

# Whorl-Specific Expression of the *SUPERMAN* Gene of *Arabidopsis* Is Mediated by *cis* Elements in the Transcribed Region

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## Summary

The *SUPERMAN* (*SUP*) gene of *Arabidopsis* is involved in controlling cell proliferation in stamen and carpel primordia and in ovules during flower development [1–5]. The *SUP* gene encodes a transcription factor with a C2H2-type zinc finger motif, a serine/proline-rich domain, a basic domain, and a leucine-zipper-like domain and is expressed in a very limited region in stamen primordia and in the developing ovary during flower development [3, 5]. The *SUP* gene is susceptible to methylation, resulting in epigenetic gene silencing [6–11]. To understand how the *SUP* gene is expressed spatially and temporally in its restricted domain, and why methylation of the transcribed region affects early-stage *SUP* expression, we have identified the *SUP cis* regulatory elements by characterizing *SUP* gene fusions. These studies show that the *SUP* gene has discrete upstream promoter elements required for expression in stamen primordia in early stages and in the ovary in later stages. The promoter activity for stamen primordia is modulated by several positive and negative elements located in the transcribed and translated regions. Several regulatory elements in the transcribed region correlate with the areas of the gene that are heavily methylated in epigenetic alleles; these data provide a possible explanation of how methylation of the transcribed region represses transcription.

## Results and Discussion

### The 5' Promoter Region Is Not Sufficient for Normal *SUP* Gene Expression

The loss-of-function *superman* (*sup*) mutant can be fully complemented by introducing a 6.7 kb *SUP* genomic fragment (8237177–8243842 of chromosome 3) that contains 5.1 kb of the 5' upstream region, 1.1 kb of the transcribed region, and 0.6 kb of the 3' nontranscribed region [3]. Preliminary work suggested that the sequences required for normal *SUP* gene expression might not simply be within the 5' upstream region (data not shown). To identify DNA sequence determinants responsible for the expression of the *SUP* gene, we newly generated 16 different *SUP*-GUS reporter gene constructs as translational fusions by adjusting the sequence to match the *uidA* gene open reading frame to

*SUP* (Figure 1 and see below) and transformed them into *Arabidopsis* (ecotype *Landsberg erecta*). Consistent with the data from *in situ* mRNA hybridization, which indicate that *SUP* RNA is far from abundant, *SUP*-GUS expression was so weak that 20–24 hr of incubation of the staining reaction was necessary to detect GUS activity. Although some variability in stain intensity between independently transformed plants was observed, the major GUS staining patterns seen in at least five independently transformed lines from at least six independent transformants are described, and these results are summarized in Figure 1 (also see below).

### *SUP*-GUS Expression at Early Stages Is Negatively Regulated by a Protein-Coding Region

Transgenic lines carrying construct #1, which has the *uidA* gene inserted 28 bp upstream (BsaBI site) from the termination codon in the complete 6.7 kb *SUP* genomic fragment, showed GUS staining in the developing ovary at floral stage 9 and later; this pattern matches the normal *SUP* expression pattern in the ovary (Figures 1 and 2A). In stage-9 flowers, *SUP*-GUS staining was observed in the inner surface of the developing ovary (Figures 2F and 2J), and, at stage 10, *SUP* continues to express in the center of the septum (Figures 2G and 2K). Later, at stages 11–12, septum expression decreases and GUS staining starts to appear in the funiculus of developing ovules (Figures 2H and 2L). At stage 14, the *SUP*-GUS construct continues to express in the funiculus of the ovules (Figure 2I). However, in these lines, no GUS staining was observed in stamen primordia at stages 3–8 (Figures 2A, 2B, and 2E).

To test if *SUP*-GUS expression at early stages is negatively regulated by a protein-coding region, a NcoI fragment (8242572–8242812 of chromosome 3) encoding the zinc finger and N-terminal half of the serine/proline-rich domain was deleted from construct #1 (Figure 1, construct #2). Transgenic plants carrying construct #2 showed GUS staining not only in the ovary, but also in stamen primordia (Figures 2C, 2D, and 2M–2Q). GUS activity was ectopically observed everywhere in whorl 3 and in neighboring whorl 4 of developing flowers from stage 3 through stage 4 (Figures 2M and 2N). At late stage 5 or stage 6, ectopic staining in whorl 4 tissue begins to be reduced (Figure 2O). At stage 7, the *SUP*-GUS staining was limited to stamen primordia and was strongly localized to their adaxial sides; this expression pattern is comparable to normal *SUP* expression (Figure 2P) [3]. Later in stage 9, *SUP*-GUS staining in the ovary was observed, first on the inner surface of the carpels, and later in the funiculus of each ovule; this staining pattern is consistent with endogenous *SUP* gene expression (Figure 2Q).

