that express *AG* (Fig. 3C). Most strikingly, precocious *AG* expression was detected in floral meristems as early as stage 1 or even in groups of cells that are about to form the stage 1 floral meristem (ie. pre-stage 1 cells) (Fig. 3C). This stage 1/pre-stage 1 expression of *AG* was never observed in *lug* or *seu* single mutants.

Removing ectopic *AG* activity restores proper organ identity and organ number but not organ shape or plant height

The above studies showed that the extent and severity of homeotic transformation and organ loss correlated with the extent of ectopic/precocious *AG* expression in *seu* and *seu lug* mutant flowers. By constructing *seu ag* double and *seu lug ag* triple mutants, we sought to determine if removing *AG* activity in the *seu* and *seu lug* background can restore proper organ identity, organ shape or organ number. We found that the organ identity and organ number of the *seu-1 ag-1* flowers are similar to those of *ag-1* flowers. Four sepals develop in whorl 1, and four petals develop in whorl 2 (Fig. 4A,B; Table 2). However, the petals and sepals of *seu-1 ag-1* flowers are narrower than those of *ag-1* or wild-type (Fig. 4B) and are similar to *seu-1* flowers. Furthermore, plant height is similar in *seu-1 ag-1* and *seu-1* plants. Therefore, removing ectopic/precocious *AG* in *seu* background can restore defects in floral organ identity and organ number but not in organ shape or plant height.

In *seu-1 lug-1 ag-1* triple mutant flowers, floral organ type and organ number in whorls 1-3 are similar to those in *ag-1* flowers (Fig. 4C; Table 2). Although whorl 1 organs of the triple mutant are narrow and canoe-shaped, their epidermal cell morphology is characteristic of sepals (Fig. 4C). Occasionally (9%), some of these whorl 1 organs are sepal/petaloid mosaics but they are never carpelloid (Table 2). This is in contrast to the high percentage (88%) of whorl 1 carpelloid organs found in *seu-1 lug-1* double mutants (Table 2; Fig. 2B-D). The whorl 2 and 3 organs of *seu-1 lug-1 ag-1* flowers are sometimes blade-like (Fig. 4C,E) with epidermal cell morphology characteristic of petals (Fig. 4D). Alternatively, the whorl 2-3 organs sometimes look like clubs (Fig. 4C,F) with epidermal cell morphology characteristic of the base of petals (data not shown). On average, 3.8 whorl 2 and 4.4 whorl 3 organs were observed in the *seu-1 lug-1 ag-1* flowers as compared to zero whorl 2 and 0.4 whorl 3 organs in the *seu-1 lug-1* double mutants (Table 2). Finally, the *seu lug ag* triple mutant (2.9 cm±0.8; n=13) is similar in height to the *seu-1 lug-1* double mutant (2.7±0.9 cm; n=20). Thus, removing ectopic/precocious *AG* activity from *seu lug* double mutants restores correct floral organ identity and organ number in whorls 1-3 but does not restore normal floral organ shape or plant height.

