Development 133, 3496 (2006) doi:10.1242/dev.02562

APETALA1 and *SEPALLATA3* interact with *SEUSS* to mediate transcription repression during flower development

Vaniyambadi V. Sridhar, Anandkumar Surendrarao and Zhongchi Liu *Development*, **133**, 3159-3166.

The authors mistakenly cited Lenhard et al., 2001 instead of Lohmann et al., 2001 in the above paper. The citation appears in the last paragraph on p. 3165. The correct reference is shown below.

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.

The authors apologise to readers for this mistake.

Development 133, 3159-3166 (2006) doi:10.1242/dev.02498

APETALA1 and *SEPALLATA3* interact with *SEUSS* to mediate transcription repression during flower development

Vaniyambadi V. Sridhar*, Anandkumar Surendrarao† and Zhongchi Liu‡

The transcriptional repression of key regulatory genes is crucial for plant and animal development. Previously, we identified and isolated two *Arabidopsis* transcription co-repressors *LEUNIG* (*LUG*) and *SEUSS* (*SEU*) that function together in a putative co-repressor complex to prevent ectopic *AGAMOUS* (*AG*) transcription in flowers. Because neither LUG nor SEU possesses a recognizable DNA-binding motif, how they are tethered to specific target promoters remains unknown. Using the yeast two-hybrid assay and a co-immunoprecipitation assay, we showed that *APETALA1* (*AP1*) and *SEPALLATA3* (*SEP3*), both MADS box DNA-binding proteins, interacted with *SEU*. The AP1-SEU protein-protein interaction was supported by synergistic genetic interactions between *ap1* and *seu* mutations. The role of SEU proteins in bridging the interaction between AP1/SEP3 and LUG to repress target gene transcription was further demonstrated in yeast and plant cells, providing important mechanistic insights into co-repressor function in plants. Furthermore, a direct in vivo association of SEU proteins with the *AG* cis-regulatory element was shown by chromatin immunoprecipitation. Accordingly, a reporter gene driven by the *AG* cis-element was able to respond to *AP1*- and *SEP3*-mediated transcriptional repression in a transient plant cell system when supplied with SEU and LUG. These results suggest that *AP1* and *SEP3* may serve as the DNA-binding partners of SEU/LUG. Our demonstration of the direct physical interaction between SEU and the C-terminal domain of SEP3 and AP1 suggests that AP1 and SEP3 MADS box proteins may interact with positive, as well as negative, regulatory proteins via their C-terminal domains, to either stimulate or repress their regulatory targets.

KEY WORDS: AGAMOUS (AG), Co-repressors, MADS box proteins, Transcription repression, Flower

INTRODUCTION

The genetic control of floral organ identity is one of the most important examples of how regulatory genes determine plant structure. Specific combinations of the A, B, C and E classes of genes direct the development of sepals, petals, stamens and carpels in four concentric floral whorls, respectively (Coen and Meyerowitz, 1991; Jack, 2004; Theissen and Saedler, 2001). Almost all ABCE genes encode the MIK-type MADS box transcription factors that can interact with each other to form multimeric complexes for gene activation (Honma and Goto, 2001; Theissen and Saedler, 2001). It was proposed that four different multimeric protein complexes consisting of A/E, A/B/E, B/C/E and C/E protein combinations control the four organ type-specific development (Theissen and Saedler, 2001). An important aspect of this model is that the A, B and C class genes are only transcribed in specific floral whorls; their expression correlates with the domain of their function. The domain or whorl-specific expression of the A, B and C genes therefore underlies the formation of whorl-specific MADS box complexes.

Previously, we identified and characterized two transcription co-repressors, *LEUNIG* (*LUG*) and *SEUSS* (*SEU*), that play crucial roles in preventing ectopic expression of the class C gene *AGAMOUS* (*AG*) (Franks et al., 2002; Liu and Meyerowitz, 1995). In wild type, *AG* is expressed in the inner two whorls of a flower to specify stamen and carpel development (Bowman and Meyerowitz, 1991; Drews et al., 1991; Yanofsky et al., 1990). The ectopic expression of *AG* in *lug* or *seu* mutants in all four floral whorls causes partial homeotic transformations of whorl 1 sepals into carpelloid sepals, and whorl 2 petals into staminoid organs or organ loss. *lug* and *seu* exhibited a synergistic genetic interaction causing a more complete homeotic transformation from sepals to carpels, and a more severe reduction of floral organs in *lug seu* double mutants (Franks et al., 2002; Liu and Meyerowitz, 1995), suggesting that *LUG* and *SEU* are partially redundant in controlling *AG* expression.

LUG encodes a nuclear protein with an overall domain structure similar to a class of functionally related transcriptional co-repressors, including Tup1 of yeast and Groucho of *Drosophila* (Conner and Liu, 2000; Hartley et al., 1988; Williams and Trumbly, 1990). Additionally, LUG possesses a conserved N-terminal 88-amino acid domain named the LUFS domain. The N-terminal half of the LUFS domain corresponds to the Lis1-homologous (LisH) domain, which was originally identified in a series of proteins associated with human disease (Emes and Ponting, 2001), and was subsequently shown to promote dimerization, tetramerization and interaction with other proteins (Cerna and Wilson, 2005). *SEU* encodes a glutamine (Q)-rich protein with a conserved domain that is similar to the dimerization domain of the LIM-domain-binding (Ldb) family of transcriptional co-regulators, such as the *Ldb1* in mouse and *Chip* in *Drosophila* (Franks et al., 2002). SEU was shown to directly interact with the LUFS domain of LUG (Sridhar et al., 2004) and may form a co-repressor complex with LUG in *Arabidopsis* (Franks et al., 2002; Sridhar et al., 2004). This complex is likely to be evolutionarily conserved, as a direct interaction between *STYLOSA* (*STY*), the ortholog of *LUG* in *Antirrhinum*, and *AmSEUSS* was reported (Navarro et al., 2004).

Strong repressor activity of *LUG* was demonstrated by tethering *LUG* to heterologous promoters of reporter genes via the GAL4 DNA-binding domain (BD) in yeast and in plant cells (Sridhar et al., 2004). The repressor activity of *LUG* was shown to depend on histone deacetylases (Sridhar et al., 2004). By contrast, *SEU* exhibited no repressor activity when it was similarly tethered to the

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA.

*Present address: Institute for Integrative Genome Biology, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA
†Present address: Section of Plant Biology, University of California, Davis, CA 95616, USA
‡Author for correspondence (e-mail: zliu@umd.edu)

Accepted 15 June 2006

heterologous promoters of reporter genes via the GAL4-BD. Because neither LUG nor SEU possesses a recognizable DNA-binding motif, how they are tethered to specific target promoters in vivo remains unknown. Furthermore, because *LUG* and *SEU* are broadly expressed in both flowers and vegetative tissues (Conner and Liu, 2000; Franks et al., 2002), how *LUG* and *SEU* confer their outer whorl-specific repressor activity on *AG* is unknown. One attractive model that addresses both of these questions is that the putative *LUG/SEU* complex interacts with DNA-binding partners that are specifically expressed in the outer two whorls of a flower. A second model is that *LUG/SEU* may regulate *AG* indirectly by repressing the expression of positive regulators of *AG*. A third model proposes that *SEU/LUG* represses *AG* in all four floral whorls, and some factors in the inner two whorls are able to antagonize the repressor effect of *LUG/SEU*.

APETALA1 (*AP1*) and *SEPALLATA3* (*SEP3*), both MADS box proteins, belong to the A and E class floral organ identity genes, respectively, and have been shown to activate the expression of B and C class genes (Castillejo et al., 2005; Gomez-Mena et al., 2005; Weigel and Meyerowitz, 1993). In this study, we demonstrate a direct SEU-AP1 and SEU-SEP3 protein-protein interaction, as well as synergistic genetic interactions between *seu* and *ap1* mutations, indicating that AP1 and SEP3 may act as the DNA-binding partners of LUG/SEU. In vivo association of *SEU* to the *AG* cis-regulatory elements shown by chromatin immunoprecipitation eliminates the second model of an indirect role of *SEU/LUG* in *AG* regulation. A revised third model is proposed illustrating how the outer whorl-specific repression of *AG* is achieved.