### Early-Stage *SUP*-GUS Expression Conferred by Construct #1 Is Suppressed at the mRNA Level

In order to determine whether the GUS reporter gene activities observed in constructs #1 and #2 correlate

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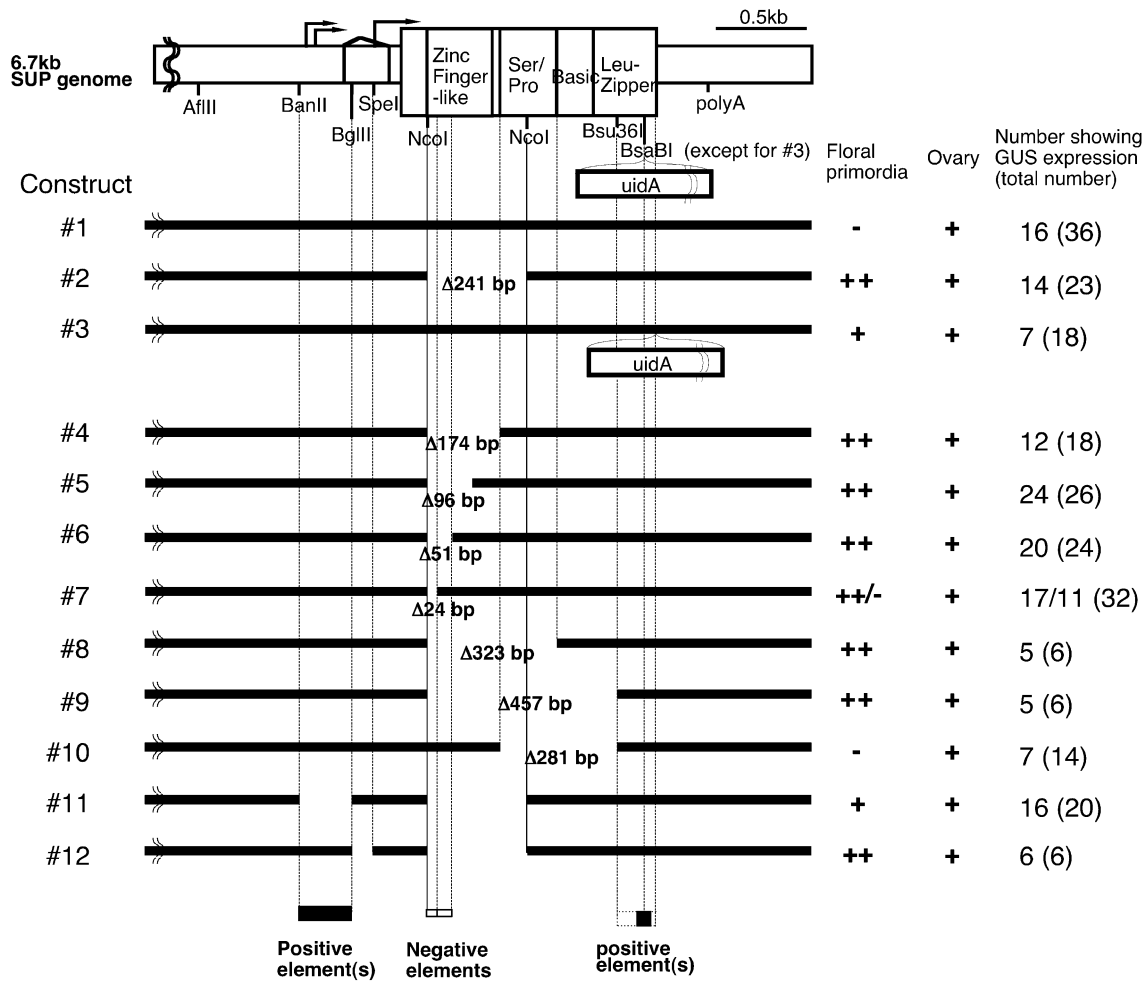


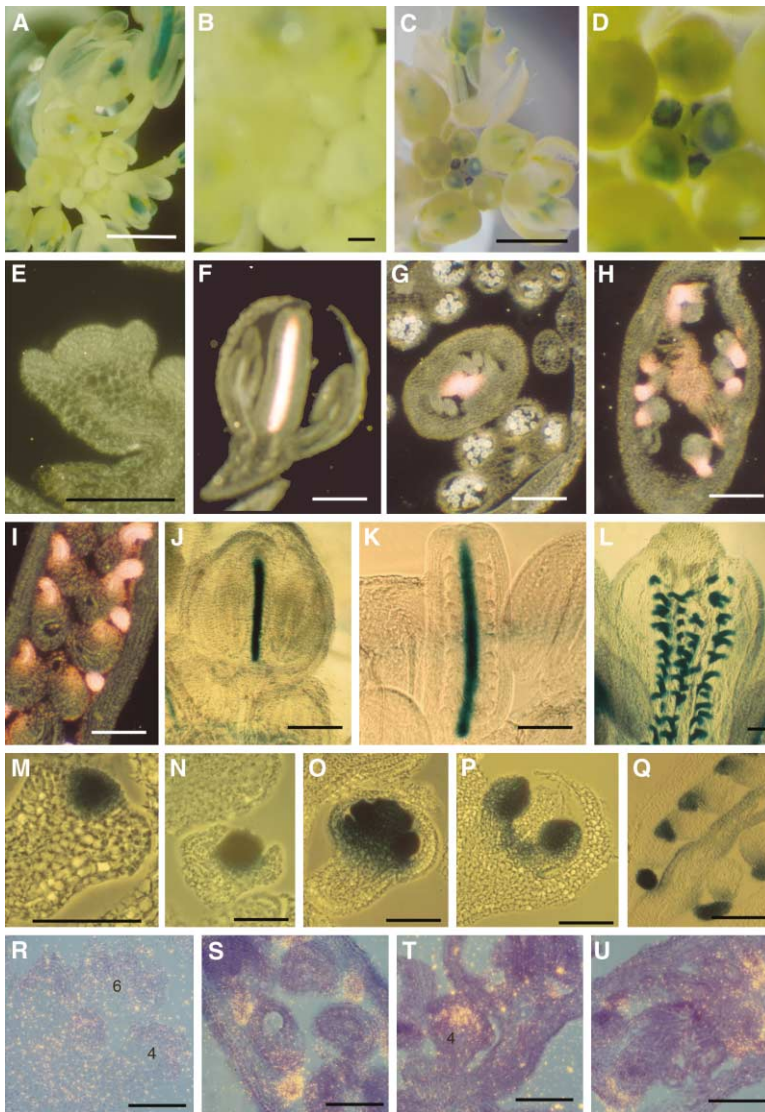
Figure 1. Diagram of Various *SUP* Gene Deletions in the Transcribed Region and a Summary of the GUS Staining Results