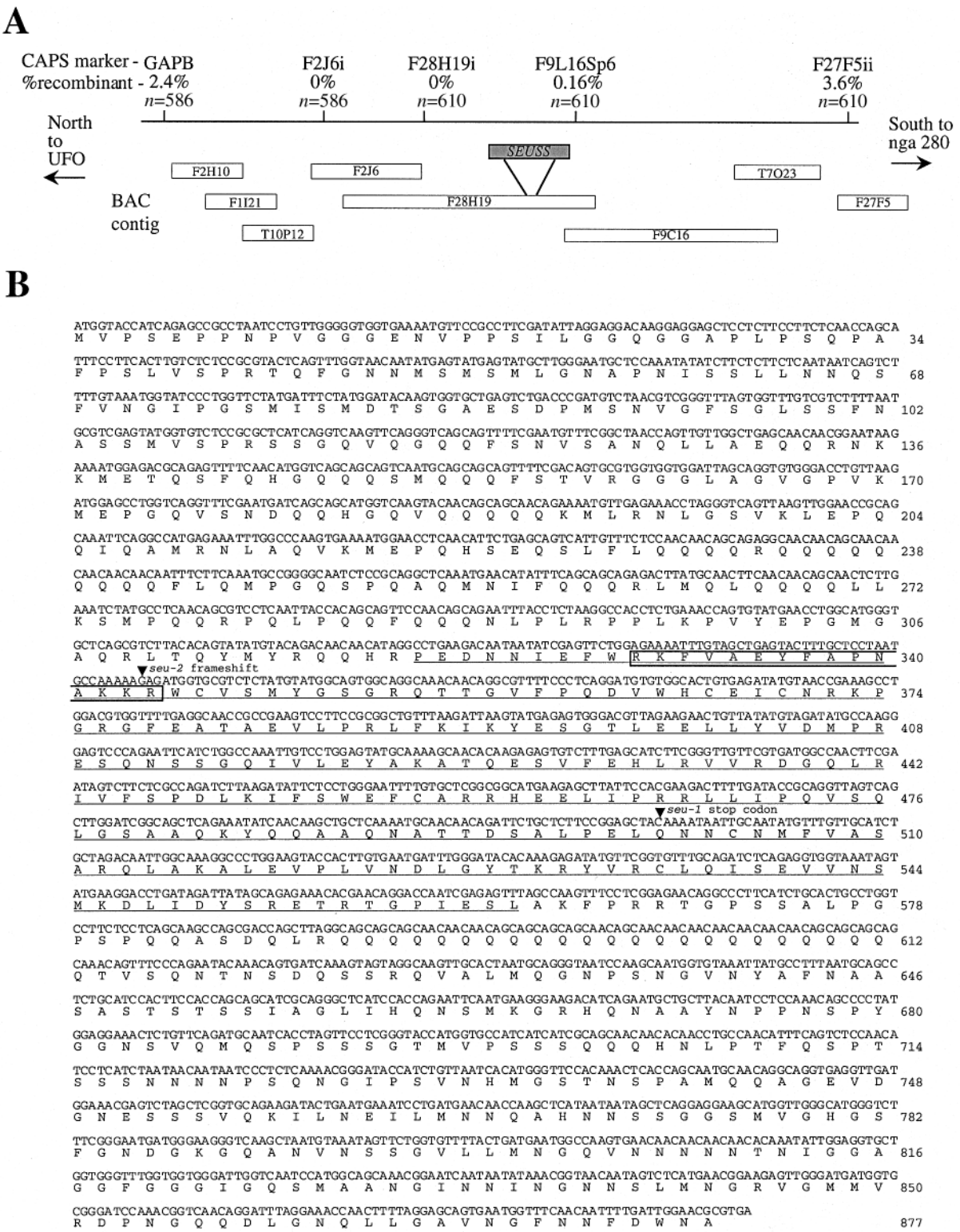

Fig. 5. Molecular cloning of the *SEUSS* gene. (A) A physical map of the *SEU* region on *Arabidopsis* chromosome I. Percentage recombination for five CAPS markers is shown. *n* indicates the number of meiotic products examined at the given marker. BAC clones are shown as open boxes. The *SEU* gene, shown as a shaded box, maps 0.16 cM north of the CAPS marker F9L16Sp6. (B) Nucleotide sequence and the predicted amino acid sequence of *SEU*. Numbers on the right indicate the amino acid residue. The boxed area encodes a bi-partite nuclear localization signal. The underlined sequence is the putative dimerization domain with similarity to the Ldb proteins. The filled triangles indicate *seu-1* and *seu-2* mutations. The *seu-1* allele is caused by a C to T transition, resulting in a 'TAA' stop codon at amino acid 501. The *seu-2* allele is caused by a single base-pair deletion of the 'G' base indicated. The full length cDNA sequence (3555 bp) including 5′ and 3′ UTR has been deposited with GenBank (AF378782).

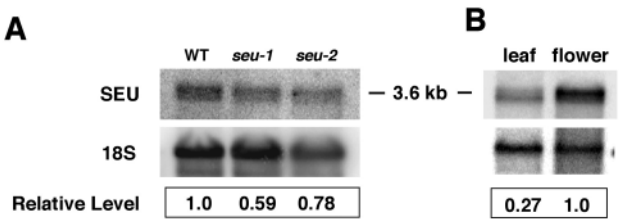

Fig. 6. *SEU* mRNA expression. (A) Northern analysis of mRNA isolated from flowers of wild-type and *seu* mutants. The doublet bands of *SEU* mRNA reflect two different transcriptional initiation sites confirmed by 5′RACE (see text). The relative mRNA level is corrected with the 18S RNA as a loading control and compared with wild-type signal level. (B) Northern analysis of mRNA isolated from leaves and flowers of wild-type plants. *SEU* mRNA is expressed at a higher level in flowers than in leaves.

