Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development

(hypomethylation/methyltransferase/flower development/homeotic transformations)

E. JEAN FINNEGAN*, W. JAMES PEACOCK, AND ELIZABETH S. DENNIS

Commonwealth Scientific and Industrial Research Organization, Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia

ABSTRACT   *Arabidopsis* plants transformed with an antisense construct of an *Arabidopsis* methyltransferase cDNA (*METI*) have reduced cytosine methylation in CG dinucleotides. Methylation levels in progeny of five independent transformants ranged from 10% to 100% of the wild type. Removal of the antisense construct by segregation in sexual crosses did not fully restore methylation patterns in the progeny, indicating that methylation patterns are subject to meiotic inheritance in *Arabidopsis*. Plants with decreased methylation displayed a number of phenotypic and developmental abnormalities, including reduced apical dominance, smaller plant size, altered leaf size and shape, decreased fertility, and altered flowering time. Floral organs showed homeotic transformations that were associated with ectopic expression of the floral homeotic genes *AGAMOUS* and *APETALA3* in leaf tissue. These observations suggest that DNA methylation plays an important role in regulating many developmental pathways in plants and that the developmental abnormalities seen in the methyltransferase antisense plants may be due to dysregulation of gene expression.

Methylation of cytosine residues, the most common modification of DNA in higher eukaryotes, has been implicated in gene regulation, genomic imprinting, the timing of DNA replication, determination of chromatin structure, and as a basis for epigenetic phenomena (for reviews, see ref 1). A fundamental role for DNA methylation in mouse development was demonstrated by establishing a targeted mutation of the DNA methyltransferase gene in the germ line of mice (2). Methylation levels in mouse embryos homozygous for the mutation were reduced to ≈30% that of heterozygous or wild-type mice and the homozygous embryos spontaneously aborted in midgestation. The underlying cause for the embryo lethal phenotype has not been determined, but the decreased methylation did result in abnormal expression of imprinted genes and *Xist* (3, 4).

In the plant *Arabidopsis thaliana* mutants with decreased DNA methylation (*ddm*) (5) developed morphological abnormalities after several generations of self-fertilization (6). These plants had rounded rosette leaves, an increased number of cauline leaves, and were late-flowering. These observations suggest that hypomethylation of plant DNA can lead to abnormal development. Cytosine methylation was reduced to ≈30% of wild type in plants homozygous for a mutation at the *ddm1* locus, and methylation of cytosines in both CG and CNG motifs was reduced (5). The biochemical basis for the reduction in methylation has not been determined but DNA methyltransferase activity was comparable to wild type (6), indicating that a methyltransferase gene or genes were not affected.

The isolation of a cDNA encoding a putative DNA methyltransferase (*METI*) of *Arabidopsis* (7) has enabled us to investigate the role of DNA methylation in plant development

using a reverse genetics approach. We have directly targeted methyltransferase activity by introducing an antisense construct of this gene into wild-type *Arabidopsis*. Transgenic plants showed a reduction of up to 90% in cytosine methylation, predominantly in CG dinucleotides, suggesting that *METI* encodes a functional methyltransferase. Reduction of methylation resulted in dramatic changes in plant morphology, including homeotic transformations of floral organs. Plants with reduced methylation showed aberrant expression of floral homeotic genes; these genes, which are normally expressed only in floral tissue (8, 9), were also transcribed in leaves of transgenic antisense plants.

MATERIALS AND METHODS

Generation of the Methyltransferase Antisense Construct. The plasmid pYc8 (7) was cut with *Eco*RI and a 2.8-kb fragment of the methyltransferase cDNA was inserted between the cauliflower mosaic virus 35S promoter (10) and the nopaline synthase 3' termination signal (11). The resulting plasmid was linearized with *Sal*I, which cleaves 5' to the 35S promoter, and inserted into the unique *Sal*I site in the binary vector pBin19 (12), then mobilized into *Agrobacterium tumefaciens* strain AGL1 (13), using pRK2013 as a helper strain in a triparental mating.

Transformation of *Arabidopsis*. Transgenic *Arabidopsis* plants, ecotype C24, were obtained from root explants using the method described in Dolferus *et al.* (14).

DNA Isolation and Southern Hybridization. DNA was isolated from above-ground tissue, and Southern hybridizations were done according to the methods published in Taylor *et al.* (15).