The thick box represents the *SUP* protein-coding region. The transcription start sites and the restriction sites that were used for cloning are indicated in the top diagram. Major GUS staining patterns are represented by ++, +, ++/-, and -. ++, ectopic staining in both whorl 3 and whorl 4; +, normal *SUP* expression; ++/-, very weak but ectopic or no staining; -, no staining. The number of independent lines that showed the indicated pattern and the total number of transgenic lines obtained are indicated on the right. The rest of the plants showed no staining, with the exception that one line of construct #5 and #9 showed only late expression and two lines of construct #11 showed an ectopic expression pattern at the early stages. The *SUP* regulatory regions in the transcribed region defined by the deletion analyses are indicated on the bottom.

with the level of *SUP*-GUS transcript accumulation, in situ hybridization experiments were performed by using GUS antisense RNA as a probe. Under the conditions in which endogenous *SUP* transcript is normally detected, no signal was observed in the stamen primordia of stage-3 to stage-6 floral buds carrying construct #1, even though a signal in the developing ovary and funiculus of ovules was observed (Figures 2R and 2S). In contrast, plants carrying construct #2 showed a relatively strong hybridization signal in both stamen and carpel at stages 3-6 as well as in the funiculus of ovules at later stages (Figures 2T and 2U). The ectopic expression of the mRNA corresponds completely to the GUS staining pattern. These data demonstrate that *SUP*-GUS expression in plants containing construct #1 is affected at the mRNA level, not at the level of protein degradation.

#### Inserting the *uidA* Gene at the 3' End of the *SUP* Gene Disrupts a Positive Regulatory Element in the Leucine-Zipper-like-Coding Region

The original construct #1 with the *uidA* gene inserted 28 bp upstream from the termination codon did not induce GUS expression in early-stage floral primordia and therefore did not mimic wild-type *SUP* expression. One possible explanation for the absence of early-stage expression is that the *uidA* gene at the 3' coding region of the *SUP* gene disrupts a positive regulatory element. Therefore, construct #3 with the *uidA* gene fused to the termination codon was generated (Figure 1). The GUS expression pattern in lines carrying construct #3 exactly replicated endogenous *SUP* mRNA expression (Figures 3A-3G). The weak GUS staining can be observed as early as late stage 3 in a cylindrical domain that encloses



**Figure 2. Expression Patterns of SUP-GUS Reporter Genes with the Full-Length Gene-Coding Region or the Truncated Coding Region**

(A and B) GUS expression in the wild-type inflorescence ([B] is a close-up view) conferred by construct #1.

(C and D) GUS expression in a wild-type inflorescence transgenic for construct #2.

(E) A dark-field image of a stained late stage-3 floral bud transgenic for construct #1.

(F–I) A dark-field image of GUS expression in an ovary transgenic for construct #1 at (F) stage 9, (G) stage 10, (H) stage 12, and (I) stage 14.

(J–L) A bright-field image of GUS expression in an ovary transgenic for construct #1 at (J) stage 9, (K) stage 10, and (L) stage 12.

(M–P) A bright-field image of GUS expression in a floral bud transgenic for construct #2 at (M) late stage 3, (N) stage 4, (O) stage 6, and (P) stage 7.

(Q) GUS expression in the funiculus of ovules in construct #2.

(R and S) In situ hybridization of *uidA* mRNA in plants transgenic for construct #1. Longitudinal sections of (R) floral buds and the (S) pistil of a stage-14 flower.

(T and U) In situ hybridization of *uidA* mRNA in plants transgenic for construct #2. Longitudinal sections of (T) floral buds and the (U) pistil of a stage-14 flower.

The scale bars in (A) and (B) represent 1 mm; the scale bars in (C)–(U) represent 100  $\mu$ m. The numbers indicate the floral stages.

