Development 134, 1901-1910 (2007) doi:10.1242/dev.003103

Specification of *Arabidopsis* floral meristem identity by repression of flowering time genes

Chang Liu[1,*], Jing Zhou[1,*], Keren Bracha-Drori[2], Shaul Yalovsky[2], Toshiro Ito[1] and Hao Yu[1,†]

Flowering plants produce floral meristems in response to intrinsic and extrinsic flowering inductive signals. In *Arabidopsis*, the floral meristem identity genes *LEAFY* (*LFY*) and *APETALA1* (*AP1*) are activated to play a pivotal role in specifying floral meristems during floral transition. We show here that the emerging floral meristems require *AP1* to partly specify their floral identities by directly repressing a group of flowering time genes, including *SHORT VEGETATIVE PHASE* (*SVP*), *AGAMOUS-LIKE 24* (*AGL24*) and *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*). In wild-type plants, these flowering time genes are normally downregulated in emerging floral meristems. In the absence of *AP1*, these genes are ectopically expressed, transforming floral meristems into shoot meristems. By post-translational activation of an AP1-GR fusion protein and chromatin immunoprecipitation assays, we further demonstrate the repression of these flowering time genes by induced AP1 activity and in vivo AP1 binding to the *cis*-regulatory regions of these genes. These findings indicate that once *AP1* is activated during the floral transition, it acts partly as a master repressor in floral meristems by directly suppressing the expression of flowering time genes, thus preventing the continuation of the shoot developmental program.

KEY WORDS: Floral meristem identity, *APETALA1*, Flowering time gene, Transcriptional regulation, *Arabidopsis*

INTRODUCTION

During floral transition in *Arabidopsis*, a complex regulatory network in response to endogenous and environmental signals mediates the activity of the floral meristem identity genes *LEAFY* (*LFY*) and *APETALA1* (*AP1*) to specify floral meristems on the flanks of the shoot apical meristem (Bowman et al., 1993; Ferrandiz et al., 2000; Huala and Sussex, 1992; Irish and Sussex, 1990; Mandel and Yanofsky, 1995b; Parcy et al., 2002; Ratcliffe et al., 1999; Weigel et al., 1992). When the activity of either gene is lost or upregulated, meristems that would normally develop into flowers are partly converted into shoot-like structures or vice versa. This implies that both genes are crucial regulators mediating the specification of floral meristems. It has been suggested that *LFY* and *AP1* antagonize the activity of the shoot identity gene *TERMINAL FLOWER 1* (*TFL1*) to establish floral meristems, which might be affected indirectly by the *TFL1* function in modulating the rate of shoot apical phase transitions in the *Arabidopsis* life cycle (Bradley et al., 1997; Liljegren et al., 1999; Parcy et al., 2002; Ratcliffe et al., 1998).

LFY plays dual roles in determining floral meristem identity and floral organ patterning via *AP1* and other floral homeotic genes (Parcy et al., 1998; Wagner et al., 1999). *AP1* is specifically expressed in young floral meristems, marking the start of flower development (Mandel et al., 1992). During floral transition, activation of *AP1* by *LFY* and a complex of *FLOWERING LOCUS T* (*FT*), a flowering time integrator, and *FD*, a bZIP transcription factor, indicates an important regulatory function of *AP1* in the specification of floral meristem identity (Abe et al., 2005; Huang et al., 2005; Wagner et al., 1999; Wigge et al., 2005).

[1]Department of Biological Sciences and Temasek Life Sciences Laboratory, National University of Singapore, Singapore 117543, Singapore. [2]Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel.

*These authors contributed equally to this work
†Author for correspondence (e-mail: dbsyuhao@nus.edu.sg)

Accepted 13 March 2007

It has been shown that *AP1* is involved in the regulation of genes promoting either floral organ formation or inflorescence commitment in floral meristems (Hill et al., 1998; Ng and Yanofsky, 2001; Tilly et al., 1998; Yu et al., 2004). Several lines of evidence suggest that *AP1* activates B class homeotic genes, especially *APETALA3* (*AP3*), to determine the identity of petals and stamens. First, whereas *AP3* expression is quite normal in *ap1* mutants and reduced in *lfy* mutants, its expression is almost undetectable in *lfy ap1* double mutants (Weigel and Meyerowitz, 1993), indicating that *AP1* can function with *LFY* to regulate *AP3* expression. Second, in vitro experiments have demonstrated the potential binding of AP1 protein to the *AP3 cis*-regulatory elements (Hill et al., 1998; Riechmann et al., 1996). Mutations in these elements abolish the AP3-specific expression (Tilly et al., 1998), suggesting that *AP1* may directly regulate *AP3* expression via these *cis*-acting regions. Lastly, expression of translational fusions of AP1 with the strong transcriptional activation domain of VP16 has revealed that AP1 can activate the expression of *AP3* and another B class gene, *PISTILLATA*, in spatially specific domains through an F-box-containing protein, *UNUSUAL FLORAL ORGANS*, in early-arising flowers (Ng and Yanofsky, 2001). While these studies suggest that *AP1* acts as transcriptional activator in floral meristem development, repression of *AGAMOUS-LIKE 24* (*AGL24*), a promoter of inflorescence identity, by *AP1* implies that *AP1* could also be a repressor (Yu et al., 2004). Thus, *AP1* may play dual roles in regulating the floral meristem development by activating or repressing different sets of genes that would determine the different fate of a floral meristem.

In this study, we show that the emerging floral meristems require *AP1* to directly repress a group of flowering time genes to partly specify their floral identities. Without *AP1* activity, the ectopic expression of these genes transforms floral meristems into various shoot structures. Therefore, *AP1* partly acts as a repressor in the floral meristem to suppress the genes required for the control of flowering time. As *AP1* expression in floral stage 1 indicates the outcome of the integration of flowering inductive signals (Mandel

Table 1. Primers used in this study

Primers used for genotyping, cloning and expression analyses		
P1	5′-GTGATCACTGTTCTCAACCAGCT-3′	
P2	5′-TGGTATATTGTCGGTGTTTACAT-3′	
P3	5′-CGGAATTCGTTGTGATGGCGAGAGAAAAGATTC-3′	
P4	5′-CGGGATCCTTCCATCTCTAACCACCATACGGT-3′	
P5	5′-CAAGGACTTGACATTGAAGAGCTTCA-3′	
P6	5′-CTGATCTCACTCATAATCTTGTCAC-3′	
P7	5′-AGCTGCAGAAAACGAGAAGCTCTCTG-3′	
P8	5′-GGGCTACTCTCTTCATCACCTCTTCC-3′	
P9	5′-GAAGCAGCAAACATGATGAAG-3′	
P10	5′-TTGCGTCTCTACTTCAGAACTTGGG-3′	
P11	5′-GTGACAAGATTATGAGTGAGATCAG-3′	
P12	5′-GAATTCACTACTTAGACATTGTCTC-3′	
P13	5′-ATCCGTGAAGAGTACCCAGAT-3′	
P14	5′-AAGAACCATGCACTCATCAGC-3′	
P15	5′-GAGGCTTTGGAGACAGAGTCGGTGA-3′	
P16	5′-AGATGGAAGCCCAAGCTTCAGGGAA-3′	
P17	5′-AACTGCAGTCGTTCCTTATAGCGGTGGAT-3′	
P18	5′-GGACTAGTTTCCCAAGATGGAAGCCTAACCAAC-3′	
P19	5′-ACAAGAGTTTTGTAATTTTCGGCCCATTATTCTCA-3′	
P20	5′-TGAGAATAATGGGCCGAAAATTACAAAACTCTTGT-3′	
Primer pairs used for ChIP assays		
AGL24-1	5′-ACAAGTTCGAAATTTGGGCCA-3′	5′-TTCACGTTTTACCATTTGCCGT-3′
AGL24-2	5′-TGCTGTTCATCAGTTCATCTACC-3′	5′-CTTATCAGGTGTCGCATCTAG-3′
AGL24-3	5′-ATCCCCAATCATACCAAGTGAC-3′	5′-GTACTGGGAAATAAGAGAGCAG-3′
AGL24-4	5′-AGTTCAATCCATCAAGATCCTCTC-3′	5′-TCTTTGGTAGACCTACTGAACA-3′
SVP-1	5′-ATGGGTTTGTAGTAGTTGCGTGGAGTA-3′	5′-TTGGGACACGATCCATTGTCCGTACAG-3′
SVP-2	5′-TTCAGTGATGATTGATACCCCC-3′	5′-CACTAATTTGGAAAGTTTGTCATGC-3′
SVP-3	5′-TCCATTTCAGTCGTCTTGTCAC-3′	5′-GAAGAGATGGAGGAGGAGGAAG-3′
SVP-4	5′-CTGATACATAGGAGTTTACTGTATC-3′	5′-GAATATTACCGTAGTTAGATACC-3′
SVP-5	5′-GATCAACCACTATCATTTTCTAACTG-3′	5′-TCTAGCTGCTGAAGCTCTTCAATGTC-3′
SOC1-1	5′-TATATCGGGAGGAGGACCACAC-3′	5′-ATCCATACAGATTTTCGGACCT-3′
SOC1-2	5′-TCTCGTACCTATATGCCCCCACT-3′	5′-TTTATCTGTTGGGATGGAAAGA-3′
SOC1-3	5′-GCAAAAGAAGTAGCTTTCCTCG-3′	5′-AGCAGAGAGAGAAGAGACGAGTG-3′
SOC1-4	5′-GGATGCAACCTCCTTTCATGAG-3′	5′-ATATGGGTTTGGTTTCATTTGG-3′
SOC1-5	5′-ATCACATCTCTTTGACGTTTGCTT-3′	5′-GCCCTAATTTTGCAGAAACCAA-3′
SOC1-6	5′-TGTTTCAGACATTTGGTCCATTTG-3′	5′-AGTCTTGTACTTTTTCCCCCTATTTTAG-3′
ACTIN	5′-CGTTTCGCTTTCCTTAGTGTTAGCT-3′	5′-AGCGAACGGATCTAGAGACTCACCTTG-3′
TUB2	5′-ATCCGTGAAGAGTACCCAGAT-3′	5′-AAGAACCATGCACTCATCAGC-3′