In *ag-1* flowers, the whorl 4 gynoecium is replaced by an indeterminate flower that repeats the $(\text{sepal-petal-petal})_n$ pattern, generating an average of 43±3.5 organs interior to whorl 3. In contrast, the *seu-1 lug-1ag-1* triple mutant averaged only 16.9±5.7 organs interior to whorl 3 (Table 2). The reduced floral organ number in whorl 4 of the triple mutant likely results from an additive effect of the floral indeterminancy caused by *ag-1* and the reduced whorl 4 primordium caused by the *seu-1 lug-1* (Fig. 2B-D; Fig. 3C).

The *SEU* gene encodes a glutamine-rich protein with a putative dimerization domain

We isolated the *SEU* gene by positional cloning (Fig. 5; Materials and Methods). *SEU* was first mapped to the centromeric region of chromosome 1, approximately 2.4 cM south of the marker GAPB. Finer mapping using additional CAPS markers (Fig. 5A; Table 1) indicated that *SEU* resides 0.16 cM north of the CAPS marker F9L16Sp6. Our recombination data from this region of chromosome I indicate that 0.16 cM represents approximately 40 kb, thus *SEU* likely resides on BAC clone F28H19. Sequencing and annotation of F28H19 (AC006423) by the *Arabidopsis* Genome Initiative indicated the presence of 13 ORFs on F28H19 that were north of the marker F9L16Sp6. Six of these appeared to encode portions of transposable elements. The seven remaining ORFs are: three hypothetical proteins, one unknown protein, one putative acyl-acyl carrier protein desaturase, one serine carboxypeptidase, and one glutamine-rich (Q-rich) protein (F28H19.10). Because Q-rich sequences are found in many transcriptional regulators including *LUG*, F28H19.10 ORF was a likely candidate for *SEU*. Sequence analysis of the F28H19.10 ORF in *seu-1* and *seu-2* genomic DNA identified mutations in both alleles. In the *seu-1* allele, a C to T transition results in the change of a glutamine codon to a stop codon at amino acid 501 (Fig. 5B). In the *seu-2* allele, a single base pair deletion in codon 343 leads to a frame shift (adding 54 novel amino acid residues) and a subsequent stop codon (Fig. 5B). The nature of the mutational changes found in *seu-1* and *seu-2* strongly supports that F28H19.10 encodes the *SEU* gene.

Using the F28H19.10 ORF sequence as a query in a TBlastN search, six *SEU Arabidopsis* EST clones were identified. Based on sequence analysis of these EST clones and 5′ RACE, full length *SEU* cDNA is represented by two transcripts of 3555 bp and 3406 bp respectively. The shorter transcript initiates 149 bp downstream from the longer transcript. Northern analyses showed that the transcript levels in *seu-1* and *seu-2* mutants are reduced to 59% and 78% wild-type level respectively (Fig. 6A). This reduced mRNA level likely reflects a reduced mRNA stability in the two *seu* mutants, both of which are predicted to produce truncated SEU protein. Consistent with diverse developmental roles played by *SEU*, *SEU* mRNA is expressed in all tissues examined including flowers and leaves (Fig. 6B) as well as seedlings (data not shown).

SEU encodes a Q-rich (15% Q overall) protein of 877 amino acid (Fig. 5B). Two major Q-rich regions reside in residues 179-289 (42% Q) and 582-632 (61%Q), respectively (Fig. 5B, Fig. 7A). Within the second Q-rich region there is a stretch of 24 Q residues. A putative bipartite nuclear localization signal (Robbins et al., 1991) found between amino acids 330 and 344 (Fig. 5B) suggests that SEU likely resides in the nucleus. In addition, a 243 amino acid central domain (residues 321-563) is highly conserved. Between 21% and 81% amino acid sequence identity was found within this domain when compared with other SEUSS-LIKE plant proteins and animal Ldb proteins (Fig. 7A,B). Protein secondary structure prediction indicates that this domain of SEU likely forms an α helix (Fig. 7B). In addition, four hydrophobic residues spaced 7 residues apart within this region (Fig. 7B) suggest that this region may form a hydrophobic zipper.

While the Ldb proteins are similar to SEU only in the conserved central domain, the SEUSS-LIKE proteins from plants are similar to SEU in the entire protein. *Arabidopsis* genome has two *SEUSS-LIKE* genes, *M7J2.110 (CAA18174)* and *MTG10.12 (BAB10171)*; both are 55% identical to SEU in the putative dimerization domain and 33% identical over the entire protein (Fig. 7A). A rice gene (AAF34437) has an overall 48% identity to SEU and a 81% identity in the putative dimerization domain (Fig. 7A). A second rice gene (BAA90807) is more closely related to the *Arabidopsis M7J2.110* and *MTG10.12* than it is to *SEU*. A large number of EST sequences from other plant species such as *Gossypium arboreum* (BG442742), *Zea mays* (AW066123), *Lycopersicon esculentum* (AW031470), *Glycine max* (AF100167), and *Pinus taeda* (AW043184) also share high levels of sequence similarity with *SEU*. Because of limited sequence information from these EST clones, only portions of these genes can be compared with *SEU*. Between 48% and 85% sequence identity within the dimerization domain is found among these SEUSS-LIKE plant proteins. However, the function of these *SEUSS-LIKE* genes is unknown.