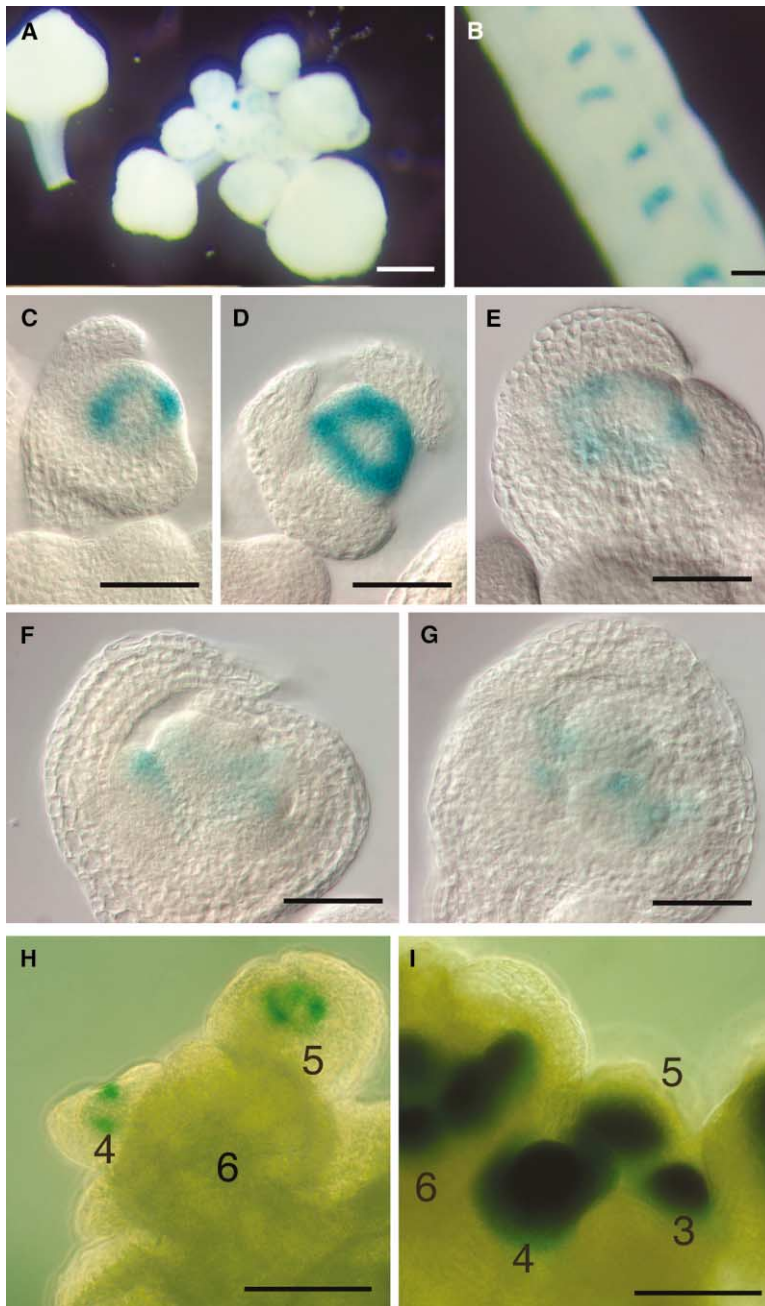
the floral meristem in whorl 4, and it continues to express in the adaxial sides of the stamen primordia until stage 6 (Figures 3C–3G). At later stages, the GUS activity was localized in the funiculus of ovules (Figure 3B). These results demonstrate that there is a positive element in the 3'-end coding sequence (around 8243094 of chromosome 3), which encodes the leucine-zipper-like domain of the SUP protein. Plants transgenic for construct #3 seemed to show not only a narrower domain, but also weaker GUS staining than construct #2, although the accurate comparison of staining strength is difficult, due to the variability in the expression level typically seen between independent transformants (Figures 2C, 2M–2P, 3A, and 3C–3G).

This 3'-end coding region does not contain any known binding sequence of transcription factors. This region is a target site for methylation that correlates with the epigenetic silencing of the SUP expression. Around the C-terminal end of the SUP-coding region, there are three asymmetric cytosines, three CpNpG, and 1 CpG, all of which are highly methylated in the *clk-st* allele, and the

methylations are lost in the *cmt3-7* revertant [11]. Blocking this region by methylation may have the same effect as the deletion shown in our analyses.

#### The Negative Region Consists of Two Elements Located in the N-Terminal Zinc Finger Domain-Coding Region

The deletion of a 241 bp region (NcoI fragment) from construct #1 induces SUP mRNA expression ectopically at early stages (Figure 2T, construct #2). This negative regulatory region corresponds to the sequence that encodes the zinc finger domain and the N-terminal half of the serine/proline-rich domain. To determine which sequences are necessary for this negative effect, we narrowed down the deleted region (Figure 1, constructs #4–#7). The deletions of 174 bp, 96 bp, or 51 bp in the N-terminal part of the zinc finger region (Figure 1, constructs #4–#6) result in a similar level of GUS expression, spatially and temporally, to plants transgenic for construct #2, which has the NcoI fragment deletion ( $\Delta$  241 bp) (Figures 1 and S1A in the Supplemental Data



**Figure 3. Expression Patterns of SUP-GUS Reporter Genes with or without Deletion of the Positive Elements in the Transcribed Region**

(A) Inflorescence with construct #3.