et al., 1992), *AP1* stands out to be a key coordinator providing feedback regulation of flowering time genes in the switch from vegetative to reproductive growth in *Arabidopsis*.

MATERIALS AND METHODS

Plant materials

All transgenic plants or mutants of the same Columbia background were used for calculating flowering time, while those in the Landsberg *erecta* (L*er*) background were used for the examination of floral phenotypes. The L*er* near-isogenic *svp-41* line was obtained by three backcrosses of the *svp-41* Columbia line (Hartmann et al., 2000) into L*er*. The *35S:SVP* construct was transformed into wild-type Columbia and L*er* plants. *35S:SOC1* and *35S::AGL24* was described elsewhere (Lee et al., 2000; Yu et al., 2002). Genotyping of *ap1-1*, *agl24-1* and *soc1-2* were performed as previously described (Ferrandiz et al., 2000; Michaels et al., 2003). For genotyping of *svp-41*, genomic DNA was amplified by PCR using the primers P1 and P2 (Table 1). The restriction enzyme *Nla*IV cleaved only the mutant DNA, generating two fragments, of 105 and 374 bp.

Plasmid construct

To produce *35S:SVP* construct, the coding region of *SVP* was amplified using primers P3 and P4 (Table 1). The resulting PCR products were cut with *Eco*RI and *Bam*HI and cloned into the corresponding sites of pGreen-0229 (Yu et al., 2004). To construct Pro_{AGL24}*:GUS*, the 4.7 kb *AGL24* genomic sequence (Fig. 7A) was amplified with the primer pair P17 and P18 (Table 1). The amplified products were digested by *Pst*I and *Spe*I and cloned into the corresponding sites of pHY107. This construct was further mutagenized to produce the mutated AP1-binding site (Fig. 7A) using the QuikChange II XL-Site-Directed Mutagenesis Kit (Stratagene) with the primer pair P19 and P20 (Table 1). A derivative pGreen-35S vector (Yu et al., 2004) was cut by *Kpn*I and *Xho*I to remove the 35S promoter, filled in the ends by T4 DNA polymerase, and self-ligated to generate a promoterless pGreen vector pHY105. A GUS fragment was then amplified from pBI101 and cloned into the *Xba*I site of pHY105 to generate pHY107.

Quantitative real-time RT-PCR

For the timecourse experiments, inflorescence apices of *ap1-1 35S:AP1-GR* containing floral buds of stages 1-10 were collected 0, 2, 4, 6 and 8 hours after a single mock- or DEX treatment. For examining the effect of the inhibition of translation on gene expression, inflorescence apices were collected 4 hours after a single mock-, DEX-, cycloheximide- and cycloheximide plus DEX treatment. Inflorescence apices of wild-type and *ap1-1* plants containing floral buds of stages 1-10 were also collected for detecting the expression of *AGL24*, *SVP* and *SOC1*. Total RNAs were extracted by RNeasy Plant Mini Kit (Qiagen) and reverse-transcribed by the ThermoScript RT-PCR system (Invitrogen). Quantitative real-time PCR assays were performed in triplicates on a 7900HT Fast Real-Time PCR system (Applied Biosystems) using tubulin (*TUB2*) as an internal standard. Diluted aliquots of the reverse-transcribed cDNAs were used as templates in quantitative PCR reactions containing the SYBR Green PCR Master Mix (Applied Biosystems). The difference between the cycle threshold (Ct) of

the target gene and the Ct of *TUB2* ($\Delta Ct=Ct_{target\ gene}-Ct_{tubulin}$) was used to obtain the normalized expression of target genes, which corresponds to $2^{-\Delta Ct}$. The expression of *AGL24*, *SVP*, *SOC1* and *TUB2* was examined by the primer pairs P15 and P16, P5 and P6, P7 and P8, and P13 and P14, respectively (Table 1).

In situ hybridization and GUS activity analysis

Non-radioactive in situ hybridization was carried out according to a published protocol (Long and Barton, 1998). For synthesis of *SOC1* and *SVP* RNA probes, the 3′ end gene-specific regions were amplified by P9 and P10 primers and P11 and P12 primers (Table 1), respectively, and cloned into pGEM-T Easy vector (Promega) to produce plasmids pHY303 (*SOC1*) and pHY305 (*SVP*). They were used as templates for in vitro transcription by the DIG RNA Labeling Kit (Roche Molecular Biochemicals). In situ localization of GUS activity was performed as previously described (Yu et al., 2002).

ChIP assays

A peptide EQWDQQNQGHNMPPPLPPQQ corresponding to amino acid residues 184-203 of AP1 was synthesized in the Peptide Synthesis Facility of the Weizmann Institute of Science, Rehovot, Israel. Synthetic peptide was conjugated to keyhole limpet hemocyanin (KLH) with the linker maleimide (Pierce) and used as an antigen for generating the anti-AP1 serum in rabbits, and for affinity purification of the antibody.

Chromatin immunoprecipitation (ChIP) assays were carried out as described previously (Ito et al., 1997; Wang et al., 2002) with minor modifications. Inflorescence tissues were collected and fixed with 1% formaldehyde for 40 minutes under vacuum. Chromatin was isolated and sonicated to produce DNA fragments under 500 bp. The solubilized chromatin was pre-cleared by incubating with normal rabbit IgG (sc-2027, Santa Cruz Biotechnology) and Protein G-Plus agarose beads (sc-2002, Santa Cruz Biotechnology) for 1 hour at 4°C. After centrifugation, the supernatant was divided equally into two parts. One part was incubated with anti-AP1 serum for 4 hours, while the other part was mixed with normal rabbit IgG as a negative control. Protein G-Plus agarose beads were then added for incubation for another hour under the same conditions. The beads were washed five times and incubated with the elution buffer supplemented with 1 μl RNase A (1 mg/ml) for 30 minutes at 37°C. Precipitated DNAs were subsequently recovered and used for enrichment test by real-time PCR assays. For ChIP assay of mock- or DEX treated samples, inflorescences of *ap1-1 35S:AP1-GR* containing floral buds of stages 1-10 were collected 4 hours after a single treatment.

We performed two fully independent ChIP experiments using samples collected separately. For each ChIP experiment, real-time PCR assay of immunoprecipitated DNAs with selected primer pairs (Table 1) was conducted in triplicate. To calculate ChIP fold enrichment, the relative amount of a target DNA fragment was first normalized against a *TUB2* genomic fragment to get the difference between the cycle threshold ($\Delta Ct=Ct_{target\ gene}-Ct_{tubulin}$). The fold enrichment was then obtained by comparing the values between DNAs immunoprecipitated by anti-AP1 serum and IgG ($\Delta\Delta Ct=\Delta Ct_{anti\text{-}AP1}-\Delta Ct_{IgG}$). The enrichment of another unrelated DNA sequence from the *ACTIN 2/7* (*ACT7* – TAIR) gene (Johnson et al., 2002) that is constitutively expressed in *Arabidopsis* was also used as a negative control. The results of the first set of ChIP experiments are shown in Fig. 6, which were also replicated in the second set of ChIP experiments (data not shown).

RESULTS

Ectopic expression of flowering time genes mimics *ap1-1* phenotypes

To elucidate the regulatory networks during floral transition, we performed a series of genetic crossings among a group of known flowering time genes. Three members of the MADS-box family of DNA-binding transcription factors, *SHORT VEGETATIVE PHASE* (*SVP*) (Hartmann et al., 2000), *AGL24* (Michaels et al., 2003; Yu et al., 2002) and *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*; also known as *AGL20* – TAIR) (Borner et al., 2000; Lee et al., 2000; Samach et al., 2000), exhibit close relationships in terms of flowering time control. While *AGL24* and *SOC1* regulate each other (Michaels et al., 2003; Yu et al., 2002), almost complete suppression of *agl24-1* and *35S:AGL24* by *svp-41* and *35S:SVP* (Gregis et al., 2006) (data not shown), respectively, suggests that *SVP* is an important repressor located downstream of *AGL24* in the regulatory network mediating floral inductive signals from multiple promotion pathways.