DISCUSSION

SEU, together with *LUG* and *AP2*, regulates the spatial and temporal pattern of *AG* expression

We report the identification and characterization of a novel mutant *seu* in flower development. We showed that the partial homeotic transformation of floral organ identity and a slight reduction of floral organ number in *seu* single mutants are caused by ectopic and precocious *AG* expression. The sepal/petal and sepal/stamen mosaics observed in *seu* whorl 1 organs also suggest an ectopic B activity. However, this ectopic B activity may be mediated by the ectopic *AG* activity because *ag seu* double mutants no longer exhibited such sepal/petal or

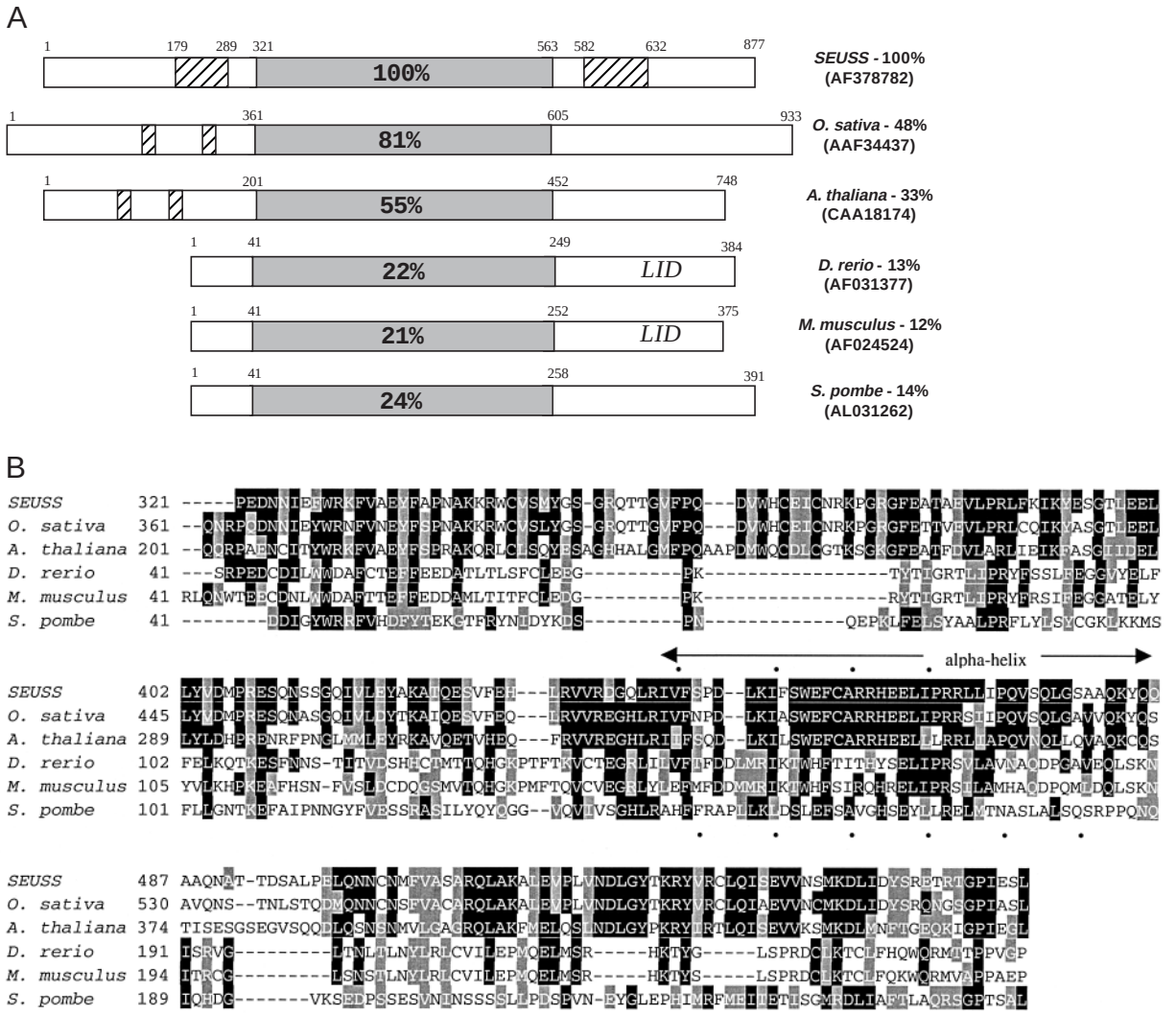

Fig. 7. Sequence similarity between SEU, SEUSS-LIKE proteins and Ldb proteins. (A) Diagramatic representation of the open reading frames of SEU, SEUSS-LIKE proteins from *Oleracea sativa* (AAF34437) and *A. thaliana* (CAA18174), representative Ldb family members from *Danio rerio* (AF031377), and *Mus musculus* (AF024524) and a putative Ldb family member from *Saccharomyces pombe* (AL031262). Numbers indicate amino acid residues. The shaded portion represents the putative dimerization domain for each protein. Percentages shown within the putative dimerization domain indicate amino acid sequence identity between SEU and the respective protein. Percentages shown to the right are percentage identity to SEU over the entire open reading frame. Glutamine-rich regions are shown as hatched boxes. LID: LIM interaction domain. (B) Sequence alignment of SEU with representative SEUSS-LIKE proteins, and Ldb family proteins in the putative dimerization domain. Identical residues are shaded black. Similar residues are shaded gray. The predicted alpha-helical portion of SEU is indicated with a two-headed arrow. Four hydrophobic or non-polar amino acids (•) in SEU are spaced seven residues apart in this region. Six hydrophobic or non-polar amino acids (•) in the *M. musculus* Ldb1 protein are also spaced seven residues apart, suggesting a hydrophobic zipper structure (Jurata and Gill, 1997).

sepal/stamen mosaics (Table 2). Hence, *SEU* is mainly involved in the negative regulation of *AG* in flowers. The relative weak phenotype of *seu* single mutants could be explained by several alternative but non-exclusive hypotheses. First, *SEU* may encode a co-regulator of LUG. In the presence of intact LUG, a defective SEU may only slightly reduce the activity of the LUG-SEU complex. Second, none of our *seu* alleles is a null as the truncated SEU protein in *seu-1* or *seu-2* might still possess partial function. Third, *SEU* may encode a member of a gene family whose function may be partially redundant with other family members.