Thin Layer Chromatography Assay. In this assay (5, 16, 17), DNA (1 μg) was cleaved with *Taq*I, which recognizes the sequence TCGA and cleaves 5' to the cytosine, whether or not the cytosine is methylated. Cleavage with *Taq*I results in fragments of <500 bp, on average. The methylation status of the terminal cytosines was determined by labeling with [γ-³²P]ATP. Enzymatic digestion cleaved DNA to deoxynucleotide monophosphates, which were separated by thin layer chromatography (16). Radioactivity in individual deoxynucleotide monophosphates was quantitated using a Molecular Dynamics PhosphorImager and IMAGEQUANT software. Uncut DNA, for each sample, was treated in parallel to determine background radioactivity incorporated into deoxy-5-methylcytosine monophosphate (d^{m}CMP) and deoxycytosine monophosphate (dCMP) due to the presence of sheared DNA; this background was subtracted before calculating 5-methylcytosine levels, by the formula d^{m}CMP/d^{m}CMP + dCMP, which were then normalized to wild type.

Root Development. Surface-sterilized seeds from C24 and T$_3$ line 10.5 were placed on Murashige–Skoog medium solidified with 1.5% agar (18), ≈1 cm from one edge of 10-cm square culture plates. The sealed plates were placed vertically in a

*To whom reprint requests should be addressed.

Table 1. Methylation levels in T_2 plants from five independent methyltransferase antisense families

Plant identification (n)	Copies of T-DNA*	Methylation level,[†] %
Family 4 (1)	3	82
Family 8 (2)	1	100.5 ± 3.4
Family 10 (3)	3	17.9 ± 6.5
Family 11 (2)	1	89.3 ± 7.3
Family 22-6 (2)	4	77.0 ± 0.83

All plants tested were from the T_2 generation; n, number of plants sampled.
*Number of copies of T-DNA inserted into the genome, as determined by number of T-DNA left border fragments
[†]% wild-type methylation $\pm$ SE.

controlled environment at 22°C under long days (16 hr fluorescent light, 8 hr dark). After 4 weeks, the length of the primary root and the number of roots initiating at the root–hypocotyl junction were determined.

Detection of the Transgene by PCR. The presence of the antisense transgene was detected using a polymerase chain reaction (PCR) assay for the selectable *Npt*II marker that was cotransformed on the same T-DNA. DNA was prepared from a single leaf from individual plants (19). DNA template (1 μl) was added to a reaction mix (20 μl) containing 10 mM Tris (pH 8.8), 50 mM KCl, 1.5 mM MgCl$_2$, 200 μM each dNTP, 1 μM primer 1 (GAGGCTATTCGGCTATGA), 1 μM primer 2 (ACTTCGCCCAATAGCAG), and 1 unit *Taq* DNA polymerase (Perkin–Elmer). Cycling conditions were 94°C for 4 min, 50°C for 30 sec, 72°C for 30 sec (one cycle), and then 34 cycles of 94°C for 10 sec, 50°C for 10 sec, and 72°C for 30 sec. The reaction products were resolved by electrophoresis on a 2% agarose gel—the expected size of the amplified fragment is 238 bp.

RNA Isolation and Northern Analyses. RNA was isolated from leaf tissue of plants grown in sterile culture or from plants grown in soil in controlled environment cabinets as described (14). Northern hybridizations were performed according to Dolferus *et al.* (14).

RESULTS

Arabidopsis (ecotype C24) was transformed with a *METI* antisense construct encoding the putative methyltransferase domain (520 aa) and $\approx$330 aa of the amino terminal domain (7) under the control of a constitutive promoter (10). Fifty independent transgenic (T_0) plants were regenerated. Progeny from a random subset of five of these plants were used for this study in which we measured the level of DNA methylation and

Table 2. Methylation levels in T_3 lines for methyltransferase antisense family 10