In addition to their effects on flowering time, *SVP*, *AGL24* and *SOC1* were distinguished from most other flowering time genes because transgenic plants overexpressing these three genes singly or in combination showed significant defects in floral meristem development. *35S:AGL24* plants often generated a central primary flower with extra secondary flowers in the axils of leaf-like sepals (Fig. 1A). The base of the ovaries of *35S:AGL24* flowers elongated

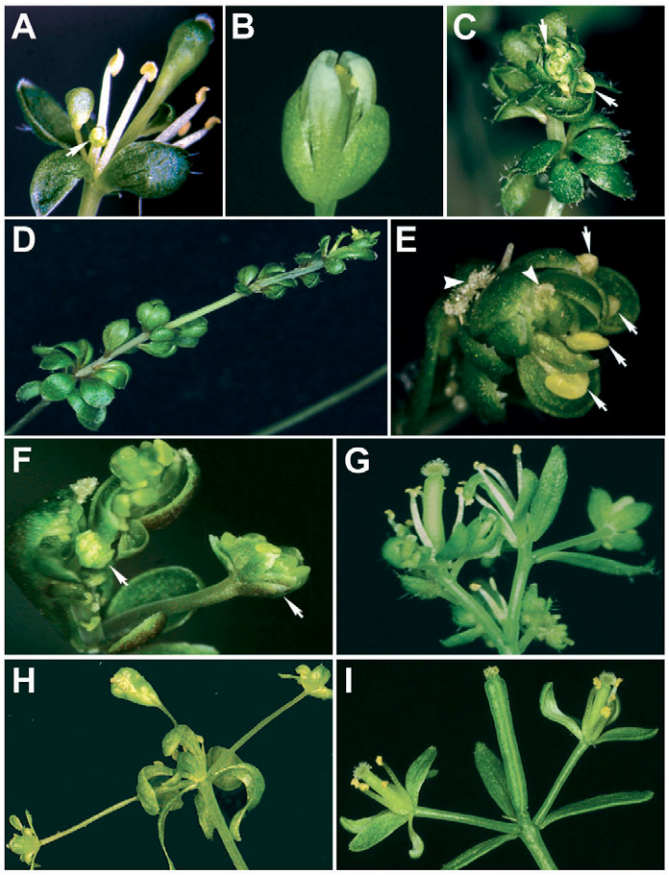

Fig. 1. Phenotypes of constitutive expression of *AGL24*, *SVP* and *SOC1*. (**A**) The *35S:AGL24 Arabidopsis* flower had leaf-like sepals and a secondary flower (arrow) without petals. (**B**) The *35S:SOC1* flower had light green sepaloid petals. (**C**) The *35S:SVP* flower was converted into a shoot-like structure. Note the formation of stamens (arrows). (**D**) Internode elongation in a *35S:SVP* flower. (**E**) The elongated *35S:SVP* flower terminated with a chimeric structure of leaves, carpelloid leaves (arrowheads) and stamens (arrows). (**F**) The *35S:AGL24 35S:SVP* flower developed like an inflorescence meristem. Note the formation of secondary flowers (arrows). (**G**) A floral structure arising from an individual floral meristem at a basal position in the main inflorescence of *ap1-1*. (**H**) The *35S:AGL24 35S:SOC1* had an increased production of secondary flowers in floral meristems at basal positions in the main inflorescence. The main inflorescence of *35S:AGL24 35S:SOC1* terminated soon after the generation of several floral structures. (**I**) A floral structure arising from an individual floral meristem at a median position in the main inflorescence of *ap1-1*.

Table 2. Number of flowers per pedicel/peduncle* in mutants and transgenic plants†

Genotype	Basal position of flowers after cauline leaf production			No. of plants scored
	1-5	6-10	11-15	
Wild type (L*er*)	1.0±0	1.0±0	1.0±0	20
ap1-1	7.8±1.5	3.7±1.2	1.9±0.3	15
soc1-2	1.0±0	1.0±0	1.0±0	20
svp-41	1.0±0	1.0±0	1.0±0	18
agl24-1	1.0±0	1.0±0	1.0±0	20
ap1-1 agl24-1	2.8±0.6	1.7±0.5	1.3±0.3	25
ap1-1 soc1-2‡	3.2±0.4	1.6±0.3	1.1±0.2	25
ap1-1 svp-41	3.6±0.8	1.8±0.4	1.2±0.2	18
ap1-1 soc1-2 agl24-1	2.0±0.2	1.2±0.3	1.1±0.2	15
ap1-1 soc1-2 svp-41	2.5±0.4	1.4±0.4	1.1±0.3	12
ap1-1 svp-41 agl24-1	2.3±0.2	1.4±0.1	1.2±0.3	15
35S::SOC1§	1.0±0	1.0±0	–	20
35S::AGL24	3.2±0.8	1.9±0.6	1.7±0.1	20
35S::SVP	1.7±0.7	1.3±0.4	1.1±0.2	20

*The term pedicel/peduncle is used to define the floral stem for either true flowers or the flowers bearing ectopic flowers or inflorescences. A floral-like structure terminated by a gynoecium is considered a flower (Bowman et al., 1993).
†Each value represents the mean±s.d.
‡Ectopic floral-like structures generated from the floral meristems at basal positions of the main inflorescences usually developed like secondary inflorescences.
§In our culture conditions, 35S::SOC1 inflorescences terminated after producing less than ten flowers.

like an inflorescence stem, and ectopic inflorescences eventually formed in the swollen ovaries (Yu et al., 2004). These phenotypes indicate the partial conversion of the floral meristem into the inflorescence meristem. *35S:SOC1* flowers were relatively normal with light green sepaloid petals (Fig. 1B). *35S:SVP* flowers usually initiated shoot-like structures with chimeric characteristics of a vegetative shoot and a flower (Fig. 1C,D). Continuous growth of *35S:SVP* flowers demonstrated that leaves emerged continuously on the substantially elongated internodes in either a whorled or a spiral mode (Fig. 1D), and stamens occasionally arose in the axils of leaves (Fig. 1C). The elongated *35S:SVP* flowers eventually terminated with a mixture of leaves, carpelloid leaves and stamens without clear carpel structures (Fig. 1E). These phenotypes demonstrated that when ectopically expressed in flowers, *SVP* promoted the shoot identity. A comparison of the average number of flowers produced in each pedicel or peduncle of *35S:AGL24*, *35S:SVP* and *35S:SOC1* revealed that overexpression *AGL24* produced more ectopic floral structures in floral meristems than *SVP* and *SOC1*, suggesting that *AGL24* plays a main role in promoting the inflorescence characteristics (Table 2).

Further combinations of the above single transgenic plants showed that the interactions among *AGL24*, *SVP* and *SOC1* transformed floral meristems into various shoot-like structures that were partially similar to the inflorescence-like floral structures in loss of function of *AP1* (Bowman et al., 1993). Flowers of double transgenic plants for *35S:AGL24 35S:SVP* developed like inflorescence shoots with the continuous production of leaves and the corresponding secondary flowers in their axils on the elongated internodes (Fig. 1F), which partially mimicked the severe defects observed in floral structures arising at basal positions of the inflorescence of *ap1-1* (Fig. 1G). Although *35S:SOC1* produced nearly normal flowers, it enhanced the production of secondary flowers in floral meristems at basal positions of the inflorescence of *35S:SOC1 35S:AGL24* (Fig. 1H), which mimicked the intermediate defects observed in floral structures arising at median positions of the inflorescence of *ap1-1* (Fig. 1I). Flowers of *35S:SOC1 35S:SVP* developed like the initial floral structures of *35S:SVP* (Fig. 1C). The internode in *35S:SOC1 35S:SVP* was not elongated and generation of secondary floral structures were only sometimes observed (data not shown). The phenotypes described above indicate that *AP1* may be a potential upstream regulator of these genes, and that misexpression of *AGL24*, *SVP* and *SOC1* in floral meristems affects normal flower development.