The effects of *seu* mutations are most striking when combined with *lug.* More complete homeotic transformation in floral organs and a greater extent of floral organ loss are observed in the *seu lug* double mutants and are shown to be mediated by enhanced *AG* mis-expression. In particular, precocious *AG* expression was observed at stages as early as stage 1 and even pre-stage 1 in *seu-1 lug-8* double mutant flowers. This stage 1/pre-stage 1 expression of *AG* was never observed in *seu-1*, *lug-1*, and *ap2-2* single mutants, which cause precocious *AG* expression starting at stage 2 floral meristems (Drews et al., 1991; Liu and Meyerowitz, 1995; Liu et al., 2000). The stage 1/pre-stage 1 *AG* expression may underlie the dramatic reduction of floral organ number in the *seu lug* double mutants. Increased *AG* activity is known to repress floral organ initiation, particularly in whorls 2-3, and

AG was postulated to prevent organ primordial initiation by inhibiting cell proliferation (Bowman et al., 1991). In addition, dominant genetic interactions between *seu-1* and *ap2-2* and among *seu-1*, *ap2-1* and *lug-1* were also observed. Dominant genetic interactions have been reported previously between *lug* and strong *ap2-9* (Liu and Meyerowitz, 1995) and may indicate direct physical interactions or a common activity threshold among the interacting proteins.

SEU regulates other developmental processes independently of *AG*

Both *LUG* and *AP2* have functions that are independent of *AG*. *AP2* specifies sepal and petal identity, while *LUG* regulates floral organ and leaf shape and gynoecium and ovule development (Bowman et al., 1991; Liu and Meyerowitz, 1995; Roe et al., 1997; Schneitz et al., 1997; Chen et al., 2000; Liu et al., 2000). With the exception of defects in floral organ identity and organ number, many of the *seu* defects are not suppressed by removing *AG* activity. For example, *seu ag* double mutants still display reduced plant height and form narrow sepals and petals. In addition, in *seu lug* double mutants, a small mound of tissue develops in whorl 4 (Fig. 2B-D). This reduced whorl 4 phenotype appears *AG*-independent as the *ag seu lug* triple mutants have a much reduced number of whorl 4 organs in the indeterminate flower. Finally, the *seu lug ag* triple mutants develop canoe-shaped sepals and blade- or club-shaped petals (Fig. 4C,E,F), suggesting a synergistic interaction between *seu* and *lug* in regulating organ shape. Hence, in addition to repressing *AG*, *SEU*, together with *LUG*, may regulate additional target genes that determine plant height, organ shape and whorl 4 primordium formation.