Plant identification* (n)	Transgene status[†]	Methylation level,[‡] %
10.1 (1)	Homozygous	23.9[§]
10.1 (3)	Hemizygous	20.8 ± 1.3
10.1 (4)	Null	42.8 ± 1.8
10.2 (1)	Hemizygous	29.7 ± 2.0
10.2 (2)	Null	64.0 ± 4.1
10.5 (6)	Homozygous	13.3 ± 1.8
10.13 (4)	Homozygous	9.3 ± 0.83

n, number of plants sampled.
*T_3 progeny of individual T_2 plants, 10.1, 10.2, etc., from family 10.
[†]Transgene status was determined by testing for presence of the transgene in 24 progeny by PCR amplification of a fragment of the *Npt*II selectable marker. Data for individual plants with same transgene status, within each line, were pooled.
[‡]% wild-type methylation $\pm$ SE.
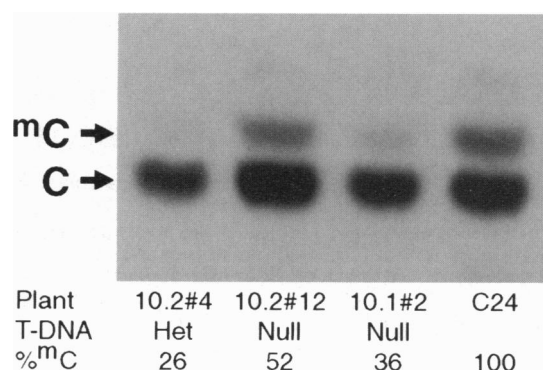[§]Value for single plant, measured in duplicate and averaged.

FIG. 1. Quantitation of DNA methylation by thin layer chromatography. 5-Methylcytosine levels, in a subset of CG dinucleotides, were estimated by quantitation of radioactivity, using a Molecular Dynamics PhosphorImager, then calculated by the formula d^{m}CMP/(d^{m}CMP + dCMP) and then normalized to wild-type levels.

monitored growth and development of plants in the T_2 and subsequent generations.

METI Antisense Reduces DNA Methylation. The level of cytosine methylation in DNA from antisense plants was compared with control C24 plants in a thin layer chromatography assay (5, 16), which measures methylation in the subset of CG dinucleotides that occur in *Taq*I sites. Methylation in the five antisense transgenic families ranged from $\approx$10% to 100% of wild type (Tables 1 and 2). Family 10, with the lowest level of cytosine methylation, has three copies of the transgene, inherited as a single locus; family 4 and family 22–6, which had 70–80% of normal methylation, contained three and four copies of the transgene, respectively. Family 8 and family 11, in which methylation was unchanged, contained only one copy of the antisense transgene.

T_2 and T_3 antisense plants from family 10 had 10–30% of wild-type methylation (Fig. 1; Tables 1 and 2). Methylation levels varied between progeny of sibling T_2 plants and within T_3 lines (Table 2). In general, plants homozygous for the transgene construct had the lowest levels of DNA methylation (Table 2), suggesting that methyltransferase activity was inversely correlated with antisense expression. Methylation levels in homozygous plants of line 10.5 remained the same over three generations (Table 3).

T_3 plants from family 10 that did not inherit the antisense construct also showed reduced levels of DNA methylation relative to wild type (Table 2, plants 10.1 null and 10.2 null), although the methylation level, at 40–65% of normal, was higher than in hemizygous sibs (20–30% of wild type). When these antisense-null plants were selfed, the methylation levels in progeny were still lower than normal.

Methylation in plant DNA occurs predominantly in cytosines located in either CG dinucleotides or CNG trinucleotides (20, 21). We determined the specificity of *METI* antisense demethylation in centromeric and ribosomal DNA sequences. In wild-type *Arabidopsis*, the centromeric repeat is heavily methylated (20) and is not cleaved by *Hpa*II (Fig. 2A). In DNA from the antisense plants, this sequence was cleaved

Table 3. Methylation levels in three successive generations of plants in line 10.5 homozygous for the transgene

Plant identification* (n)	Methylation level,[†] %
T_2 10.5 (1)	14.8
T_3 10.5 (6)	13.3 ± 1.8
T_4 10.5 (6)	9.6 ± 0.5