Inflorescence character of *ap1-1* is reduced by *agl24*, *svp* and *soc1*

We then tested if the floral phenotypes of loss of function of *AP1* were partially caused by the activity of *SVP*, *AGL24* and *SOC1*. The *ap1-1* strong mutants exhibited at least two types of defects in floral meristem specification and perianth floral organ specification. The disturbed specification of floral meristems in *ap1-1* was manifested by the phenotypes showing that flowers arising at basal positions of the *ap1-1* inflorescence generated secondary flowers or inflorescences in the axils of the leaf-like first whorl organs on the elongated internodes, whereas flowers arising at median or apical positions generated fewer or no secondary flowers in the axils of first whorl organs without internode elongation (Bowman et al., 1993). The generation of secondary flowers or inflorescences in floral structures arising from individual floral meristems at basal positions of the main inflorescence was significantly reduced in the double mutants of *ap1-1 agl24-1*, *ap1-1 svp-41* and *ap1-1 soc1-2* compared with that in *ap1-1* single mutants (Table 2 and Fig. 2B,D,F,H), and the phenotype of supernumerary inflorescence of *ap1-1* was suppressed accordingly (Fig. 2A,C,E,G). In *ap1-1 agl24-1* and *ap1-1 svp-41*, most of the flower meristems developed as single flowers occasionally with secondary flowers, but without internode elongation (Fig. 2D,F). In the flowers of *ap1-1 soc1-2*, the number of secondary floral structures was reduced compared with that in *ap1-1*, but these floral structures usually developed like inflorescences with internode elongation (Table 2 and Fig. 2H). A close examination of the mean number of floral structures produced in each pedicel or peduncle of *ap1-1 agl24-1*, *ap1-1 svp-41*, and *ap1-1 soc1-2* showed that loss of these genes function caused almost similar effect on reducing the ectopic floral structures in *ap1-1* (Table 2). Triple mutants created by genetic crossing of the above double mutants further decreased the mean number of floral structures in each pedicel or peduncle (Table 2). These observations indicate that all these three genes partly contribute to the shoot characteristics in *ap1-1* floral meristems, and that *AP1* activity may be required for the regulation of expression of these genes.

Another striking phenotype in *ap1-1* was the disruption of perianth floral organ development (Bowman et al., 1993). The first whorl sepals of *ap1-1* flowers developed into bract-like structures, whereas the second whorl petals were usually absent, especially in the floral structures derived from basal positions of the inflorescence. The sepal defect of *ap1-1* was not obviously rescued by *agl24-1*, *svp-41* and *soc1-2*, because bract-like structures are still present in the double mutants. However, *agl24-1*, rather than *svp-41* and *soc1-2*, alleviated the second whorl defect of *ap1-1* flowers as one or two petals were often observed in the flowers at basal positions of the *ap1-1 agl24-1* inflorescence (Fig. 2D,F,H).

Ectopic expression of *SVP* and *SOC1* in *ap1-1* floral meristems

In wild-type plants, *AP1* was strongly expressed in emerging floral meristems and perianth organ primordia of floral meristems after stage 3 (Fig. 3A). To examine if *AP1* acts as a repressor of flowering time genes in floral meristems, we compared the expression patterns

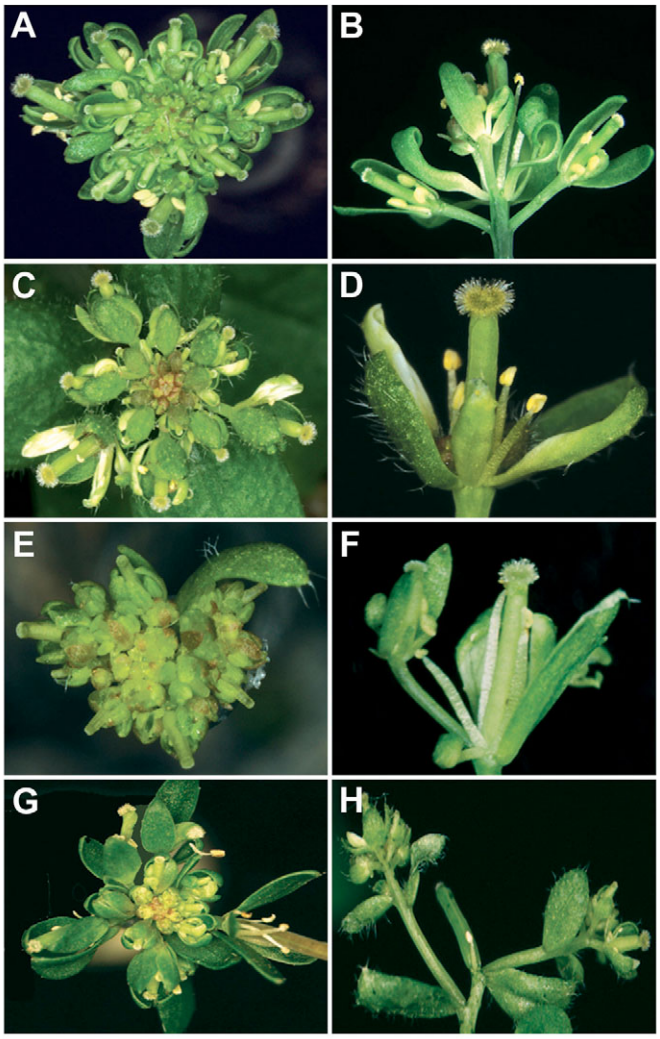

Fig. 2. Rescue of *ap1-1* by loss-of-function of *AGL24*, *SVP* and *SOC1*. (**A**,**B**) Phenotypes of *Arabidopsis ap1-1*. (**C**,**D**) Phenotypes of *ap1-1 agl24-1*. (**E**,**F**) Phenotypes of *ap1-1 svp-41*. (**G**,**H**) Phenotypes of *ap1-1 soc1-2*. Top view of a developing inflorescence is shown in A,C,E,G, while side view of a floral structure arising from an individual floral meristem at a basal position in the main inflorescence is shown in B,D,F,H.

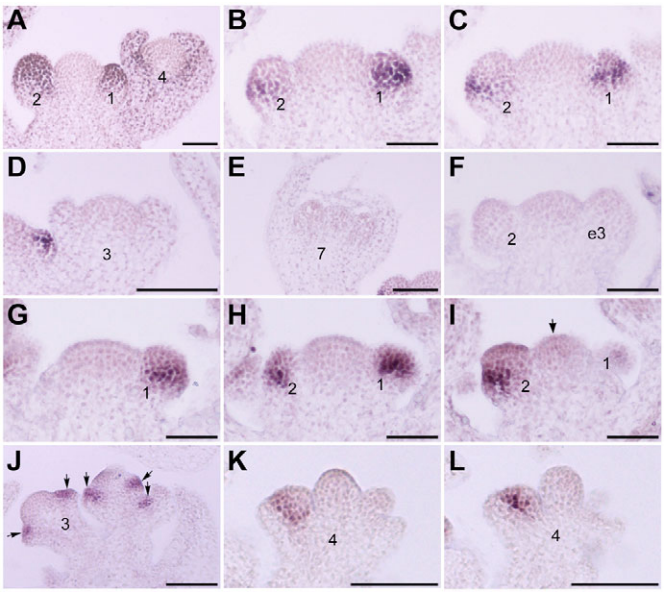

Fig. 3. In situ localization of *SVP* in wild-type and *ap1-1 Arabidopsis* plants. (**A**) A longitudinal section of a wild-type inflorescence apex hybridized with *AP1* antisense probe. (**B**,**C**) Two successive longitudinal sections of a wild-type inflorescence apex hybridized with *SVP* antisense probe. (**D**,**E**) Longitudinal sections of wild-type floral meristems at stage 3 (D) and stage 7 (E) hybridized with *SVP* antisense probe. (**F**) A longitudinal section of an *ap1-1* inflorescence apex hybridized with *SVP* sense probe. (**G-I**) Serial longitudinal sections of an *ap1-1* inflorescence apex hybridized with *SVP* antisense probe. An arrow in I indicates another floral meristem appearing at the edge of the inflorescence meristem. (**J**) Longitudinal section through *ap1-1* floral meristems at stage 3 or later stages hybridized with *SVP* antisense probe. Arrows indicate the ectopic expression of *SVP*. (**K**,**L**) Two successive longitudinal sections of an *ap1-1* stage-4 floral meristem hybridized with *SVP* antisense probe. Note the ectopic expression of *SVP* in the adaxial surface of the first whorl organs. Numbers in A-L indicate floral stages (Smyth et al., 1990). Scale bars: 100 μm for all panels.

of *SVP* and *SOC1* in floral meristems arising at basal positions of the inflorescences of wild-type and *ap1-1* plants by in situ hybridization. In wild-type plants during floral transition, *SVP* was expressed in the shoot apex and the corresponding cauline leaf (Hartmann et al., 2000). *SVP* expression was barely detectable in the inflorescence meristem, but strongly localized in the stage 1 floral meristem (Fig. 3B,C). Its expression was mainly confined to a lower part of the stage 2 floral meristem (Fig. 3B,C) and not detectable in the floral meristems after stage 3 (Fig. 3D,E). In *ap1-1* mutants, by comparing in situ hybridization results with *SVP* sense (Fig. 3F) and antisense (Fig. 3G-I) probes, we detected strong *SVP* expression in both stage 1 and stage 2 floral meristems. In *ap1-1* floral meristems at stages 3 and 4, ectopic *SVP* expression was detectable in the adaxial surface of the first whorl organs (Fig. 3J-L). At later stages of floral development, *SVP* was ectopically expressed in certain regions that might potentially emerge as new shoot meristems or floral meristems in *ap1-1* basal flowers (Fig. 3J).