(B) A pistil of a stage-15 flower transgenic for construct #3.

(C–G) Floral buds of a plant transgenic for construct #3 at (C) late stage 3, (D) stage 4, (E) stage 5, (F) late stage 5, and (G) stage 6. (H and I) Inflorescence transgenic for (H) construct #11 and (I) construct #12.

The numbers indicate the floral stages. The scale bars in (A), (B), (H), and (I) represent 100  $\mu\text{m}$ ; the scale bars in (C)–(G) represent 50  $\mu\text{m}$ .

available with this article online). These results demonstrate that the negative element can be disrupted without affecting the N-terminal half of the serine/proline-rich region or the C-terminal half of the zinc finger region. However, lines carrying construct #7 with a 24 bp deletion in the N-terminal part of the zinc finger region showed weak or no ectopic expression at early stages (Figures 1 and S1B in the Supplemental Data). Eleven out of 32 lines with construct #7 showed only late-stage ovary expression, and 17 out of the remaining 21 lines showed weak but distinct GUS expression in whorls 3 and 4 of the floral primordia in addition to ovary expression (Figure 1). These results suggest that there are two separable or dosage-dependent negative elements at

+128–+152 and +153–+178 from the most abundant transcription site (+295–+345 bp from the longest cDNA; 8242575–8242625 of chromosome 3), which encodes the N-terminal part of the zinc finger domain.

We further examined the effect of the protein-coding region comprising the C-terminal half of the serine/proline-rich domain, the basic domain, and the N-terminal half of the leucine-zipper-like domain on construct #1 by extending the deleted area toward the 3' end of the gene (Figure 1, constructs #8 and #9). The extra deletion of these regions results in an ectopic expression pattern similar to that of construct #2 (Figure S1C in the Supplemental Data). In contrast, transgenic lines carrying construct #10, which deletes an overlapping region encod-

ing the serine/proline-rich domain, the basic domain, and the N-terminal half of the leucine-zipper-like domain, but contains the region coding for the full zinc finger domain, showed no GUS staining in early floral buds just as lines with construct #1 (Figures 1 and S1D in the Supplemental Data). These results demonstrate that the regions encoding the serine/proline-rich domain, the basic domain, and the N-terminal half of the leucine-zipper-like domain (8242751–8243031 of chromosome 3) have no additional negative *cis* elements.

We narrowed down the negative elements into two separable, 51 bp-long elements, which correspond to +128–+178 from the most abundant transcription site. One possible explanation for this negative function is that deletion of the 5' SUP protein-coding region might affect nucleosome positioning in the core promoter region and result in ectopic expression. For example, repositioning of the nucleosome (from a position at –15–+132 to a new position at +20–+167) spanning the human interferon- $\beta$  gene promoter by an artificial nucleosome-positioning signal resulted in enhanced expression and loss of specificity [12]. The 51 bp-long negative elements of the SUP gene contain one short consensus sequence for the nucleosome-positioning signal TAANNGCC [13]. Via this element, the negative elements may enhance nucleosome condensation around the transcription start sites; this nucleosome condensation may regulate the level of initiation of transcription. In vivo studies of nucleosome formation at the SUP promoter remain to be done, although chromatin changes that relate to nucleosome function have been shown to have a role in SUP gene expression [14].

#### The SUP Promoter Has Discrete Regions Required for SUP Expression in Stamen Primordia at Early Stages and in Ovary at Later Stages

In order to define the upstream promoter elements required for the distinct phases of SUP expression in early-stage stamen primordia and late-stage ovaries, four different 5' promoter deletions were generated (Figure 4, constructs #13–#16). Each construct was made, based not on construct #3, which exactly replicated endogenous SUP expression, but on construct #2, which confers strong early and late expression. This was done because construct #3 confers barely detectable early expression (Figure 1). The 5' deletions up to –3000 (construct #13, ClaI), –2000 (construct #14, PvuII), or –1200 (construct #15, A<sub>va</sub>II) resulted in GUS activity in the ovary at later stages but failed to exhibit GUS staining in the floral primordia at early stages, while construct #2 confers GUS expression at both early and late stages (Figures 4, 5A–5C, 5E, and 5F). In contrast, transgenic lines for construct #16, with a deletion from –3000 to –280 (A<sub>fi</sub>II fragments), showed early-stage expression in floral primordia, but did not show GUS expression in the ovary (Figures 5D, 5G, and 5H). These results show that the upstream promoter element necessary for ovary expression is located within –1200 to –280 (8240783–8242020 of chromosome 3) and that a distinct 2.2 kb region further upstream from –5200 to –3000 (8237177–8239001 of chromosome 3) contains the promoter for early-stage expression in floral organ primordia.

#### The Region around the Transcription Start Sites Functions as a Positive Element

The TCTCTCT sequences near the transcription start sites (8242293–8242299 and 8242310–8242325 of chromosome 3) are highly methylated in the epigenetically silenced alleles of the SUP locus [6–11]. Especially in the 99 bp region of +3–+101 for the longest cDNA (8242283–8242381 of chromosome 3), all of the 27 cytosines (24 asymmetric, 0 CpG, and 3 CpNpG) on the top strand were heavily methylated in all *sup* epigenetic alleles tested, *clk-3*, *clk-1*, and antisense cytosine methyltransferase (AMT) lines [6, 11].