MATERIALS AND METHODS

Yeast two-hybrid assay

The yeast strains PJ69-4A (James et al., 1996) and *SEU-BD* have been described previously (Sridhar et al., 2004). *AP1-AD* and *SEP3-AD* in *pACT2* (Honma and Goto, 2001), *PI-AD* (*pD1293*) in *pGAL4-C* and *AP3-BD* (*pD1294*) in *pGBDU-C* (Yang et al., 2003) were gifts from Drs K. Goto and T. Jack, respectively.

To construct *AP3-AD*, *AP3* was excised from *pD1294* as a *Bam*HI/*Pst*I fragment and inserted into *pGAL4-C* at the same sites. *AP1-MIK* and *SEP3-MIK* were excised from the *AP1-AD* and *SEP3-AD* in *pACT2* (Honma and Goto, 2001) as an *Nco*I/*Sac*I fragment and cloned into *pET30a* (Novagen) at the same sites. Subsequently, the respective MIK fragment was excised as an *Nco*I/*Xho*I fragment from above *pET30a-MIK* and inserted into *pGADT7* (Clontech). *AP1-C* and *SEP3-C* were excised from the *AP1-AD* and *SEP3-AD* in *pACT2* (Honma and Goto, 2001) as a *Sac*I/*Xho*I fragment, and cloned into *pET30a* and *pET30c* at the same sites, respectively. Subsequently, *AP1-C* and *SEP3-C* were excised as an *Nco*I/*Xho*I fragment and cloned into *pGADT7* (Clontech).

The yeast two-hybrid assay was performed as previously described (Sridhar et al., 2004). β-galactosidase activity was measured in triplicate using the Galacto Light Plus Kit (Applied Biosystems) and normalized with the OD of the culture.

Yeast repression assay

Full-length *SEU* cDNA was excised from *HFFL#7* (Sridhar et al., 2004) with *Hin*dIII/*Xho*I and inserted into *p426GALL* (Mumberg et al., 1994). As a result, *SEU* was driven by the *GALL* promoter and its expression is induced by galactose. *LUG* and $LUG_{delta}LUFS$ were expressed from *pGAD424* (Clontech) but with the GAL4-AD domain removed. Specifically, *Kpn*I and *Sma*I were used to digest *LUG-AD* and $LUG_{delta}LUFS$-*AD* in *pGAD424* (Sridhar et al., 2004), and religated to delete the *GAL4-AD*. The $pGAD_{delta}424$ control vector was similarly treated to remove the *GAL4-AD* from *pGAD424* (Clontech). *AP1-BD* in *pAS2-1* was a gift from Dr K. Goto (Honma and Goto, 2001). *SEP3-BD* was constructed by digesting *SEP3* in *pACT2* (Honma and Goto, 2001) with *Nco*I/*Xho*1. The *Nco*I/*Xho*I fragment was cloned into *pGBTK7* (Clontech) at the *Nco*I/*Sal*I sites.

For yeast strain PJ69-4A (James et al., 1996), yeast transformation and β-galactosidase assay were similarly performed as the yeast two-hybrid assay. *AP1-BD* or *SEP3-BD* was selected by –Trp, and *LUG* or $LUG_{delta}LUFS$ was selected by –Leu. *SEU* was selected by Ura3, and its expression was induced by the addition of galactose instead of glucose in the media. Data shown in Fig. 3A are averages of triplicates, and the experiment was repeated twice.

In vitro pulldown assay

His-tagged *AP1* and *AP3* were gifts from Dr X. Chen (U.C. Riverside). Full-length *AP1* and *AP3* cDNAs were cloned into the *Eco*RI/*Bam*HI sites in pRSET (Invitrogen). *API-C* and *SEP3-C* were excised from *AP1-AD* and *SEP3-AD* in *pACT2* (Honma and Goto, 2001) as a *Sac*I/*Xho*I fragment and cloned into *pET30a* and *pET30c* (Novagen), respectively. Full-length *SEP3* was excised from *SEP3-AD* in *pACT2* as an *Nco*I/*Xho*I fragment and cloned into the same sites of *pET30a* (Novagen).

Plasmid templates were used for synthesizing ^{35}S-radiolabeled AP1, SEP3 and AP3 proteins with the TnT Quick Coupled Transcription/Translation System (Promega). The TnT reaction mix (5 μl) was loaded directly onto a NuPAGE gel (Invitrogen) as the input control. For two-protein pulldown, GST-SEU protein was purified from bacteria as previously described (Sridhar et al., 2004). GST-SEU protein (5 μg) bound on the GST.Bind resin (Novagen) was incubated with 15 μl ^{35}S reaction mix from TnT for 2 hours at room temperature. The GST-SEU bound resin was washed five times with cold 1×PBS, resuspended in protein gel loading buffer, heated and loaded directly to the NuPAGE gel (Invitrogen), and run for 3 hours at 70 V.

For three-protein pulldown, MBP and MBP-LUFS proteins were purified from bacteria as previously described (Sridhar et al., 2004). MBP-LUFS proteins were bound to amylose beads. After an extensive wash with cold 1×PBS, 10 μl MBP-LUFS/amylose beads were estimated for protein concentration. MBP-LUFS/amylose beads (5 μg) were then incubated at 4°C for two hours with 5 μg SEU-GST eluted from the GST.Bind resin, as well as 15 μl ^{35}S-labeled AP3, SEP3 or AP1 from TnT. The MBP-LUFS/amylose resin was washed five times with cold 1×PBS, resuspended in protein gel loading buffer, heated and then loaded onto the NuPAGE gel.

Repression assays in plants

To construct the *pAG3′I::LUC* reporter, primer pair AGdF (5′-TGGTCTGCCTTCTACGATCC-3′) and AGdR (5′-TTAATTTCTG-CCACCGATCC-3′) was used to amplify the ~900 bp *AG* 3′ enhancer using Columbia genomic DNA as a template. The PCR product was TA-cloned into pCRII-TOPO (clone #27). The ~900 bp *AG* fragment was excised from clone #27 with *Kpn*I/*Xho*I and inserted into *–58/LUC*, a gift from Dr Krizek. *–58/LUC* was constructed by inserting the –58/+6 fragment of the 35S promoter into the *Bgl*II site of *pGL3* (Promega), destroying the *Bgl*II site (Krizek and Sulli, 2006).

Full-length *AP1* and *SEP3* were excised as an *Nco*I/*Xho*I fragment from their respective *pACT2* plasmids (Honma and Goto, 2001) and inserted into the *Nco*I/*Xho*I sites of *pSPUTK* (Stratagene). *AP1* and *SEP3* were subsequently excised from *pSPUTK* as a *Hin*dIII/*Xba*I fragment, and cloned into *pART7* (Gleave, 1992) at the same sites to yield *35S::AP1* and *35S::SEP3*. *35S::LUG* was described previously (Sridhar et al., 2004). *35S::SEU* was constructed by excising *SEU* from *HFFL#7* (Sridhar et al., 2004) as a *Sal*I/*Xba*I fragment and inserted in *pART7* at the same sites.

One microgram of *pAG3′I::*LUC, 100 ng *35S::Renilla LUC*, 1 μg *35S::AP1* (or *35S::SEP3*), 1 μg *35S::SEU* and 2 μg of *35S::LUG* were mixed and introduced into onion epidermal cells by particle bombardment following the protocol of Padmanabhan et al. (Padmanabhan et al., 2005). Total DNA was maintained constant at 5 μg per transformation by using the *pART7* vector to make up the difference between transformations. Diced onion was bombarded and followed by incubation at room temperature for 16-20 hours in a petri dish containing wet 3MM paper. Epidermal peels were removed from the onion, grounded, lysed with Passive Lysis Buffer and assayed with the Dual-Luciferase Reporter Assay System (Promega). The data shown in Fig. 3C are averages of triplicate assays. The experiment was repeated twice.