The ectopic expression of *SOC1* was also observed in *ap1-1* floral meristems. In wild-type plants, *SOC1* expression was strong in the inflorescence meristem, but almost absent in the floral meristems before stage 3 (Fig. 4A-D). Its expression was again detectable in the centre of the floral meristem after stage 3 (Fig. 4E) and in the stamen and carpel primordia of later-stage floral meristems (Fig. 4F). These expression patterns are comparable to previously published results showing the subtle change of *SOC1* expression in early-stage floral meristems (Borner et al., 2000; Lee et al., 2000; Samach et al., 2000). In *ap1-1*, in situ hybridization with *SOC1* sense (Fig. 4G) and antisense (Fig. 4I-N) probes revealed that *SOC1* was ectopically expressed throughout young floral meristems from stages 1 to early stage 3 compared with its expression in wild-type floral meristems (Fig. 4A-D). *SOC1* expression was also detectable with the antisense probe in the whole zone of the floral meristems at stage 3 (Fig. 4O) or later stages (Fig. 4P) compared with background staining with the sense probe (Fig. 4G,H). These results, together

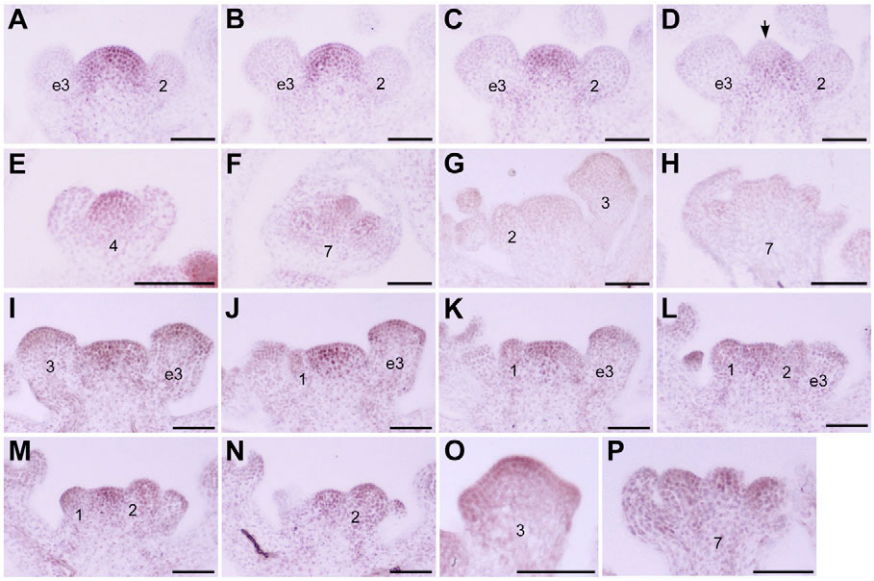

Fig. 4. In situ localization of *SOC1* in wild-type and *ap1-1 Arabidopsis* plants. (**A-D**) Serial longitudinal sections of a wild-type inflorescence apex hybridized with *SOC1* antisense probe. An arrow in D indicates another floral meristem appearing at the edge of the inflorescence meristem. (**E**,**F**) Longitudinal sections of wild-type floral meristems at stage 4 (E) and stage 7 (F) hybridized with *SOC1* antisense probe. (**G**) A longitudinal section of an *ap1-1* inflorescence apex hybridized with *SOC1* sense probe. (**H**) A longitudinal section of an *ap1-1* stage-7 floral meristem hybridized with *SOC1* sense probe. (**I-N**) Serial longitudinal sections of an *ap1-1* inflorescence apex hybridized with *SOC1* antisense probe. Note the ectopic expression of *SOC1* in floral meristems at stage 1 to early stage 3. (**O**,**P**) Longitudinal sections of *ap1-1* floral meristems at stage 3 (O) and stage 7 (P) hybridized with *SOC1* antisense probe. Note the *SOC1* expression throughout the stage 3 and stage 7 floral meristems. Numbers in A-P indicate floral stages (Smyth et al., 1990). Scale bars: 100 μm for all panels.

with the previous report showing the ectopic expression of *AGL24* in the floral meristems of *ap1-1* (Yu et al., 2004), suggest that *AP1* may be required for the repression of a group of flowering time genes in floral meristems, at least including *AGL24*, *SVP* and *SOC1*.

Induced *AP1* activity represses the expression of *SVP* and *SOC1*

In a previous study, we have established a transgenic line of *ap1-1 35S:AP1-GR*, where the biologically functional *AP1-GR* fusion could rescue *ap1-1* phenotypes in a steroid-dependent manner (Yu et al., 2004). By using this line, we found that *AGL24* expression in inflorescence apices was repressed by induced AP1 activity even in the presence of cycloheximide, an efficient inhibitor of protein synthesis, suggesting that *AGL24* is an immediate target of transcriptional repression by *AP1*.

To test whether AP1 activity is also able to repress the expression of *SVP* and *SOC1*, we used the same transgenic line of *ap1-1 35S:AP1-GR* to quantify the expression levels of both genes upon the induction of AP1 activity. Quantitative real-time RT-PCR analyses showed that dexamethasone treatment of inflorescence apices of *ap1-1 35S:AP1-GR* for 2 hours or longer resulted in continuous reduction of *SVP* and *SOC1* RNA levels relative to mock-treated controls (Fig. 5A,B). In the experiment with combined treatment of dexamethasone and cycloheximide at the 4 hour time point, the repression of *SVP* and *SOC1* by induced AP1 activity was not blocked by cycloheximide (Fig. 5C). These results suggest that both *SVP* and *SOC1*, like *AGL24*, are immediate targets of transcriptional repression by *AP1*.

We further compared the expression of *AGL24*, *SVP* and *SOC1* in wild-type and *ap1-1* inflorescence apices containing floral buds of stage 1-10 and found that the expression of these three genes was much elevated in *ap1-1* (Fig. 5D). This substantiates that *AP1* may transcriptionally repress these three genes in young floral meristems.

AGL24, *SVP* and *SOC1* are direct targets of *AP1*

To determine whether *AP1* is a direct repressor of these flowering time genes, we performed ChIP assays to detect the in vivo binding of AP1 protein to the regulatory regions of *AGL24*, *SVP* and *SOC1*. We have found that a P_{AGL24}*:GUS* reporter gene containing 4.7 kb of sequence upstream of the *AGL24* stop codon and a P_{SVP}*:GUS* reporter gene containing 5.1 kb of sequence upstream of the *SVP* stop codon could recapitulate the endogenous *AGL24* and *SVP* mRNA expression patterns (C.L., D. Li and H.Y., unpublished). Also, it has been reported that a *SOC1* genomic DNA fragment including 1.4 kb of sequence upstream of the transcriptional start site was able to complement the *soc1* mutation (Samach et al., 2000). Thus, we scanned the sequences encompassing the above regulatory regions of *AGL24*, *SVP* and *SOC1* for the CC(A/T)$_6$GG (CArG) motif (Riechmann and Meyerowitz, 1997), a canonical binding site for MADS-domain proteins, with a maximum of one nucleotide mismatch (Fig. 6A). Gene-specific primers flanking the regions near the identified CArG motifs (Table 1) were designed for quantification of the enrichment of the DNA sequences associated with the AP1 or AP1-GR fusion proteins, both of which were specifically precipitated by the anti-AP1 serum in ChIP assays (Fig. 6B-D). Real-time PCR assay of ChIP-enriched DNAs revealed that the fragments of AGL24-4, SVP-4, SOC1-1 and SOC1-2 were more significantly enriched than other fragments by the specific anti-AP1 serum over IgG in wild-type inflorescences, while all the tested DNA fragments of *AGL24*, *SVP* and *SOC1* as well as a control genomic fragment of *ACTIN2/7* gene were not enriched in *ap1-1* (Fig. 6E-G).