What underlies these *AG*-independent defects of *seu*? Examination of petal cells in *seu* single and *seu lug ag* triple mutants by scanning electron microscopy indicated that the petal cell size is similar to that of wild type (R.G. F. and Z. L., unpublished data). Hence, the narrow organ shape, reduced plant height, and reduced whorl 4 organ primordia are consistent with a general reduction of cell number, and, perhaps, reflect a role of *SEU* in promoting cell proliferation. *LUG* was similarly proposed to have such a role in cell proliferation control (Liu et al., 2000).

SEUSS encodes a putative transcriptional co-repressor

seu mutants are similar to *lug* mutants in their phenotype, their synergistic and dominant interaction with *ap2*, their ability to negatively regulate *AG*, and their role in regulating organ shape and gyneocium development. These similarities suggest that *SEU* may function similarly to *LUG*. We showed that *SEU* does not encode a protein with significant sequence similarity to *LUG*. Rather, *SEU* encodes a Q-rich protein with a conserved domain that is similar to the dimerization domain of Ldb family of transcriptional co-regulators. Our finding that both the *seu-1* and *seu-2* mutation results in the truncation of the SEU protein in this conserved domain suggests that this domain is important for *SEU* function.

Ldb protein family members regulate transcription via direct physical interactions with DNA-binding transcription factors such as the LIM-homeodomain proteins (Agulnick et al., 1996; Bach et al., 1997; Jurata and Gill, 1997). In the *M. musculus* Ldb1 protein, the domain conserved between Ldb1 and SEU was predicted to form an amphipathic α helix and mediate homo-dimerization (Jurata and Gill, 1997). In addition, Ldb proteins contain a second domain, the LIM Interaction Domain (LID) (Fig. 7A), which mediates the interaction between Ldb proteins and the LIM-homeodomain proteins. However, there is no sequence similarity in the LID domain between SEU and members of the Ldb family.

SEUSS-LIKE genes are found in *Arabidopsis*, rice, soybean, corn, pine and other plant species and define a novel family of plant regulatory proteins. With the exception of *SEU*, the function of other family members is not known. Our genetic and molecular analysis of *seu* is beginning to shed light on the function of this novel family of plant regulators. Furthermore, using a reverse genetic approach, we will be able to test whether the two *Arabidopsis SEUSS-LIKE* genes play redundant roles with *SEU*.

A proposed model

Based on our genetic and molecular analyses, we propose that *SEU* is a co-regulator of *LUG*. The domain structure of *LUG* is similar to that of a class of functionally related transcriptional co-repressors including *Tup1* of yeast, *Groucho* of *Drosophila* and *TLE* (Transducin-like Enhancer of split) in mammals (Hartley et al., 1988; Williams and Trumbly, 1990; Parkhurst, 1998; Conner and Liu, 2000). In yeast, the Tup1 co-repressor is brought to target promoters by sequence-specific DNA-binding proteins and regulates a wide array of independent sets of genes such as **a**-cell specific genes, glucose-repressed genes, flocculation genes, and DNA-damage-induced genes (Roth, 1995; Teunissen et al., 1995). The N-terminal portion of Tup1 forms a repression complex with Ssn6, a tetratricopeptide repeat protein (Keleher et al., 1992), which is needed to facilitate the interaction between Tup1 and the corresponding DNA-binding transcription factors (Tzamarias and Struhl, 1994).

If *LUG* acts via a mechanism similar to Tup1, could *SEU* be the *Arabidopsis* equivalent of Ssn6? Although *SEU* does not share sequence similarity with Ssn6, both SEU and Ssn6 possess Q-rich domains, lack a DNA-binding motif, and contain a putative protein-protein interaction domain. Both *seu* and *ssn6* mutants are pleiotropic in phenotype. The distinct molecular identity but similar genetic function between *SEU* and *LUG* also support that *SEU* and *LUG* may work together by forming a co-repressor complex. Preliminary experiments indicate that *SEU* interacts with *LUG* in the yeast two-hybrid system (A. Surendrarao, R. G. F. and Z. L., unpublished data). Hence, our current working model predicts that by interacting with DNA-binding transcription factors that bind to *AG* cis-elements, the putative SEU/LUG co-repression complexes are recruited to repress *AG* expression in the outer two whorls of a flower. Candidate DNA-binding factors include, but are not limited to, *AP2*. This model could explain the synergistic and dominant genetic interactions among *ap2*, *seu* and *lug*. The molecular isolation of *LUG* (Conner and Liu, 2000), *AP2* (Jofuku et al., 1994), and *SEU* (reported here) will allow us to further test these hypotheses. Other molecular and biochemical analyses will increase our understanding of the transcriptional repression mechanism in higher plants.