Genetic analyses

seu-1 ap1-1 and *seu-1 ap1-3* double mutants were constructed by crossing *ap1-1* or *ap1-3* with *seu-1*. Seeds of *ap1*-like F2 plants were individually collected. These *ap1*-like plants can be divided into three types. Type I plants were *ap1* single mutants, which produced 100% *ap1* in F3. Type II plants exhibited a more severe phenotype than *ap1* single mutants and segregated three types of progeny in F3, suggesting that type II were homozygous for *ap1* but heterozygous for *seu-1*. Type III plants exhibited the most severe phenotype and produced F3 progeny that all resembled their parent, indicating that type III is homozygous for *ap1-1* and *seu-1*. The segregation patterns of these mutants were verified in F4.

Chromatin immunoprecipitation

The anti-SEU chicken antibody (AA126-139A), a gift of Dr Franks, was raised by Gallus Immunotech against a peptide (CNQLLAEQ-QRNKKMEKLH) located at the N-terminal domain of SEU. To purify the anti-SEU antibody, 20 μg purified SEU-GST from bacteria was spotted onto nitrocellulose membrane and then blocked by 10% milk in 0.01% Tween-TBS (TTBS) for 1 hour at room temperature. The membrane was washed five times with TTBS and incubated with 1 ml crude anti-SEU serum overnight at 4°C. After an extensive wash with TTBS, the bound antibody was eluted with 500 μl 0.2 M Glycine (pH 2.8) and immediately neutralized with 50 μl 1 M Tris-HCl (pH 8.0). The purified antiserum was concentrated to 20 μl using the Micon centrifugal filter device (Millipore).

Chromatin immunoprecipitation was performed essentially as described by Kwon et al. (Kwon et al., 2005). Wild-type (L*er*, 1.8 g) and *seu-3* (1.8 g) inflorescences were fixed with 1% formaldehyde for 2 hours. One-twentieth of the eluted DNA was used for PCR (94°C for 1 minute; then 35 cycles of 94°C for 15 seconds, 55°C for 40 seconds and 72°C for 40 seconds). The AG-3 primer pair (5′-CTATGTACAAGTACATATACAGGAAACTC-3′ and 5′-GATAGGGTCAAATCGACCACTTGCACAG-3′) amplifies the 3′ *AG* second intron. The AG-5 primer pair (5′-GCCGTGGTCGT-CTCTATGAGTACTCTAAC-3′ and 5′-CTCCACATTAGAAAAAACC-CTGATGG-3′) amplifies the 5′ *AG* second intron. The control E1F4P primer was based on that described by Kwon et al. (Kwon et al., 2005).

RESULTS

SEU interacts with *AP1* and *SEP3* in yeast and in vitro

To test our first model on how the putative LUG/SEU complex may interact with a whorl-specific floral gene product with DNA-binding activities, we performed a yeast two-hybrid assay against several DNA-binding transcription factors in flower development, including *AP1*, *APETALA2* (*AP2*), *LEAFY* (*LFY*) and *BELLRINGER* (*BRL*) (Bao et al., 2004; Jofuku et al., 1994; Mandel et al., 1992; Weigel et al., 1992). Although *LUG* failed to interact with any of the proteins tested (data not shown), *SEU* interacted strongly with *AP1* (Fig. 1A). To test the specificity of the interaction between *SEU* and *AP1*, other MADS box genes belonging to the B and E classes were subsequently tested. A strong interaction between *SEU* and *SEP3* was found (Fig. 1A). However, an interaction between *SEU* and *AP3* or *PI* was not detected (Fig. 1A). The interaction between SEU and AP1 or SEP3 protein was confirmed by the in vitro pull-down assay. ^{35}S-labeled AP1, SEP3 or AP3 was bound to SEU-GST resins. After washing, AP1 and SEP3, but not AP3, were retained by the SEU-GST resins (Fig. 1C).

To determine the domain of AP1 or SEP3 that interacts with SEU, AP1 and SEP3 were each divided into the N-terminal MIK (MADS-box, Intervening region, and K-box) domain and the C (carboxyl) domain. In yeast, SEU failed to interact with either AP1-MIK or SEP-MIK but interacted with AP1-C and SEP3-C (Fig. 1B). The interaction of SEU with the C terminus of AP1 or SEP3 is weaker than with the full-length AP1 or SEP3 (compare Fig. 1A with 1B). This weaker interaction is confirmed by the in

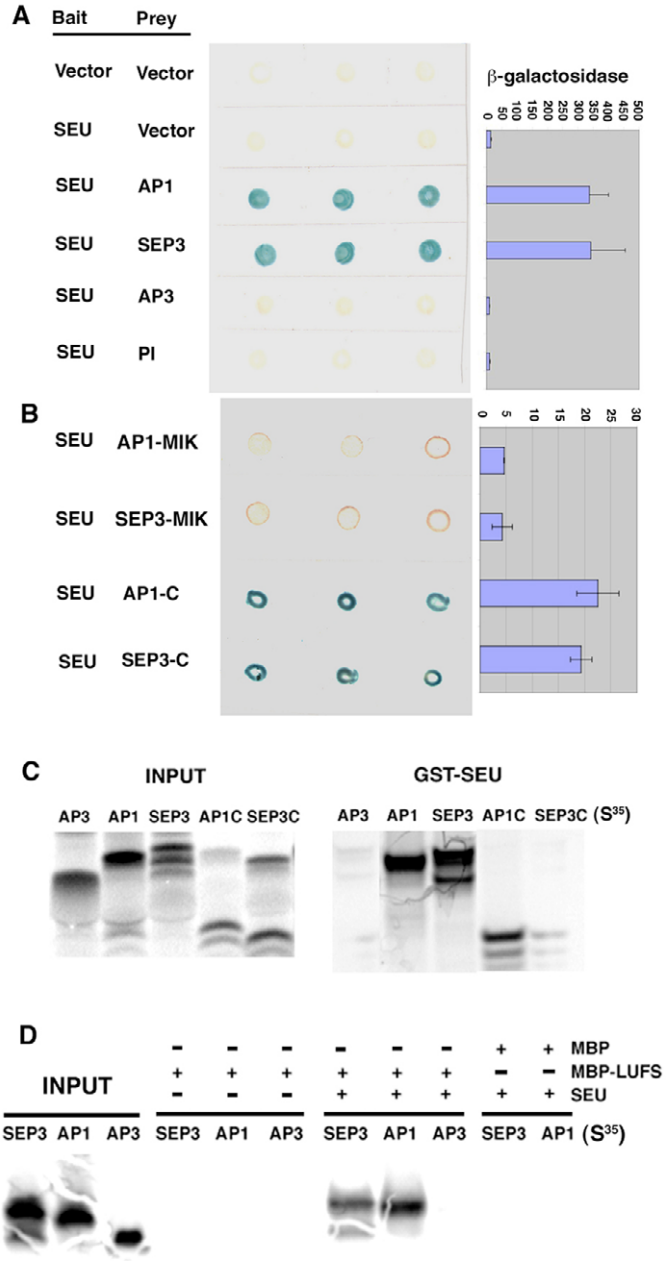

Fig. 1. Interactions between *SEU* and *AP1/SEP3* in yeast and in vitro. (**A**) A yeast two-hybrid interaction assay showing positive interactions between the *SEU-BD* bait and the *AP1-AD* or *SEP3-AD* prey. A truncated *SEU* without its Q2 and C-terminal domains was used in constructing *SEU-BD*, which no longer self-activates in yeast (Sridhar et al., 2004). Full-length *AP1*, *SEP3*, *AP3* and *PI* were fused to *GAL4-AD*. Activation of *HIS3* and *lacZ* is indicated by growth on –HIS media and by the blue color, respectively. Red colonies indicate a lack of *ADE2* reporter activation (James et al., 1996). The relative level of *lacZ* (β-galactosidase) activity is shown to the right. (**B**) A similar yeast two-hybrid interaction assay showing positive interactions between *SEU-BD* and the C-terminal domain of AP1 and SEP3 (AP1-C and SEP3-C). (**C**) An in vitro pull-down assay showing ^{35}S-labeled AP1 and SEP3 proteins retained by GST-SEU (right). Equal amounts of in vitro translated products were loaded onto the NuPAGE gel (INPUT lanes). GST alone failed to retain any of the ^{35}S proteins (data not shown). (**D**) A three-protein pull-down assay with SEU-GST serving as a bridging protein. The ability of MBP-LUFS/amylose beads to retain ^{35}S-labeled AP1, SEP3 or AP3 was tested in the presence (+) or absence (–) of SEU-GST. MBP was used as a negative control.

vitro pull-down assay (Fig. 1C). There are two possible explanations for the weak interaction between SEU and AP1-C or SEP3-C. First, AP1-C or SEP3-C protein truncations may be less stable than full-length proteins. Second, AP1 or SEP3 may require homo- or heterodimerization in order to strongly interact with SEU. Dimerization is likely to be absent for these C-terminal truncations.