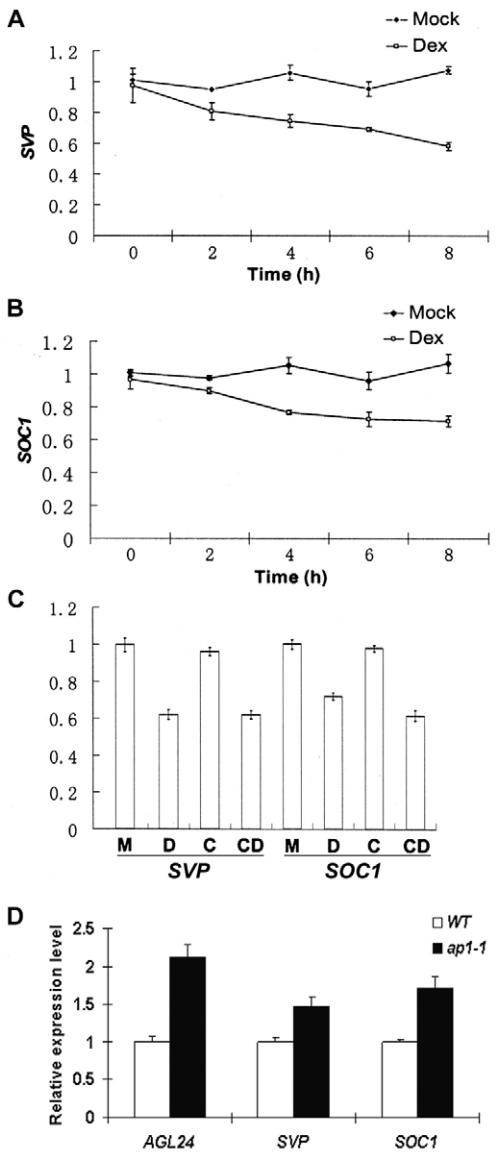

Fig. 5. Induced AP1 activity can transcriptionally repress *SVP* and *SOC1*. Transcript levels were determined by quantitative real-time PCR analyses of three independently collected replicates. Results were normalized against the expression of *TUB2*, then against the value of the first set of samples. Error bars indicate SD. (**A**,**B**) Timecourse expression of *SVP* (A) and *SOC1* (B) in inflorescence apices of *ap1-1 35S:AP1-GR* plants mock-treated (Mock) or treated with 10 μM dexamethasone (Dex). (**C**) Expression of *SVP* and *SOC1* in inflorescence apices of *ap1-1 35S:AP1-GR*, which were mock-treated (M), treated with 10 μM dexamethasone (D), with 10 μM cycloheximide (C) and with 10 μM cycloheximide plus dexamethasone (CD). Expression analyses were performed after 4 hours of treatment. (**D**) Expression of *AGL24*, *SVP* and *SOC1* in inflorescence apices of wild-type and *ap1-1* plants. Gene expression in wild-type *Arabidopsis* plants was set to one.

We further tested if these identified DNA fragments could also be specifically enriched in the dexamethasone-treated *ap1-1 35S:AP1-GR* lines, where the AP1-GR fusion protein is translocated from the cytoplasm to the nucleus and performs its function as a DNA-binding regulator. ChIP results showed that in mock-treated inflorescences of *ap1-1 35S:AP1-GR*, none of the fragments tested was enriched by the specific anti-AP1 serum over IgG, whereas in dexamethasone-treated samples, four fragments (AGL24-4, SVP-4, SOC1-1 and SOC1-2) demonstrated again highest enrichment (Fig. 6E-G). These results show that both the endogenous AP1 protein and the biologically functional AP1-GR protein interact directly with the regulatory sequences of *AGL24*, *SVP* and *SOC1* genes.

To evaluate whether the CArG motif near the identified AP1-binding site is responsible for the regulation of *AGL24* expression in floral meristems, we made a translational fusion between an *AGL24* genomic fragment containing 4.7 kb of sequence upstream of the stop codon and the GUS reporter gene (Fig. 7A). This construct provided a similar pattern of GUS staining to endogenous *AGL24* expression (Fig. 7B) (Michaels et al., 2003; Yu et al., 2004), which is strong in the inflorescence shoot apical meristem but decreased in young floral meristems. Based on this construct, we created another reporter gene cassette where the CArG motif near the identified AP1-binding site was mutated (Fig. 7A). The transformants bearing this construct exhibited ectopic GUS staining in young floral meristems (Fig. 7C). These results confirm that AP1 directly binds to the tested site to repress *AGL24* expression in young floral meristems.

DISCUSSION

Here we have shown that ectopic expression of several flowering time genes partially mimics the phenotypes of *ap1-1* floral structures (Fig. 1). Overexpression of *AGL24* transformed the floral meristem into the inflorescence meristem that had the potential to generate new floral meristems, while overexpression of *SOC1* enhanced the production of secondary flowers in flower meristems of *35S:AGL24*. Overexpression of *SVP* produced chimeric floral structures bearing the typical features of vegetative shoots, such as the continuous generation of leaves instead of floral organs. These phenotypes suggest that *SVP* functions in the maintenance of vegetative shoot identity, while *AGL24*, enhanced by *SOC1*, mainly promotes inflorescence identity. This conclusion is consistent with the respective function of *AGL24* and *SOC1* as flowering activators and of *SVP* as a flowering repressor (Borner et al., 2000; Hartmann et al., 2000; Lee et al., 2000; Michaels et al., 2003; Samach et al., 2000; Yu et al., 2002).

AP1 activity is required for repressing the ectopic expression of *AGL24*, *SVP* and *SOC1* at different floral developmental stages. Both *AGL24* (Yu et al., 2004) and *SOC1* (Fig. 4I-N) are ectopically expressed throughout the emerging *ap1-1* floral meristems, which coincides with the loss of *AP1* activity in the same regions (Mandel et al., 1992), indicating that *AP1* represses *AGL24* and *SOC1* early in emerging floral meristems. However, *SVP* expression was regulated in a different pattern. In wild-type plants, *SVP* expression is still detectable in stage 1 and 2 floral meristems despite the presence of *AP1* activity (Fig. 3B,C). In *ap1-1*, *SVP* expression slightly increases in the stage-2 floral meristem and is only detectable in the adaxial surface of the first whorl floral organs or the regions that could potentially emerge as new meristems in floral meristems after stage 2 (Fig. 3J-L). Thus, *AP1* specifically repress *SVP* mainly in floral meristems after stage 2, which may be temporally and spatially mediated by other *AP1* co-factors. In support of the suggestion that *AP1* is necessary for repression of these flowering time genes, loss of function of *AGL24*, *SVP* and *SOC1* or their combinations reduces the inflorescence character of *ap1-1* flowers at various degrees (Table 2, Fig. 2).