In order to investigate the function of this region on SUP transcription, a 132 bp region surrounding the 99 bp fragment was cut out by BanII (with a site at –15 relative to the longest cDNA) and BglII (+113 for the longest cDNA) restriction enzymes from construct #2, which shows strong early and late-stage expression (Figure 1, construct #11). As a control, we made construct #12 with a deletion of a 53 bp region, adjacent to the deletion in construct #11, by use of BglII (+113 for the longest cDNA) and SpeI (+162 for the longest cDNA) (Figure 1). This 53 bp region contains 12 cytosines, 10 of which are asymmetric (10% highly methylated, 20% half-methylated, 60% no methylation in epigenetically repressed lines) and 2 of which are CpNpG (100% completely methylated) [6, 11]. Transgenic plants carrying construct #11 recapitulated the initiation phase expression of SUP (Figure 3H), while the lines carrying construct #12 showed strong and ectopic GUS expression at the early stages, like the lines with the original construct #2 (Figures 3H and 3I). In lines carrying construct #11, weak GUS expression was observed only in whorl 3 at the boundary with whorl 4 of stage-3/4 floral buds (Figure 3H). This GUS expression disappeared at the end of stage 5, although the endogenous SUP gene continues to express until stage 8. At later stages, weak GUS expression conferred by construct #11 was observed in the transmitting tract and in the funiculus of ovules (data not shown). These results showed that endogenous SUP expression at initiation phase is recapitulated by deletion of the BanII-BglII region, but that the region is necessary to maintain the SUP expression from stage 6 through stage 8. These results suggest that the BanII-BglII region (8242265–8242393 of chromosome 3) functions as a positive element for early-stage SUP expression, while the neighboring BglII-SpeI fragment (8242393–8242442 of chromosome 3) has no, or redundant, function.

5' race PCR and cDNA clones of SUP showed that multiple transcription start sites are used in the wild-type SUP gene from 8242281, 8242309, and 8242448 of chromosome 3 (Steve Jacobsen, personal communication). These data suggest that construct #11 only maintains one of the three transcription start sites, while construct #12 maintains all three.

These results suggest that in *sup* epigenetic alleles, methylation of the BanII-BglII region as well as the C-terminal part-coding region might block the positive elements in these regions and silence SUP expression at early stages. Demethylation of cytosines in these regions would reactivate these elements for early-stage SUP expression. The negative elements may not be se-

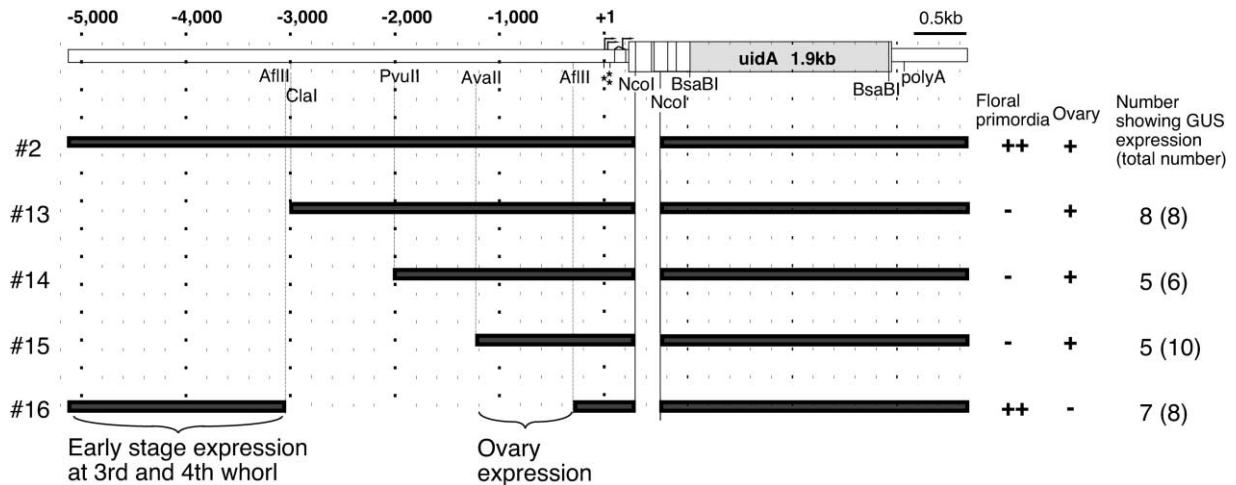


Figure 4. Diagram of Various *SUP* Promoter Deletions and a Summary of GUS Staining Results

The thick box represents the *SUP* protein-coding region (in white) and the *uidA* gene (shaded). The transcription start sites and the restriction sites used for cloning are indicated. Major GUS staining patterns are represented by ++, +, and -. ++ represents ectopic staining in both whorl 3 and whorl 4; + is normal *SUP* expression; and - represents no staining. The number of independent lines that showed the indicated pattern and the total number of transgenic lines obtained are indicated on the right. The rest of the plants showed no staining. The asterisk shows the CT cluster. The *SUP* promoter region defined by the deletion analyses is indicated on the bottom.

verely affected by methylation in epigenetic alleles, as there are only a few methylated cytosines in the negative elements [6, 11].