The C-terminal domain is the most divergent domain of MADS box proteins with no obvious sequence similarity among AP1, SEP3, AP3 and PI except that AP1-C and SEP3-C are both rich in glutamine (Q). It remains to be seen if the glutamines in AP1-C and SEP3-C are important for the interaction with SEU, which is also Q-rich. The absence of interaction between *SEU* and the B class proteins AP3 or PI suggests that SEU may specifically interact with a subset of MADS box proteins, including *AP1* and *SEP3*. However, our assay could not exclude the possibility that the AP3/PI heterodimers maybe able to interact with SEU.

SEU bridges an interaction between *LUG* and *AP1/SEP3*

If SEU but not LUG interacts with AP1 and SEP3, could SEU bridge the interaction between LUG and AP1 or SEP3? This was tested by in vitro pull-down assays using the LUFS domain of LUG tagged by Maltose Binding Protein (MBP). The LUFS domain of LUG was previously shown to be necessary and sufficient for interacting with SEU (Sridhar et al., 2004). The interaction between LUFS-MBP and ^{35}S-labeled AP1, SEP3 or AP3 was tested in the presence or absence of purified SEU (Fig. 1D). In the absence of SEU, LUFS-MBP/amylose resin failed to retain any of the ^{35}S-labeled AP1, SEP3 and AP3 proteins. By contrast, when SEU was added, ^{35}S-labeled AP1 and SEP3, but not ^{35}S-labeled AP3, were retained by the LUFS-MBP/amylose resin. The interaction is specific to LUFS, as MBP alone failed to interact with AP1 or SEP3, even in the presence of SEU (Fig. 1D).

Genetic interactions between *ap1* and *seu*

If AP1 is a DNA-binding partner of LUG/SEU in *AG* repression, and partial functional redundancies exist among *AP1*, *LUG* and *SEU*, *ap1* mutations may exhibit a synergistic genetic interaction with *lug* and *seu*. A synergistic genetic interaction between *lug-1* and *ap1-1* was previously reported (Liu and Meyerowitz, 1995). A more dramatic transformation from sepals to carpels and a more severe reduction of whorl 2-3 organs were observed in *lug-1 ap1-1* double mutants. The enhanced phenotype correlated with an increased ectopic *AG* expression in the *lug-1 ap1-1* double mutants (Liu and Meyerowitz, 1995).

We constructed *seu-1 ap1* double mutants using weak *ap1-3* (Fig. 2C) and strong *ap1-1* (Fig. 2G,H) alleles. The genetic synergy is more striking between the weak *ap1-3* and *seu-1*. Both *seu-1* and *ap1-3* single mutants are fertile and develop petals in whorl 2 (Fig. 2A-C). In *ap1-3 seu-1* double mutant flowers, whorl 1 organs are transformed into carpelloid organs with horn-like projections and whorl 2 organs are absent (Fig. 2E). The double mutants form small siliques and exhibit reduced fertility. In addition, *ap1-3/ap1-3* plants heterozygous for *seu-1* (i.e. *ap1-3/ap1-3; seu-1/+*) exhibited a much stronger floral phenotype than did *ap1-3* single mutants (Fig. 2F). In addition to the floral phenotype, plant height is also affected, with *ap1-3/ap1-3; seu-1/+* being 75% of the height of *ap1-3* single mutants and *ap1-3 seu-1* double homozygotes being 50% of the height of *ap1-3* single mutants (Fig. 2D). *seu-1* single mutants are similar to *seu-1 ap1-3* in height. Overall, *seu-1 ap1-3* double mutants showed a floral phenotype similar to but more severe than *seu-1*, suggesting that *ap1-3* enhances *seu-1* in floral organ identity specification.

By contrast, the *ap1-1 seu-1* double mutants (Fig. 2I,J) exhibited a more dramatic enhancement in the meristem defects with inflorescences resembling, but being less severely affected than, those of *ap1 cal* double mutants. Each floral meristem gave rise to a secondary floral meristem, which generated tertiary and higher order

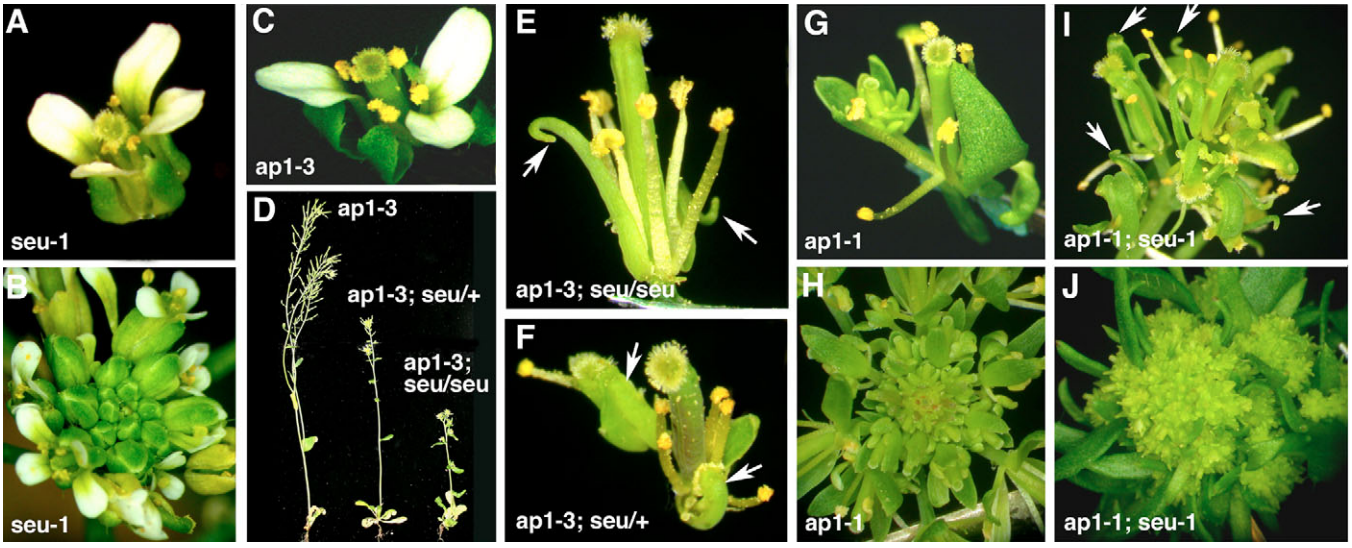

Fig. 2. Synergistic genetic interactions between *seu* and *ap1*. (**A**) A *seu-1* flower. (**B**) An inflorescence of *seu-1*. (**C**) An *ap1-3* flower. (**D**) Comparing the height of *ap1-3* plants heterozygous or homozygous for *seu-1*. (**E**) An *ap1-3 seu-1* double mutant flower. Note the complete absence of petals and the carpelloid whorl 1 organs with horn-like projections (arrows). Secondary flowers are absent. (**F**) An *ap1-3* flower heterozygous for *seu-1*. Note the complete loss of petals and the formation of carpelloid whorl 1 organs (arrows) in the primary and the secondary flowers. (**G**) An *ap1-1* flower. (**H**) An inflorescence of *ap1-1*. (**I**) An *ap1-1 seu-1* double mutant flower that produced secondary and higher order flowers. Many of the flowers exhibit carpelloid sepals (arrows). (**J**) The inflorescence of an *ap1-1 seu-1* double mutant plant. The inflorescence resembles cauliflowers, with many more higher order floral meristems.

meristems (Fig. 2J). Eventually, these floral meristems differentiated into flowers with carpelloid first whorl organs (Fig. 2J) and reduced fertility. *seu-1/+* heterozygotes also enhanced *ap1-1* giving rise to more pronounced meristem defects than did *ap1-1* single mutants (data not shown). Our data strongly support a functional relevance of *AP1-SEU* interaction, not only in organ identity specification, but also in meristem identity specification.