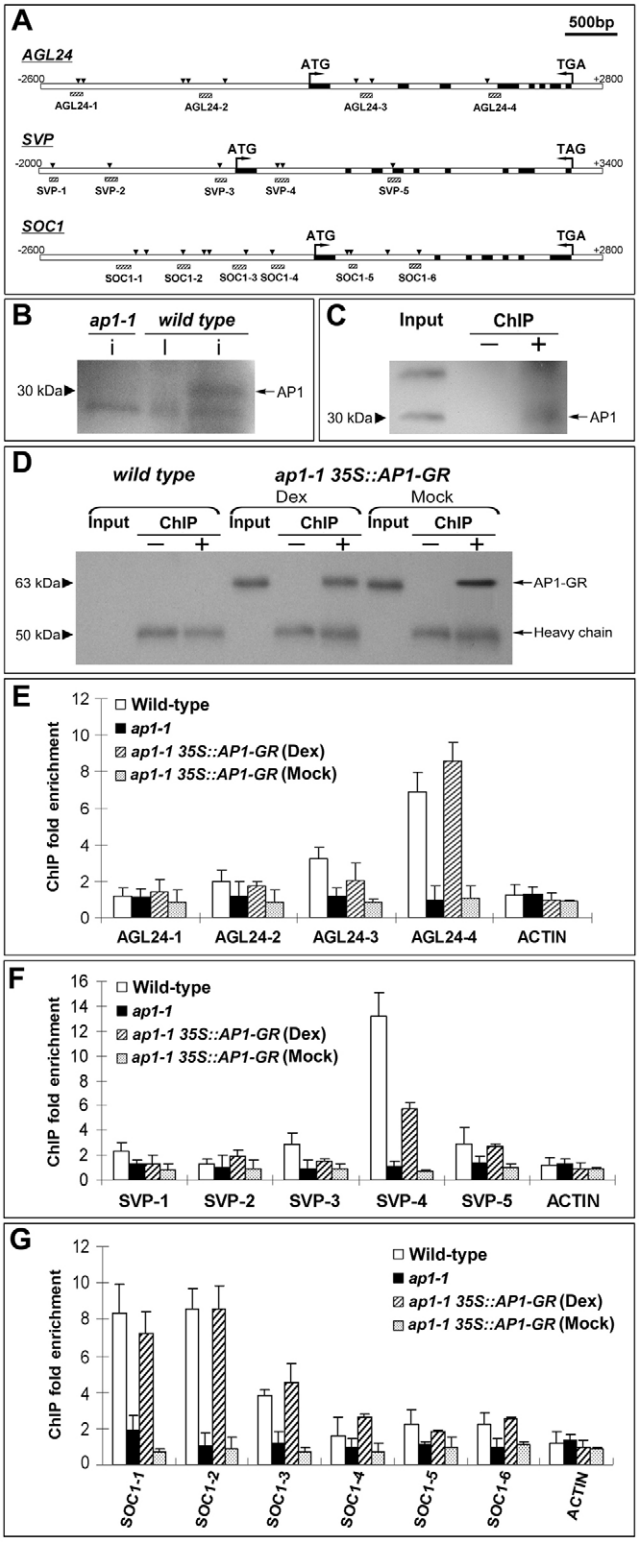

Fig. 6. AP1 directly binds to the regulatory regions of *AGL24*, *SVP* and *SOC1*. (**A**) Schematic of the genomic regions of *Arabidopsis AGL24*, *SVP* and *SOC1*. Bent arrows denote translational start sites and stop codons. Exons and introns are shown by black and white boxes, respectively. The arrowheads indicate the sites containing either single mismatch or perfect match from the consensus binding sequence (CArG box) for MADS-domain proteins. The hatched boxes represent the DNA fragments amplified in the ChIP assay. (**B**) Western analysis of nuclear extracts from inflorescences (i) of *ap1-1*, and inflorescences (i) and leaves (l) of wild-type plants probed with the purified AP1 antibody. AP1 protein was only detectable in wild-type inflorescences. (**C**) Western analysis of the specificity of anti-AP1 serum in the ChIP procedure. After sonication, the supernatant containing solubilized chromatin from wild-type inflorescence served as an input for immunoprecipitation either with IgG(–) or with anti-AP1 serum (+). Anti-AP1 serum could specifically precipitate AP1 protein. (**D**) Western analysis of the specificity of anti-AP1 serum to precipitate AP1-GR fusion protein in the ChIP procedure. After sonication, the supernatant containing solubilized chromatin from inflorescences of wild-type and *ap1-1 35S:AP1-GR* (Dex- or Mock-treated) plants served as an input for immunoprecipitation either with IgG(–) or with anti-AP1 serum (+). Anti-AP1 serum could specifically precipitate AP1-GR protein. (**E-G**) ChIP analysis of AP1 binding to regulatory sequences of *AGL24* (E), *SVP* (F) and *SOC1* (G). Real-time PCR assay of immunoprecipitated DNAs was conducted in triplicate. Relative enrichment of each target DNA fragment was calculated first by normalizing the amount of a target DNA fragment against a *TUB2* genomic fragment, and then by normalizing the value for anti-AP1 serum against the value for IgG. The enrichment of an *ACTIN 2/7* gene fragment was used as a negative control. Error bars indicate the standard error of the mean.

By post-translational activation of *AP1-GR*, we further demonstrated the repression of *SVP* and *SOC1* by induced AP1 activity (Fig. 5A,B). Moreover, downregulation of *SVP* and *SOC1* by dexamethasone treatment of *AP1-GR* inflorescence apices was not affected by cycloheximide, indicating that repression of both genes by *AP1* is independent of protein synthesis (Fig. 5C). These results, together with the previous finding (Yu et al., 2004), suggest that *AGL24*, *SVP* and *SOC1* are all early targets of transcriptional repression by *AP1*. ChIP assays using specific anti-AP1 antibodies further revealed in vivo AP1 binding to the *cis*-regulatory regions of these genes (Fig. 6), thus suggesting that *AP1* acts as a direct regulator repressing a group of flowering time genes, including *AGL24*, *SVP*, and *SOC1* in the floral meristem. A further experiment by promoter mutagenesis substantiates the idea that AP1 represses *AGL24* expression in young floral meristems by directly binding to its genomic region (Fig. 7). In our previous study (Yu et al., 2004), *AGL24* was suggested as an early target of transcriptional repression by *AP1*. However, this study could not establish whether or not *AP1* is a real transcriptional repressor, because the effect of *AP1* on *AGL24* could be mediated by other molecules such as miRNAs, which could not be revealed by applying the translation inhibitor cycloheximide in our AP1-GR inducible system. The results shown here demonstrate that *AP1* at least functions as a transcriptional repressor in wild-type floral meristems and directly represses three flowering time genes to prevent the reversion of floral meristems into shoot meristems.

Although *AP1* and *LFY* function together as major floral meristem identity genes, *LFY* and *AP1* may specify floral meristem identity by distinct mechanisms. In a previous study, we have suggested that *LFY* could repress indirectly *AGL24* expression in the floral meristem possibly via other mediators, including *AP1* (Yu et al., 2004). Unlike *AGL24*, *SOC1* and *SVP* was not ectopically expressed in *lfy-6* floral meristems (data not shown). The remaining *AP1* expression in *lfy-6* floral meristems

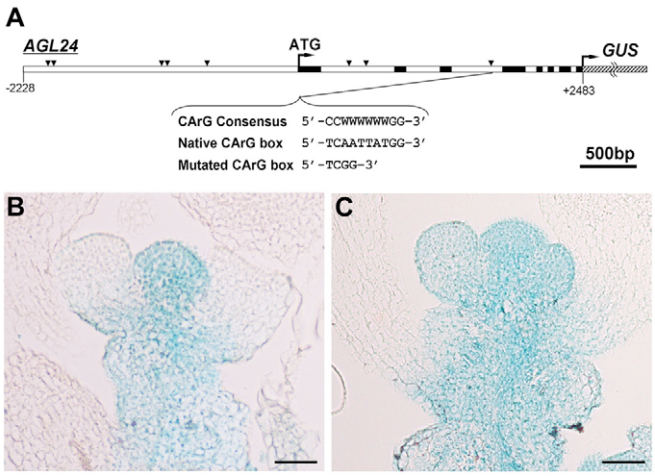

Fig. 7. Mutagenesis of AP1-binding site causes ectopic expression of *AGL24* in young floral meristems. (**A**) Schematic of the *Pro$_{AGL24}$:GUS* construct where the 4.7 kb *Arabidopsis AGL24* genomic sequence was translationally fused with the GUS gene. The native CArG box near the AGL24-4 fragment identified in Fig. 6E was mutated. (**B**,**C**) GUS staining in inflorescence apices of the transformants containing *Pro$_{AGL24}$:GUS* (B) and its derived construct with the mutated CArG box (C). At least 12 independent lines for each construct were analyzed and representative images are shown. Scale bars: 100 μm for B,C.

(Liljegren et al., 1999; Yu et al., 2004) could be sufficient to repress the ectopic expression of *SVP* and *SOC1*, but not *AGL24*, suggesting that different threshold levels of *AP1* are required for repression of different target genes. As *LFY* directly upregulates *AP1* (Wagner et al., 1999), *LFY* may partly specify floral meristem identity via mediating the expression levels of *AP1*. It has recently been shown that *AP1* is activated by a flowering complex of *FT* and *FD* that is independent of *LFY* activity (Abe et al., 2005; Huang et al., 2005; Wigge et al., 2005). Activation of *AP1* for direct repression of flowering time genes in the floral meristem could be a key regulatory pathway that is parallel with activation of *LFY* for promoting floral organ identity genes (Parcy et al., 1998; Weigel et al., 1992). Direct regulation of *AP1* by *LFY* may provide an essential channel to coordinate these two events during the specification of the floral meristem identity.

CAULIFLOWER (*CAL*) and *FRUITFULL* (*FUL*; also known as *AGL8* – TAIR) are other two regulators involved in floral meristem formation, as *ap1 cal* mutants show complete transformation of floral meristems into inflorescence meristems and *ap1 cal ful* mutants show even stronger phenotypes with more vegetative traits in the transformed meristems (Bowman et al., 1993; Ferrandiz et al., 2000; Mandel and Yanofsky, 1995a). It is possible that the flowering time genes in this study are controlled redundantly by *AP1* and *CAL*, because *AP1* and *CAL* have overlapping expression patterns and act redundantly to specify floral meristems (Bowman et al., 1993; Kempin et al., 1995). On the contrary, *FUL* may not be directly involved in the regulation of flowering time genes, as it is not expressed in floral meristems at early stages (Mandel and Yanofsky, 1995a).

Interestingly, *AP1* has shown dual functions as either an activator or a repressor in the floral meristem. Previous studies have revealed that *AP1* acts as a transcriptional activator mediating the specification of petals by regulating B class homeotic genes (Hill et al., 1998; Ng and Yanofsky, 2001), and the current study has uncovered a new facet of *AP1* as an important transcriptional repressor in preventing the reversion of floral meristems into shoot meristems. The fascinating variety of activities ascribed to *AP1* implies that it may be a part of different protein complexes or subject to various post-translational modifications that lead to different developmental regulations. One example is that AP1 protein could be farnesylated both in vitro and in vivo and that the non-prenylated form of AP1 could generate novel phenotypes when ectopically expressed in *Arabidopsis* (Yalovsky et al., 2000), implying that protein farnesylation plays a role in modulating AP1 function.

It is noteworthy that yeast two-hybrid assays have revealed broad protein interactions between three flowering time regulators examined in this study (SOC1, AGL24 and SVP) and some floral organ identity genes (de Folter et al., 2005). In particular, the protein interaction of AP1 and AGL24 or AP1 and SVP may mediate flower development at early stages (de Folter et al., 2005; Gregis et al., 2006; Pelaz et al., 2001). When the double mutants *svp agl24* were grown at 30°C, their flowers exhibited homeotic transformation in all four whorls of floral organs due to ectopic expression of function B and C homeotic genes. The similar floral defects were also observed under normal growth conditions (22°C) in *ap1 svp agl24* (Gregis et al., 2006). These phenotypes were similar to those observed in the single or double mutants of *leunig* (*lug*) and *seuss* (*seu*) (Franks et al., 2002; Liu and Meyerowitz, 1995). In vitro assays further revealed that the MADS-box dimers AP1-AGL24 and AP1-SVP weakly interacted with the *LUG-SEU* co-repressor in yeast, indicating that AP1, together with AGL24 and SVP, is involved in the recruitment of *LUG-SEU* repressor complex for the regulation of flower development (Gregis et al., 2006). Transcriptional regulation of flowering time genes by *AP1* mediates the specification of floral meristems, and possibly affects the components involved in the protein interactions required for further floral organ development. An intriguing aspect is to investigate whether recruitment of different components into an AP1 protein complex would cause distinct setting of transcriptional activities of AP1.