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REFERENCES

Agulnick, A. D., Taira, M., Breen, J. J., Tanaka, T., Dawid, I. B. and Westphal, H. (1996). Interactions of the LIM-domain-binding factor Ldb1 with LIM homeodomain proteins. *Nature* **384**, 270-272.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1991). *Current Protocols in Molecular Biology.* New York: Wiley and Sons.

Bach, I., Carriere, C., Ostendorff, H. P., Andersen, B. and Rosenfeld, M. G. (1997). A family of LIM domain-associated cofactors confer transcriptional synergism between LIM and Otx homeodomain proteins. *Genes Dev*. **11**, 1370-1380.

Bomblies, K., Dagenais, N. and Weigel, D. (1999). Redundant enhancers mediate transcriptional repression of *AGAMOUS* by *APETALA2*. *Dev. Biol.* **216**, 260-264.

Bowman, J. L., Smyth, D. R. and Meyerowitz, E. M. (1989). Genes directing flower development in *Arabidopsis*. *Plant Cell* **1**, 37-52.

Bowman, J. L., Smyth, D. R. and Meyerowitz, E. M. (1991). Genetic interactions among floral homeotic genes of *Arabidopsis*. *Development* **112**, 1-20.

Busch, M. A., Bomblies, K. and Weigel, D. (1999). Activation of a floral homeotic gene in *Arabidopsis*. *Science* **285**, 585-587.

Chen, C., Wang, S. and Huang, H. (2000). *LEUNIG* has multiple functions in gynoecium development in *Arabidopsis*. *Genesis* **26**, 42-54.

Coen, E. S. and Meyerowitz, E. M. (1991). The war of the whorls: genetic interactions controlling flower development. *Nature* **353**, 31-37.

Conner, J. and Liu, Z. (2000). *LEUNIG*, a putative transcriptional corepressor that regulates *AGAMOUS* expression during flower development. *Proc. Natl. Acad. Sci. USA* **97**, 12902-12907.

Deyholos, M. K. and Sieburth, L. E. (2000). Separable whorl-specific expression and negative regulation by enhancer elements within the *AGAMOUS* second intron. *Plant Cell* **12**, 1799-1810.

Drews, G. N., Bowman, J. L. and Meyerowitz, E. M. (1991). Negative regulation of the *Arabidopsis* homeotic gene *AGAMOUS* by the *APETALA2* product. *Cell* **65**, 991-1002.

Eshed, Y., Baum, S. F. and Bowman, J. L. (1999). Distinct mechanisms promote polarity establishment in carpels of *Arabidopsis*. *Cell* **99**, 199-209.

Goto, K. and Meyerowitz, E. M. (1994). Function and regulation of the *Arabidopsis* floral homeotic gene *PISTILLATA*. *Genes Dev.* **8**, 1548-1560.

Hartley, D. A., Preiss, A. and Artavanis-Tsakonas, S. (1988). A deduced gene product from the *Drosophila* neurogenic locus, *enhancer of split*, shows homology to mammalian G-protein beta subunit. *Cell* **55**, 785-795.

Huang, H., Mizukami, Y., Hu, Y. and Ma, H. (1993). Isolation and characterization of the binding sequences for the product of the *Arabidopsis* floral homeotic gene *AGAMOUS*. *Nucleic Acids Res.* **21**, 4769-4776.

Jack, T., Brockman, L. L. and Meyerowitz, E. M. (1992). The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. *Cell* **68**, 683-697.

Jofuku, K. D., den Boer, B. G., Van Montagu, M. and Okamuro, J. K. (1994). Control of *Arabidopsis* flower and seed development by the homeotic gene *APETALA2*. *Plant Cell* **6**, 1211-1225.

Jurata, L. W. and Gill, G. N. (1997). Functional analysis of the nuclear LIM domain interactor NLI. *Mol. Cell. Biol.* **17**, 5688-5698.