SEU and *LUG* modulate transcription activity of *AP1* and *SEP3* in yeast

To reveal the molecular mechanism underlying the interaction between *AP1/SEP3* and *SEU*, we tested whether direct interaction between *AP1* and *SEU*, or between *SEP3* and *SEU*, in yeast could lead to the recruitment of *LUG* and the subsequent repression of reporter gene expression. Yeast containing an integrated *GAL7-lacZ* reporter was transformed with *AP1-BD* or *SEP3-BD* in the presence or absence of *SEU* or *LUG*. *AP1-BD* and *SEP3-BD* were previously reported to activate a *lacZ* reporter via their C-terminal domain (Honma and Goto, 2001) (Fig. 3A, lanes 3, 9). This activity of *AP1-BD* or *SEP3-BD* was reduced by 50-62% when *SEU* was induced by galactose (Fig. 3A, lanes 4, 10). This was, at first, unexpected, as *SEU-BD* was previously shown to exhibit no repressor activity when tethered to heterologous promoters by GAL4-BD (Sridhar et al., 2004). The reduction of *lacZ* by *SEU* is likely to be due to a direct physical block of the AP1 and SEP3 C-terminal domain by *SEU*. *lacZ* expression was further reduced to background level when *LUG* was introduced together with *SEU* (Fig. 3A, lanes 6, 12). In the absence of *SEU*, *LUG* did not exert any repressor activity in yeast expressing *AP1-BD* or *SEP3-BD* (Fig. 3A, lanes 5, 11). The requirement for *SEU* in mediating the effect of *LUG* was further demonstrated by showing a lack of enhanced repression when $LUG_{delta}LUFS$ (LUG lacking the LUFS domain) instead of full-length *LUG* was introduced into yeast expressing *AP1-BD* or *SEP3-BD* together with *SEU* (Fig. 3A; lanes 8, 14). LUG_{delta}LUFS could no longer interact with SEU (Sridhar et al., 2004) and was unable to be recruited to repress the reporter gene.

Transcription activation and repression of *AG* can be mediated by *AP1* and *SEP3*

The second intron of *AG* was previously shown to direct *GUS* reporter expression in a pattern identical to endogenous *AG* (Busch et al., 1999; Sieburth and Meyerowitz, 1997). This *pAG-I::GUS* reporter responded to *LUG* regulation (Sieburth and Meyerowitz, 1997). The *AG* second intron can be divided into two non-overlapping but functionally redundant 5′ and 3′ enhancers (Busch et al., 1999; Deyholos and Sieburth, 2000). The 3′ enhancer contains the binding sites for LFY and WUSCHEL (WUS), as well as two CArG boxes, the target-binding sites of MADS box proteins. To test *AP1*, *SEP3*, *LUG* and *SEU* activity in a biologically relevant context, we constructed a *LUC* reporter driven by the 3′ enhancer (*pAG3′I::LUC*; Fig. 3B).

Because a direct regulatory role of *AG* by *AP1* or *SEP3* has not been established, we first tested whether *AP1* or *SEP3* could regulate *pAG3′I::LUC* expression. In a transient assay using onion epidermal cells, *pAG3′I::LUC* responded to *AP1* and *SEP3* activation (Fig. 3C, lanes 2, 6), with *SEP3* showing a higher transcription activator activity than *AP1*. To test whether *LUG* and *SEU* could repress the positive regulatory effect of *AP1* and *SEP3* on the *pAG3′I::LUC* reporter, *35S::SEU* or *35S::LUG* was co-transfected with *35S::AP1* or *35S:SEP3*. *35S::LUG* alone does not interfere with *AP1* or *SEP3* activities (Fig. 3C, lanes 3, 7). By contrast, *35S::SEU* reduced the *LUC* expression level to 48-55% (Fig. 3C, lanes 4, 8). Simultaneous introduction of *35S::LUG* and *35S::SEU* into the onion epidermal cells further reduced reporter expression to about 24-26% (Fig. 3C, lanes 5, 9), suggesting that, through its interaction with *SEU*, *LUG* is recruited to the *pAG3′I::LUC* reporter to repress its expression. This result suggests that *AP1* and *SEP3* may act upon the *AG* 3′ enhancer, possibly via the two CArG boxes. Similar to the results obtained from reporter gene expression in yeast (Fig. 3A), *AP1* and *SEP3* are converted from transcription activators to repressors simply by their interaction with the SEU/LUG co-repressor.

SEU directly associates with the *AG* 3′ enhancer in vivo

Although previous genetic and molecular analyses indicated that *LUG* and *SEU* exert their negative regulatory effect on *AG* via the second intron of *AG* (Sieburth and Meyerowitz, 1997), our second

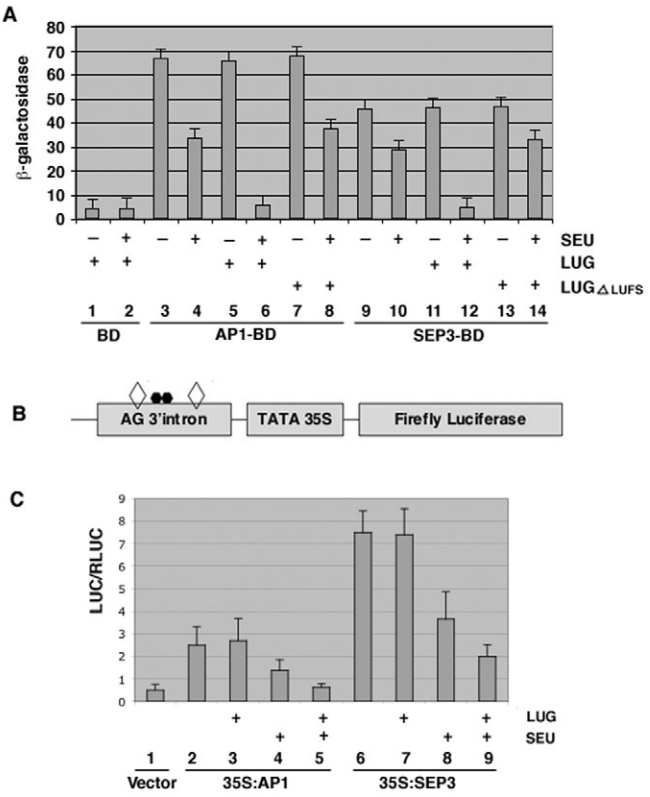

Fig. 3. *AP1* and *SEP3* mediate the repressor activity of *SEU/LUG*. (**A**) *AP1-BD* and *SEP3-BD*, with *GAL4 BD* at the N terminus of the fusion proteins, mediate the repressor activity of *SEU/LUG* in yeast, as indicated by the β-galactosidase activity from the GAL7-*lacZ* reporter. *AP1-BD* (lane 3) and *SEP3-BD* (lane 9), but not BD (pGBT9 vector; lanes 1-2), activate *lacZ* expression. This activity is reduced in the presence of *SEU* (lanes 4, 10). Full-length *LUG* (lanes 5, 6, 11, 12) and *LUG* without its LUFS domain ($LUG_{delta}LUFS$; lanes 7, 8, 13, 14) were tested in the presence or absence of *SEU*. Error bars show the standard deviation of means of triplicate assays. (**B**) Diagram of the *pAG3′I::LUC* reporter. An ~900 bp fragment from the 3′ *AG* enhancer is inserted upstream of the TATA box of the 35S promoter that drives firefly luciferase (*LUC*). The two *LFY/WUS*-binding sites (black circles) and the two CArG boxes (diamonds) are indicated. (**C**) *AP1* and *SEP3* mediate the repression of the *pAG3′I::LUC* reporter in onion cells. The ratio of *pAG3′I::LUC* and *35S::Renilla LUC* expression is shown. Onion epidermal cells were transiently transformed with *35S::AP1* or *35S::SEP3*, together with *35S::SEU* or *35S::LUG,* or *35S::SEU* plus *35S::LUG*. The *pART7* vector was a negative control. Error bars show standard deviation of means of triplicate assays.