The repressive function of *AP1* seems crucial for determining the identities of perianth floral organs, because ectopic expression of several flowering time genes in the absence of *AP1* is sufficient to transform perianth organs into new flowers or inflorescences with or without internode elongation. This significantly affects the structure of floral perianth organs. The orthologs of *Arabidopsis AP1*, termed eu*AP1* gene clade, are only present in the core eudicots that comprise the majority of extant angiosperm species (Litt and Irish, 2003). The fixed floral perianth structures in these plants are in contrast to the plastic ones in non-eudicot and non-core eudicot species. It will be interesting to examine if the orthologs of the flowering time genes revealed in this study are normally expressed in the flowers of non-eudicot and non-core eudicot species that lack eu*AP1* genes. This will be important for addressing the puzzle of whether repression of flowering time genes by *AP1* orthologs contributes to the variation of floral perianth structures in flowering plants.

We thank Peter Huijser for providing *svp-41* seeds; Prakash P. Kumar, Yuehui He and members of the laboratory of Hao Yu for critical reading of the manuscript. This work was supported by Academic Research Funds R-154-000-232-101 from the National University of Singapore and R-154-000-263-112 from the Ministry of Education, Singapore, and the intramural research funds from Temasek Life Sciences Laboratory.

References

Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y., Ichinoki, H., Notaguchi, M., Goto, K. and Araki, T. (2005). FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* **309**, 1052-1056.

Borner, R., Kampmann, G., Chandler, J., Gleissner, R., Wisman, E., Apel, K. and Melzer, S. (2000). A MADS domain gene involved in the transition to flowering in Arabidopsis. *Plant J.* **24**, 591-599.

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.

Bradley, D., Ratcliffe, O., Vincent, C., Carpenter, R. and Coen, E. (1997). Inflorescence commitment and architecture in Arabidopsis. *Science* **275**, 80-83.

de Folter, S., Immink, R. G., Kieffer, M., Parenicova, L., Henz, S. R., Weigel, D., Busscher, M., Kooiker, M., Colombo, L., Kater, M. M. et al. (2005). Comprehensive interaction map of the Arabidopsis MADS Box transcription factors. *Plant Cell* **17**, 1424-1433.

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.

Gregis, V., Sessa, A., Colombo, L. and Kater, M. M. (2006). AGL24, SHORT VEGETATIVE PHASE, and APETALA1 redundantly control AGAMOUS during early stages of flower development in Arabidopsis. *Plant Cell* **18**, 1373-1382.

Hartmann, U., Hohmann, S., Nettesheim, K., Wisman, E., Saedler, H. and Huijser, P. (2000). Molecular cloning of SVP: a negative regulator of the floral transition in Arabidopsis. *Plant J.* **21**, 351-360.

Hill, T. A., Day, C. D., Zondlo, S. C., Thackeray, A. G. and Irish, V. F. (1998). Discrete spatial and temporal cis-acting elements regulate transcription of the Arabidopsis floral homeotic gene APETALA3. *Development* **125**, 1711-1721.

Huala, E. and Sussex, I. M. (1992). LEAFY interacts with floral homeotic genes to regulate Arabidopsis floral development. *Plant Cell* **4**, 901-913.

Huang, T., Bohlenius, H., Eriksson, S., Parcy, F. and Nilsson, O. (2005). The mRNA of the Arabidopsis gene FT moves from leaf to shoot apex and induces flowering. *Science* **309**, 1694-1696.

Irish, V. F. and Sussex, I. M. (1990). Function of the apetala-1 gene during Arabidopsis floral development. *Plant Cell* **2**, 741-753.

Ito, T., Takahashi, N., Shimura, Y. and Okada, K. (1997). A serine/threonine protein kinase gene isolated by an in vivo binding procedure using the Arabidopsis floral homeotic gene product, AGAMOUS. *Plant Cell Physiol.* **38**, 248-258.

Johnson, L., Cao, X. and Jacobsen, S. (2002). Interplay between two epigenetic marks. DNA methylation and histone H3 lysine 9 methylation. *Curr. Biol.* **12**, 1360-1367.

Kempin, S. A., Savidge, B. and Yanofsky, M. F. (1995). Molecular basis of the cauliflower phenotype in Arabidopsis. *Science* **267**, 522-525.

Lee, H., Suh, S. S., Park, E., Cho, E., Ahn, J. H., Kim, S. G., Lee, J. S., Kwon, Y. M. and Lee, I. (2000). The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in Arabidopsis. *Genes Dev.* **14**, 2366-2376.

Liljegren, S. J., Gustafson-Brown, C., Pinyopich, A., Ditta, G. S. and Yanofsky, M. F. (1999). Interactions among APETALA1, LEAFY, and TERMINAL FLOWER1 specify meristem fate. *Plant Cell* **11**, 1007-1018.

Litt, A. and Irish, V. F. (2003). Duplication and diversification in the APETALA1/FRUITFULL floral homeotic gene lineage: implications for the evolution of floral development. *Genetics* **165**, 821-833.

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

Long, J. A. and Barton, M. K. (1998). The development of apical embryonic pattern in Arabidopsis. *Development* **125**, 3027-3035.

Mandel, M. A. and Yanofsky, M. F. (1995a). The Arabidopsis AGL8 MADS box gene is expressed in inflorescence meristems and is negatively regulated by APETALA1. *Plant Cell* **7**, 1763-1771.

Mandel, M. A. and Yanofsky, M. F. (1995b). 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.

Michaels, S. D., Ditta, G., Gustafson-Brown, C., Pelaz, S., Yanofsky, M. and Amasino, R. M. (2003). AGL24 acts as a promoter of flowering in Arabidopsis and is positively regulated by vernalization. *Plant J.* **33**, 867-874.

Ng, M. and Yanofsky, M. F. (2001). Activation of the Arabidopsis B class homeotic genes by APETALA1. *Plant Cell* **13**, 739-753.

Parcy, F., Nilsson, O., Busch, M. A., Lee, I. and Weigel, D. (1998). A genetic framework for floral patterning. *Nature* **395**, 561-566.

Parcy, F., Bomblies, K. and Weigel, D. (2002). Interaction of LEAFY, AGAMOUS and TERMINAL FLOWER1 in maintaining floral meristem identity in Arabidopsis. *Development* **129**, 2519-2527.

Pelaz, S., Gustafson-Brown, C., Kohalmi, S. E., Crosby, W. L. and Yanofsky, M. F. (2001). APETALA1 and SEPALLATA3 interact to promote flower development. *Plant J.* **26**, 385-394.

Ratcliffe, O. J., Amaya, I., Vincent, C. A., Rothstein, S., Carpenter, R., Coen, E. S. and Bradley, D. J. (1998). A common mechanism controls the life cycle and architecture of plants. *Development* **125**, 1609-1615.

Ratcliffe, O. J., Bradley, D. J. and Coen, E. S. (1999). Separation of shoot and floral identity in Arabidopsis. *Development* **126**, 1109-1120.

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

Riechmann, J. L., Wang, M. and Meyerowitz, E. M. (1996). DNA-binding properties of Arabidopsis MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA and AGAMOUS. *Nucleic Acids Res.* **24**, 3134-3141.

Samach, A., Onouchi, H., Gold, S. E., Ditta, G. S., Schwarz-Sommer, Z., Yanofsky, M. F. and Coupland, G. (2000). Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. *Science* **288**, 1613-1616.

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

Tilly, J. J., Allen, D. W. and Jack, T. (1998). The CArG boxes in the promoter of the Arabidopsis floral organ identity gene APETALA3 mediate diverse regulatory effects. *Development* **125**, 1647-1657.

Wagner, D., Sablowski, R. W. and Meyerowitz, E. M. (1999). Transcriptional activation of APETALA1 by LEAFY. *Science* **285**, 582-584.

Wang, H., Tang, W., Zhu, C. and Perry, S. E. (2002). A chromatin immunoprecipitation (ChIP) approach to isolate genes regulated by AGL15, a MADS domain protein that preferentially accumulates in embryos. *Plant J.* **32**, 831-843.

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.

Wigge, P. A., Kim, M. C., Jaeger, K. E., Busch, W., Schmid, M., Lohmann, J. U. and Weigel, D. (2005). Integration of spatial and temporal information during floral induction in Arabidopsis. *Science* **309**, 1056-1059.

Yalovsky, S., Rodriguez-Concepcion, M., Bracha, K., Toledo-Ortiz, G. and Gruissem, W. (2000). Prenylation of the floral transcription factor APETALA1 modulates its function. *Plant Cell* **12**, 1257-1266.

Yu, H., Xu, Y., Tan, E. L. and Kumar, P. P. (2002). AGAMOUS-LIKE 24, a dosage-dependent mediator of the flowering signals. *Proc. Natl. Acad. Sci. USA* **99**, 16336-16341.

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.