The deletion of positive elements in the coding region did not silence reporter gene expression in the ovary at later stages [8]; this finding matches the fact that dense methylation in the *SUP* genomic region of the epigenetic mutants does not affect the late-stage ovule expression of *SUP* [8].

### *SUP* Regulatory Models

The effects of the described deletions on *SUP* RNA accumulation are, to our knowledge, the first detailed evidence for positive and negative regulatory elements in a protein-coding region. Various mechanisms could account for this, from the use of distal enhancers and suppressors in the compact *Arabidopsis* genome structure, to changes in mRNA stability [15, 16], to binding sites for regulatory RNAs [17], and to the spreading of

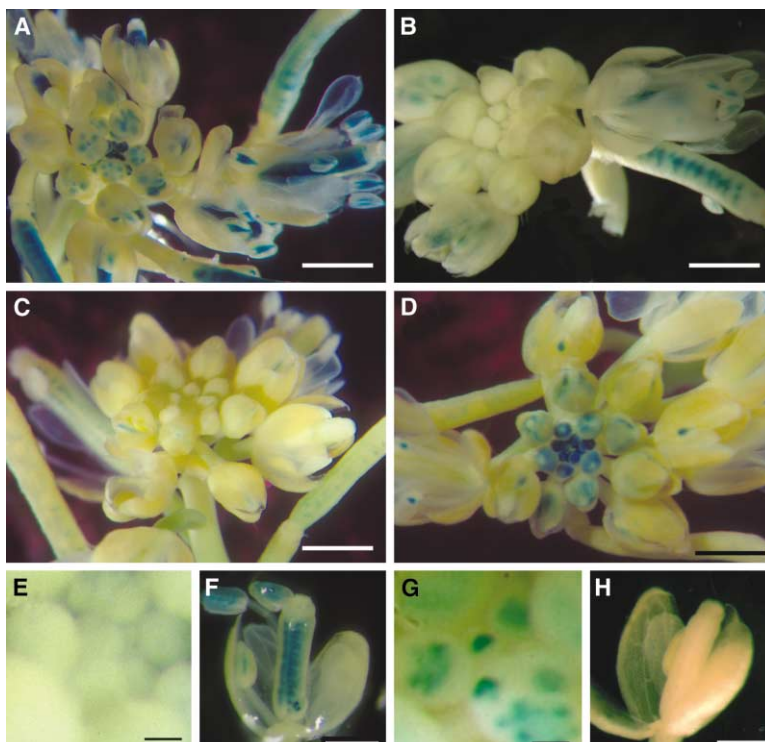


Figure 5. Expression Patterns of *SUP*-GUS Reporter Genes with Various Deletions in the *SUP* Promoter Region

(A–D) Inflorescence transgenic for (A) construct #2, (B) construct #13, (C) construct #15, and (D) construct #16.

(E) A close-up view of floral buds of transgenic lines for construct #15.

(F) A stage-10 flower with construct #15.

(G) A close-up view of floral buds of transgenic lines for construct #16.

(H) A stage-10 flower with construct #16.

The scale bars in (A)–(D) represent 1 mm, the scale bars in (E) and (G) represent 100  $\mu$ m, and the scale bars in (F) and (H) represent 0.5 mm.

an open chromatin configuration [12, 14]. One possible and plausible speculation for modulation of the upstream promoter by the transcribed region is regulation by nucleosome architecture as follows. At stage 3, *SUP* expression is initiated only in a very limited domain, and with a limited expression level, because the activity of upstream promoter elements might be modulated by a nucleosome that is blocking the transcriptional start sites (the BanII-BglII region deleted in construct #11). By late stage 5–stage 6, the nucleosome repositioning would already have been induced, and the transcription start sites (core promoter) become totally open, an effect that is mimicked by construct #2. The positive element in the C-terminal region might affect the release of the nucleosome around the transcriptional start sites directly or indirectly. At later stages, the *SUP* core promoter is totally open, and the elements in the coding region would not affect the upstream promoter activity for late stages. This chromatin model is consistent with all of our results. In addition, the recent finding that changes in chromatin structure induce ectopic *SUP* expression [14] might support this model.

### Conclusions

We have found that the *SUP* protein-coding region contains not only positive, but also negative, *cis*-acting elements required for the control of transcription. A number of studies have shown that 5' or 3' untranslated regions can contribute to the regulation of gene expression [18–22]. Our results suggest that even the protein-coding region can contain the *cis* elements for transcriptional regulation. Therefore, we suggest that including transcribed regions in promoter-reporter fusion analysis may be a necessity for thoroughly and accurately elucidating the mechanisms governing transcription.

### Supplemental Data

Supplemental Data including expression patterns of *SUP*-GUS reporter genes with various deletions (constructs #6, #7, #9, and #10) in the *SUP* gene-coding region are available at <http://www.currentbiology.com/cgi/content/full/13/17/1524/DC1/>.