model, that *LUG*/*SEU* represses *AG* by repressing the expression of positive regulators of *AG* such as *LFY* or *WUS*, could not be excluded. Using the chromatin immunoprecipitation (ChIP) assay, we investigated whether SEU directly associates with the *AG* 3′ enhancer in vivo. Chromatin isolated from the inflorescences of wild type and *seu-3* was precipitated by the anti-SEU antibody (αSEU Ab). *seu-3* is a nonsense mutation at residue 127 (Pfluger and Zambryski, 2004), which results in a truncated SEU protein lacking the epitope for the αSEU Ab. The αSEU Ab was able to immunoprecipitate sequences within the *AG* 3′ enhancer (AG-3), but not sequences within the 5′ enhancer (AG-5) (Fig. 4A,B). Furthermore, the AG-3 probe was precipitated only from wild-type chromatin, not from the *seu-3* chromatin. SEU protein is therefore associated with the 3′ enhancer of *AG*, supporting a direct regulatory role of *SEU* in *AG* repression. The association of SEU protein with the 3′ enhancer but not with the 5′ enhancer is consistent with the results shown in Fig. 3, where *AP1* and *SEP3* act upon the *AG* 3′ enhancer.

DISCUSSION

SEU interacts with the C-terminal domain of AP1 and SEP3

The molecular basis for the combinatorial action of the ABCE genes lies in the assembly of four different MADS box transcription factor complexes in four floral whorls to specify four floral organ types

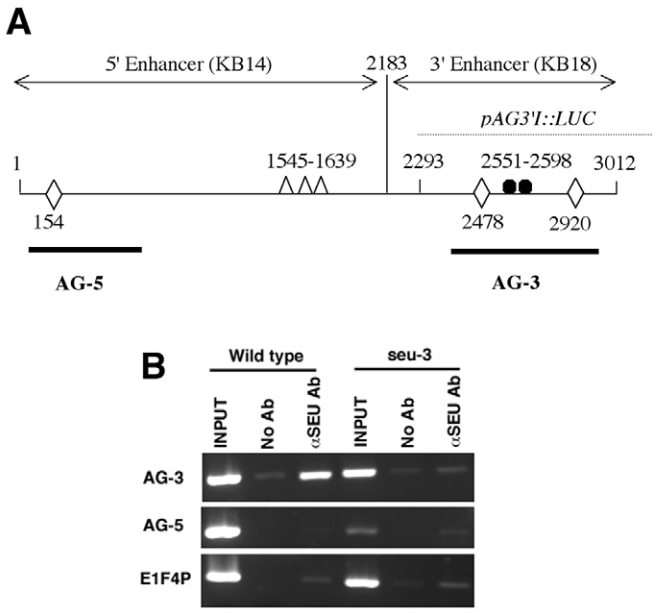

Fig. 4. In vivo association of SEU protein with *AG* 3′ enhancer. (**A**) Diagram of the *AG* second intron, which coincides with an ~3 kb *Hin*dIII fragment (Bao et al., 2004; Busch et al., 1999; Deyholos and Sieburth, 2000). Numbers indicate the nucleotide sequence, with the 5′ *Hin*dIII site designated as 1. The location of *LFY*/*WUS*-binding sites (back circles), CArG boxes (diamonds), and *BLR*-binding sites (triangles) is indicated. The two 'redundant' enhancers defined by KB14 (5′ enhancer) and KB18 (3′ enhancer) reporter lines (Busch et al., 1999), as well as the position of the *AG-5* and *AG-3* PCR products, are shown. The ~900 bp *AG* fragment in the *pAG3′I::LUC* reporter is indicated. Drawing is not to scale. (**B**) Association of SEU with the *AG* 3′ enhancer revealed by ChIP with an anti-SEU antibody. AG-5 and AG-3 are PCR products detecting immunoprecipitated wild-type and *seu-3* chromatin, respectively. The control E1F4P primer amplifies a non-regulatory target of LUG/SEU. 'No Ab' and 'αSEU Ab' correspond to chromatin treated without or with anti-SEU antibodies, respectively.

(Theissen and Saedler, 2001). Among the MADS box genes belonging to the A, B, C and E classes, *AP1* and *SEP3* are unique in that their C-terminal domain possesses strong transcription activation activity (Cho et al., 1999; Honma and Goto, 2001), which may be supplied to the ternary protein complexes. In addition, the C-terminal domains of SEP3 and SQUA, the *Antirrhinum* ortholog of *AP1*, were shown to mediate the formation of higher order MADS box protein complexes (Egea-Cortines et al., 1999; Honma and Goto, 2001).

The interaction between SEU and the C-terminal domain of AP1 or SEP3 indicates that AP1 and SEP3 also interact with non-MADS box proteins via their C-terminal domain. This observation suggests an exciting and novel mechanism in which SEU may compete with other MADS box proteins for binding to the C-terminal domain of AP1 and SEP3. Previously, SEU-BD alone was reported to exhibit no repressor activity when tethered to heterologous promoters (Sridhar et al., 2004). The reduced ability of *AP1* and *SEP3* to activate reporter gene expression in the presence of *SEU* is consistent with a mechanism in which SEU, by competing for binding to the same C-terminal domain of AP1/SEP3, prevents other transcription co-activators from interacting with AP1/SEP3. In yeast, the repressor effect of *SEU* may result from a physical block of the C-terminal domain of AP1 and SEP3 by SEU, rendering the C-terminal domain inaccessible to yeast transcription activation machineries. Therefore, the repressor effect of *SEU* is distinct from that of *LUG*, which was dependent on histone deacetylases (Sridhar et al., 2004). The recruitment of LUG by SEU further reduces target gene expression.

SEU and *AP1* may function together to regulate floral meristem identity, as well as floral organ identity

Mutations in *AP1* were previously shown to disturb two successive steps of flower development: flower meristem specification and floral organ identity specification (Bowman et al., 1993; Irish and Sussex, 1990). These two successive functions of *AP1* are reflected by the mRNA expression pattern of *AP1*, which initially is expressed throughout the young floral primordium but later (at stage 3) is only present in the outer two whorls (Mandel et al., 1992). The exclusion of *AP1* from the inner two whorls is the result of repression by *AG* (Gustafson-Brown et al., 1994).

CAULIFLOWER (*CAL*), which is highly similar to *AP1*, and *LFY*, a transcription factor, act together with *AP1* to regulate meristem identity (Ferrandiz et al., 2000; Weigel et al., 1992). *AP1*, *CAL* and *LFY* promote floral development not only by positively regulating floral organ identity genes, but also by repressing the expression of another MADS box protein, AGL24, to prevent shoot identity (Yu et al., 2004). Much of the inflorescence characters in *lfy*, *ap1* single and *ap1 cal1* double mutants were shown to result from ectopic *AGL24* expression, and *AGL24* was found to be an immediate target of transcription repression by *AP1* (Yu et al., 2004). Our observation that *ap1-1 seu-1* double mutants accumulate indeterminate inflorescence meristems similarly to *ap1-1 cal1* indicates that *SEU* may assist *AP1* in the repression of *AGL24*.

Once the floral fate is specified, *AP1* is involved in class A activity, specifying sepal and petal identity. The carpelloid floral organs in *ap1-1 seu-1* and *ap1-3 seu-1* double mutants indicate that *AP1* is involved in the negative regulation of *AG* in the outer two whorls, perhaps by its association with the SEU/LUG co-repressor. Our data indicating a role of *AP1* in *AG* repression are supported by previous genetic studies by Bowman et al. (Bowman et al., 1993), who observed staminoid or carpelloid bracts in whorl 1 at a medial

position in weak *ap1* alleles, and petalloid stamens and stamens in whorl 2 of weak and intermediate *ap1* alleles, indicating ectopic *AG* expression in weak *ap1* mutants.