Keleher, C. A., Redd, M. J., Schultz, J., Carlson, M. and Johnson, A. D. (1992). Ssn6-Tup1 is a general repressor of transcription in yeast. *Cell* **68**, 709-719.

Konieczny, A. and Ausubel, F. M. (1993). A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. *Plant J.* **4**, 403-410.

Lenhard, M., Bohnert, A., Jurgens, G. and Laux, T. (2001). Termination of stem cell maintenance in *Arabidopsis* floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. *Cell* **105**, 805-814.

Levin, J. Z., Fletcher, J. C., Chen, X. and Meyerowitz, E. M. (1998). A genetic screen for modifiers of *UFO* meristem activity identifies three novel *FUSED FLORAL ORGANS* genes required for early flower development in *Arabidopsis*. *Genetics* **149**, 579-595.

Lohmann, J. U., Hong, R. L., Hobe, M., Busch, M. A., Parcy, F., Simon, R. and Weigel, D. (2001). A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. *Cell* **105**, 793-803.

Liu, Z., Franks, R. G. and Klink, V. P. (2000). Regulation of gynoecium marginal tissue formation by *LEUNIG* and *AINTEGUMENTA*. *Plant Cell* **12**, 1879-1892.

Liu, Z. and Meyerowitz, E. M. (1995). *LEUNIG* regulates *AGAMOUS* expression in *Arabidopsis* flowers. *Development* **121**, 975-991.

Mandel, M. A., Gustafson-Brown, C., Savidge, B. and Yanofsky, M. F. (1992). Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. *Nature* **360**, 273-277.

Mizukami, Y. and Ma, H. (1997). Determination of *Arabidopsis* floral meristem identity by *AGAMOUS*. *Plant Cell* **9**, 393-408.

Neff, M. M., Neff, J. D., Chory, J. and Pepper, A. E. (1998). dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: experimental applications in *Arabidopsis thaliana* genetics. *Plant J.* **14**, 387-392.

Nole-Wilson, S. and Krizek, B. A. (2000). DNA binding properties of the *Arabidopsis* floral development protein *AINTEGUMENTA*. *Nucleic Acids Res.* **28**, 4076-4082.

Parkhurst, S. M. (1998). Groucho: making its Marx as a transcriptional co-repressor. *Trends Genet.* **14**, 130-132.

Riechmann, J. L. and Meyerowitz, E. M. (1998). The *AP2/EREBP* family of plant transcription factors. *Biol. Chem.* **379**, 633-646.

Robbins, J., Dilworth, S. M., Laskey, R. A. and Dingwall, C. (1991). Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. *Cell* **64**, 615-623.

Roe, J. L., Nemhauser, J. L. and Zambryski, P. C. (1997). *TOUSLED* participates in apical tissue formation during gynoecium development in *Arabidopsis*. *Plant Cell* **9**, 335-353.

Roth, S. Y. (1995). Chromatin-mediated transcriptional repression in yeast. *Curr. Opin. Genet. Dev.* **5**, 168-173.

Schneitz, K., Hulskamp, M., Kopczak, S. D. and Pruitt, R. E. (1997). Dissection of sexual organ ontogenesis: a genetic analysis of ovule development in *Arabidopsis thaliana*. *Development* **124**, 1367-1376.

Sieburth, L. E. and Meyerowitz, E. M. (1997). Molecular dissection of the *AGAMOUS* control region shows that *cis* elements for spatial regulation are located intragenically. *Plant Cell* **9**, 355-365.

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

Teunissen, A. W., van den Berg, J. A. and Steensma, H. Y. (1995). Transcriptional regulation of flocculation genes in *Saccharomyces cerevisiae*. *Yeast* **11**, 435-446.

Tzamarias, D. and Struhl, K. (1994). Functional dissection of the yeast Cyc8-Tup1 transcriptional co-repressor complex. *Nature* **369**, 758-761.

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

Weigel, D. and Meyerowitz, E. M. (1993). Activation of floral homeotic genes in *Arabidopsis*. *Science* **216**, 1723-1726.

Weigel, D. and Meyerowitz, E. M. (1994). The ABCs of floral homeotic genes. *Cell* **78**, 203-209.

Williams, F. E. and Trumbly, R. J. (1990). Characterization of TUP1, a mediator of glucose repression in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **10**, 6500-6511.

Yanofsky, M. F., Ma, H., Bowman, J. L., Drews, G. N., Feldmann, K. A. and Meyerowitz, E. M. (1990). The protein encoded by the *Arabidopsis* homeotic gene *AGAMOUS* resembles transcription factors. *Nature* **346**, 35-39.