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### References

- Schultz, E.A., Pickett, F.B., and Haughn, G.W. (1991). The *flo10* gene product regulates the expression domain of homeotic genes AP3 and PI in *Arabidopsis* flowers. *Plant Cell* 3, 1221–1237.
- Bowman, J.L., Sakai, H., Jack, T., Weigel, D., Mayer, U., and Meyerowitz, E.M. (1992). SUPERMAN, a regulator of floral homeotic genes in *Arabidopsis*. *Development* 114, 599–615.
- Sakai, H., Medrano, L.J., and Meyerowitz, E.M. (1995). Role of SUPERMAN in maintaining *Arabidopsis* floral whorl boundaries. *Nature* 378, 199–203.
- Gaiser, J.C., Robinson-Beers, K., and Gasser, C.S. (1995). The *Arabidopsis* SUPERMAN gene mediates asymmetric growth of the outer integument of ovules. *Plant Cell* 7, 333–345.
- Sakai, H., Krizek, B.A., Jacobsen, S.E., and Meyerowitz, E.M. (2000). Regulation of *SUP* expression identifies multiple regulators involved in *Arabidopsis* floral meristem development. *Plant Cell* 12, 1607–1618.
- Jacobsen, S.E., and Meyerowitz, E.M. (1997). Hypermethylated SUPERMAN epigenetic alleles in *Arabidopsis*. *Science* 277, 1100–1103.
- Jacobsen, S.E., Sakai, H., Finnegan, E.J., Cao, X., and Meyerowitz, E.M. (2000). Ectopic hypermethylation of flower-specific genes in *Arabidopsis*. *Curr. Biol.* 10, 179–186.
- Kishimoto, N., Sakai, H., Jackson, J., Jacobsen, S.E., Meyerowitz, E.M., Dennis, E.S., and Finnegan, E.J. (2001). Site specificity of the *Arabidopsis* MET1 DNA methyltransferase demonstrated through hypermethylation of the superman locus. *Plant Mol. Biol.* 46, 171–183.
- Lindroth, A.M., Cao, X., Jackson, J.P., Zilberman, D., McCallum, C.M., Henikoff, S., and Jacobsen, S.E. (2001). Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation. *Science* 292, 2077–2080.
- Jackson, J.P., Lindroth, A.M., Cao, X., and Jacobsen, S.E. (2002). Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* 416, 556–560.
- Cao, X., and Jacobsen, S.E. (2002). Locus-specific control of asymmetric and CpNpG methylation by the DRM and CMT3 methyltransferase genes. *Proc. Natl. Acad. Sci. USA* 99, 16491–16498.
- Lomvardas, S., and Thanos, D. (2002). Modifying gene expression programs by altering core promoter chromatin architecture. *Cell* 110, 261–271.
- Shrader, T.E., and Crothers, D.M. (1989). Artificial nucleosome positioning sequences. *Proc. Natl. Acad. Sci. USA* 86, 7418–7422.
- Tian, L., and Chen, Z.J. (2001). Blocking histone deacetylation in *Arabidopsis* induces pleiotropic effects on plant gene regulation and development. *Proc. Natl. Acad. Sci. USA* 98, 200–205.
- Ominato, K., Akita, H., Suzuki, A., Kijima, F., Yoshino, T., Yoshino, M., Chiba, Y., Onouchi, H., and Naito, S. (2002). Identification of a short highly conserved amino acid sequence as the functional region required for posttranscriptional autoregulation of the cystathionine gamma-synthase gene in *Arabidopsis*. *J. Biol. Chem.* 277, 36380–36386.
- Chiba, Y., Ishikawa, M., Kijima, F., Tyson, R.H., Kim, J., Yamamoto, A., Nambara, E., Leustek, T., Wallsgrove, R.M., and Naito, S. (1999). Evidence for autoregulation of cystathionine gamma-synthase mRNA stability in *Arabidopsis*. *Science* 286, 1371–1374.
- Rhoades, M.W., Reinhart, B.J., Lim, L.P., Burge, C.B., Bartel, B., and Bartel, D.P. (2002). Prediction of plant microRNA targets. *Cell* 110, 513–520.
- Dickey, L.F., Gallo-Meagher, M., and Thompson, W.F. (1992). Light regulatory sequences are located within the 5' portion of the *Fed-1* message sequence. *EMBO J.* 11, 2311–2317.
- Larkin, J.C., Oppenheimer, D.G., Pollock, S., and Marks, M.D. (1993). *Arabidopsis* GLABROUS1 gene requires downstream sequences for function. *Plant Cell* 5, 1739–1748.
- Fu, H., Kim, S.Y., and Park, W.D. (1995). A potato *Sus3* sucrose synthase gene contains a context-dependent 3' element and a leader intron with both positive and negative tissue-specific effects. *Plant Cell* 7, 1395–1403.
- Chen, R., Silver, D.L., and de Bruijn, F.J. (1998). Nodule parenchyma-specific expression of the *sesbania rostrata* early nodulin gene *SrEnod2* is mediated by its 3' untranslated region. *Plant Cell* 10, 1585–1602.
- Nickelsen, J., Fleischmann, M., Boudreau, E., Rahire, M., and Rochaix, J.D. (1999). Identification of *cis*-acting RNA leader elements required for chloroplast *psbD* gene expression in *Chlamydomonas*. *Plant Cell* 11, 957–970.