Dual roles of SEP3 in floral homeotic gene activation and MADS box complex formation

The *SEP1*, *SEP2* and *SEP3* floral organ identity genes were first described as being necessary, albeit redundantly, for the normal development of petals, stamens and carpels, as triple *sep1/2/3* mutants developed flowers with indeterminate whorls of sepals, a phenotype that mirrors the double mutants of B and C genes (Pelaz et al., 2000). Recently, *SEP4* has been reported, and *sep1/2/3/4* quadruple mutants develop indeterminate flowers with only leaf-like organs (Ditta et al., 2004), indicating that all A, B and C genes require the function of *SEP* genes. This requirement was reported to be for the formation of multimeric complexes with SEP proteins supplying the transcriptional activation function to the complex (Honma and Goto, 2001).

Two recent studies indicated that *SEP3* functions at other levels in addition to being a member of organ-specific MADS box protein complexes. First, *AG* was found to autoregultate its own transcription (Gomez-Mena et al., 2005), and this positive autoregulation of *AG* requires the AG/SEP3 complex. Second, *35S::SEP3* transgenic lines resulted in the homeotic transformation of sepals into carpelloid structures (Castillejo et al., 2005). Therefore, *AG* must be activated ectopically in *35S::SEP3* plants. The same studies also suggested that ectopic *SEP3* led to ectopic *AP3* expression. Therefore, SEP3 functions both as a component of the organ-specific MADS box protein complex, and as a transcription activator of the B and C class genes.

AP1 and *SEP3* may function as both activators and repressors

Many transcription factors, including MADS box proteins, could function both as activators and repressors depending on their interaction with co-activators or co-repressors. For instance, the *MYOCYTE ENHANCER FACTOR-2* (*MEF2*) MADS box protein is capable of repressing or activating transcription by association with a variety of co-repressors or co-activators in a calcium-dependent manner (Han et al., 2003). Therefore, *AP1* may positively regulate organ identity genes such as *AG* at the early phase of meristem determination and negatively regulate *AG* at the later phase of organ identity determination by associating with different co-factors. The function of *SEP3* both as an activator of *AG* (in the AG/SEP3 complex) and as a repressor of *AG* (in the putative SEP3/SEU/LUG complex) may explain why ectopic *AG* is not observed in *sep* triple or *sep* quadruple mutants, because the role of *SEP3* in *AG* activation is epistatic to the role of *SEP3* in *AG* repression.

A proposed model

How do *LUG* and *SEU* exert their outer whorl-specific repressor effect on *AG*? We had proposed three alternative models. First, the *LUG/SEU* complex might interact with DNA-binding partners that are specifically expressed in the outer two whorls of a flower. Second, the *LUG/SEU* complex may regulate *AG* indirectly by repressing the expression of a positive regulator of *AG*. Third, *SEU/LUG* might repress *AG* in all four whorls, and some factors in the inner two whorls could antagonize the repressor effect of *LUG/SEU*.

The finding of *AP1* as a DNA-binding partner of *SEU/LUG*, at the first glance, appears to support the first model. However, several previous observations are not consistent with a role of *AP1* in providing an outer whorl-specific repressor activity of *AG*. First, in situ hybridization did not detect ectopic *AG* mRNA in strong *ap1-1* mutants, and *ap1-1* whorl 1 organs are bracts rather than carpels (Gustafson-Brown et al., 1994). Second, *35S::AP1* transgenic plants have normal stamens and carpels (Gustafson-Brown et al., 1994; Mandel and Yanofsky, 1995), suggesting that ectopic *AP1* the in inner two whorls did not lead to the repression of *AG*. Furthermore, in *ag* mutants, *AP1* activity is extended into whorls 3 and 4, but *AG* mRNA expression remained expressed in the inner two whorls. Additionally, *SEP3* is expressed in the inner three whorls and does not fit the criteria for being the outer whorl-specific DNA-binding factor. The direct association between *SEU* and the *AG* 3′ enhancer (shown in Fig. 4) also helps to eliminate the second model.

Therefore, all previous and current results are consistent with the third model, which is explained in Fig. 5. *AP1* and *SEP3* (and possibly other *SEP* genes) can all function redundantly as the DNA-binding partners of SEU/LUG, conferring repressor activity in all four whorls of a flower. Although their repressor effect is enhanced in whorls 1 and 2 by the presence of other outer-specific repressors, including *AP2* (Bowman and Meyerowitz, 1991; Chen, 2004; Jofuku et al., 1994) and *BLR* (Bao et al., 2004), it is dramatically weakened and antagonized in whorls 3 and 4 by inner whorl-specific activators of *AG*. These inner whorl-specific positive regulatory factors include the combined activities of *LFY* and *WUS* (Lenhard et al., 2001), and positive autoregulation by the AG/SEP3 complex (Gomez-Mena et al., 2005). Additionally, the AG/SEP3 complex inhibits *AP1* transcription, and an interaction between AG and SEP3 may preclude the SEU/LUG co-repressors from interacting with SEP3. As a result, *AG* is de-repressed only in whorls 3 and 4. Therefore, we propose that the domain-specific expression of *AG* is regulated by multiple factors exerting opposite regulatory effects upon *AG*.

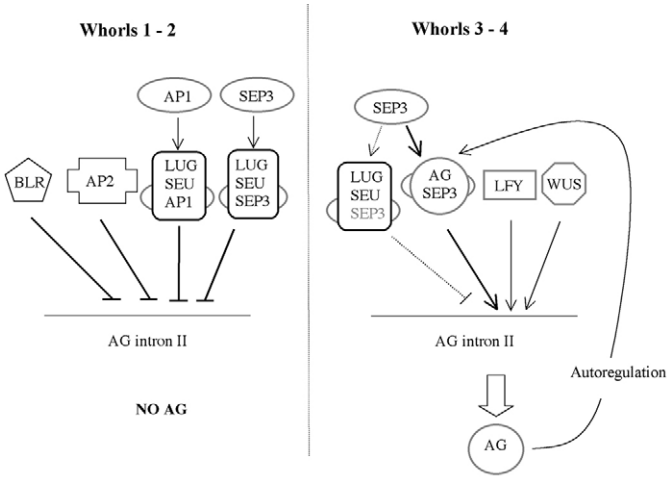

Fig. 5. A proposed model of how the inner whorl-specific activation of *AG* is achieved. In whorls 1 and 2, multiple negative regulatory activities impinge upon the *AG* cis-regulatory region (such as the *AG* intron II) to prevent *AG* transcription. These negative regulatory factors include *AP2*, *BLR* and *SEU/LUG/AP1* or *SEU/LUG/SEP3*. In whorls 3 and 4, multiple positive regulatory factors antagonize the negative effect of SEU/LUG/SEP3 to promote *AG* transcription. These positive regulatory factors include the combined activities of *LFY* and *WUS*, as well as positive autoregulation by AG/SEP3. Additionally, the AG/SEP3 complex inhibits *AP1* transcription and an interaction between AG and SEP3 may preclude the SEU/LUG co-repressors from interacting with SEP3. Arrows leading from AP1 or SEP3 to respective LUG/SEU/AP1, LUG/SEU/SEP3 or AG/SEP3 complexes indicate the incorporation of these MADS box proteins into the respective protein complexes.

Transcriptional repression is emerging as a major regulatory mechanism underlying many key developmental and signal pathways in higher plants. For example, the *Arabidopsis WUSCHEL* (*WUS*) gene was recently shown to directly interact and recruit transcription co-repressors to repress target genes involved in maintenance of the stem cell pool in the shoot apex (Kieffer et al., 2006). Similar to LUG, these *WUS*-interacting co-repressors possess an N-terminal LisH domain and C-terminal WD repeats. Therefore, insights gained from our study on transcription repression mechanisms and MADS box protein function are relevant to many areas of plant biology.

We thank Drs K. Goto, T. Jack, X. Chen and E. Krizek for plasmid constructs; D. Schubert, J. Goodrich, A. Probst, J. Paszkowski for ChIP protocols and advice; and D. Gonzalez and R. S. Conlan for advice on antiserum purification. We are indebted to Dr R. Franks for the antiserum of *SEU*. We also thank C. Li, D. Li, T. Thompson and P. Hosseini for assistance in this work; and Drs C. Chang and R. Franks for comments on the manuscript. This work was supported by U.S. Department of Agriculture Grant 2001-35304-10926 and National Science Foundation Grant IBN 0212847 to Z.L.

References

Bao, X., Franks, R. G., Levin, J. Z. and Liu, Z. (2004). Repression of AGAMOUS by BELLRINGER in floral and inflorescence meristems. *Plant Cell* **16**, 1478-1489.
Bowman, J. L. and Meyerowitz, E. M. (1991). Genetic control of pattern formation during flower development in Arabidopsis. *Symp. Soc. Exp. Biol.* **45**, 89-115.
Bowman, J. L., Alvarez, J., Weigel, D., Meyerowitz, E. M. and Smyth, D. R. (1993). Control of flower development in Arabidopsis thaliana by APETALA1 and interacting genes. *Development* **119**, 721-743.
Busch, M. A., Bomblies, K. and Weigel, D. (1999). Activation of a floral homeotic gene in Arabidopsis. *Science* **285**, 585-587.
Castillejo, C., Romera-Branchat, M. and Pelaz, S. (2005). A new role of the Arabidopsis SEPALLATA3 gene revealed by its constitutive expression. *Plant J.* **43**, 586-596.
Cerna, D. and Wilson, D. K. (2005). The structure of Sif2p, a WD repeat protein functioning in the SET3 corepressor complex. *J. Mol. Biol.* **351**, 923-935.
Chen, X. (2004). A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. *Science* **303**, 2022-2025.
Cho, S., Jang, S., Chae, S., Chung, K. M., Moon, Y. H., An, G. and Jang, S. K. (1999). Analysis of the C-terminal region of Arabidopsis thaliana APETALA1 as a transcription activation domain. *Plant Mol. Biol.* **40**, 419-429.
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.
Ditta, G., Pinyopich, A., Robles, P., Pelaz, S. and Yanofsky, M. F. (2004). The SEP4 gene of Arabidopsis thaliana functions in floral organ and meristem identity. *Curr. Biol.* **14**, 1935-1940.
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.
Egea-Cortines, M., Saedler, H. and Sommer, H. (1999). Ternary complex formation between the MADS-box proteins SQUAMOSA, DEFICIENS and GLOBOSA is involved in the control of floral architecture in Antirrhinum majus. *EMBO J.* **18**, 5370-5379.
Emes, R. D. and Ponting, C. P. (2001). A new sequence motif linking lissencephaly, Treacher Collins and oral-facial-digital type 1 syndromes, microtubule dynamics and cell migration. *Hum. Mol. Genet.* **10**, 2813-2820.
Ferrandiz, C., Gu, Q., Martienssen, R. and Yanofsky, M. F. (2000). Redundant regulation of meristem identity and plant architecture by FRUITFULL, APETALA1 and CAULIFLOWER. *Development* **127**, 725-734.
Franks, R. G., Wang, C., Levin, J. Z. and Liu, Z. (2002). SEUSS, a member of a novel family of plant regulatory proteins, represses floral homeotic gene expression with LEUNIG. *Development* **129**, 253-263.
Gleave, A. P. (1992). A versatile binary vector system with a T-DNA organisational structure conducive to efficient integration of cloned DNA into the plant genome. *Plant Mol. Biol.* **20**, 1203-1207.
Gomez-Mena, C., de Folter, S., Costa, M. M., Angenent, G. C. and Sablowski, R. (2005). Transcriptional program controlled by the floral homeotic gene AGAMOUS during early organogenesis. *Development* **132**, 429-438.
Gustafson-Brown, C., Savidge, B. and Yanofsky, M. F. (1994). Regulation of the arabidopsis floral homeotic gene APETALA1. *Cell* **76**, 131-143.
Han, A., Pan, F., Stroud, J. C., Youn, H. D., Liu, J. O. and Chen, L. (2003). Sequence-specific recruitment of transcriptional co-repressor Cabin1 by myocyte enhancer factor-2. *Nature* **422**, 730-734.
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.
Honma, T. and Goto, K. (2001). Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. *Nature* **409**, 525-529.
Irish, V. F. and Sussex, I. M. (1990). Function of the apetala-1 gene during Arabidopsis floral development. *Plant Cell* **2**, 741-753.
Jack, T. (2004). Molecular and genetic mechanisms of floral control. *Plant Cell* **16**, S1-S17.
James, P., Halladay, J. and Craig, E. A. (1996). Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* **144**, 1425-1436.
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.
Kieffer, M., Stern, Y., Cook, H., Clerici, E., Maulbetsch, C., Laux, T. and Davies, B. (2006). Analysis of the transcription factor WUSCHEL and its functional homologue in antirrhinum reveals a potential mechanism for their roles in meristem maintenance. *Plant Cell* **18**, 560-573.
Krizek, B. A. and Sulli, C. (2006). Mapping sequences required for nuclear localization and the transcriptional activation function of the Arabidopsis protein AINTEGUMENTA. *Planta* doi: 10.1007/s00425-006-0253-9.
Kwon, C. S., Chen, C. and Wagner, D. (2005). WUSCHEL is a primary target for transcriptional regulation by SPLAYED in dynamic control of stem cell fate in Arabidopsis. *Genes Dev.* **19**, 992-1003.
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.
Liu, Z. and Meyerowitz, E. M. (1995). LEUNIG regulates AGAMOUS expression in Arabidopsis flowers. *Development* **121**, 975-991.
Mandel, M. A. and Yanofsky, M. F. (1995). A gene triggering flower formation in Arabidopsis. *Nature* **377**, 522-524.
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.
Mumberg, D., Muller, R. and Funk, M. (1994). Regulatable promoters of Saccharomyces cerevisiae: comparison of transcriptional activity and their use for heterologous expression. *Nucleic Acids Res.* **22**, 5767-5768.
Navarro, C., Efremova, N., Golz, J. F., Rubiera, R., Kuckenberg, M., Castillo, R., Tietz, O., Saedler, H. and Schwarz-Sommer, Z. (2004). Molecular and genetic interactions between STYLOSA and GRAMINIFOLIA in the control of Antirrhinum vegetative and reproductive development. *Development* **131**, 3649-3659.
Padmanabhan, M. S., Goregaoker, S. P., Golem, S., Shiferaw, H. and Culver, J. N. (2005). Interaction of the tobacco mosaic virus replicase protein with the Aux/IAA protein PAP1/IAA26 is associated with disease development. *J. Virol.* **79**, 2549-2558.
Pelaz, S., Ditta, G. S., Baumann, E., Wisman, E. and Yanofsky, M. F. (2000). B and C floral organ identity functions require SEPALLATA MADS-box genes. *Nature* **405**, 200-203.
Pfluger, J. and Zambryski, P. (2004). The role of SEUSS in auxin response and floral organ patterning. *Development* **131**, 4697-4707.
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.
Sridhar, V. V., Surendrarao, A., Gonzalez, D., Conlan, R. S. and Liu, Z. (2004). Transcriptional repression of target genes by LEUNIG and SEUSS, two interacting regulatory proteins for Arabidopsis flower development. *Proc. Natl. Acad. Sci. USA* **101**, 11494-11499.
Theissen, G. and Saedler, H. (2001). Plant biology. Floral quartets. *Nature* **409**, 469-471.
Weigel, D. and Meyerowitz, E. M. (1993). Activation of floral homeotic genes in Arabidopsis. *Science* **261**, 1723-1726.
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.
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.
Yang, Y., Fanning, L. and Jack, T. (2003). The K domain mediates heterodimerization of the Arabidopsis floral organ identity proteins, APETALA3 and PISTILLATA. *Plant J.* **33**, 47-59.
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.
Yu, H., Ito, T., Wellmer, F. and Meyerowitz, E. M. (2004). Repression of AGAMOUS-LIKE 24 is a crucial step in promoting flower development. *Nat. Genet.* **36**, 157-161.