n, number of plants sampled.
*Homozygous plants from different generations of line 10.5.
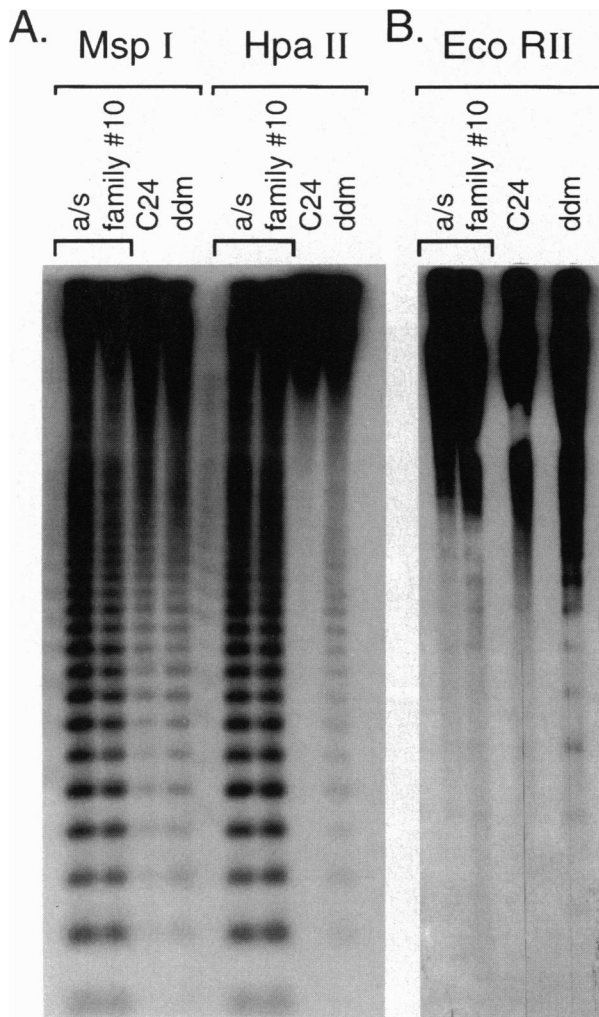[†]% wild-type methylation $\pm$ SE.

FIG. 2. Specificity of demethylation caused by *METI* antisense. (*A*) Southern analysis of DNA from individual T_3 10 methyltransferase antisense plants, homozygous *ddm1* (5), and untransformed C24, to detect changes in CG methylation. DNA was digested with either *Msp*I or *Hpa*II, which cleave the site CCGG but which differ in their sensitivity to DNA methylation. *Hpa*II will cleave this sequence only if both cytosines are unmethylated (23, 24), while *Msp*I will not cleave when the external cytosine is methylated (23, 24) but is only affected by methylation of the internal cytosine in some sequence contexts (25–27). A 180-bp repeat unit from the centromeric DNA of *Arabidopsis* was used as a probe (22). (*B*) Southern analysis of DNA from individual T_3 10 methyltransferase antisense plants, homozygous *ddm1* (5), and untransformed C24 to monitor changes in CA/TG methylation. DNA was cleaved with *Eco*RII, which cleaves the sequence CCA/TGG when the internal cytosine is not methylated (23). The Southern blot was probed with a fragment from the 5S ribosomal repeat of *Arabidopsis* (28). The apparent gap in hybridization to C24 DNA is due to incomplete DNA transfer.

by methylation sensitive *Hpa*II (Fig. 2*A*), indicating that the centromeric DNA was hypomethylated in antisense plants relative to wild type. In DNA from plants homozygous for the *ddm1* mutation (5), this sequence was also hypomethylated (Fig. 2*A*), but the pattern of demethylation differed between the antisense and *ddm1* plants. Cleavage of CG sites (tested with *Hpa*II) was greater in DNA from the antisense plants (Fig. 2*A*), whereas CAG and CTG sites (monitored with *Eco*RII) were cleaved more extensively in DNA from *ddm1* plants (Fig. 2*B*). These data suggest that the *METI* antisense preferentially reduces methylation in CG dinucleotides, whereas *ddm1* affects methylation of both CG and CNG (5).

*Msp*I cleaved centromeric DNA from both *METI* antisense and *ddm1* plants more extensively than DNA isolated from

untransformed C24 (Fig. 2*A*), showing that methylation of the 5′ cytosine in the sequence CCGG was also reduced in the antisense plants compared with wild type. Thus, *METI* antisense may reduce activity of an enzyme that methylates both ^{m}CG and ^{m}CCG, like the partially purified CG methyltransferase from pea (29), or the *METI* antisense may interact with transcripts from two members of the methyltransferase gene family (7). A third possibility is that, in the antisense plants, demethylation of the internal cytosine, in that sequence context, affected *Msp*I cleavage (25–27).

The 17S ribosomal repeat sequence (5) was also cleaved more extensively by *Hpa*II in antisense plants than in C24. Families 4, 10, and 22–6, had reduced CG methylation in these repeat sequences.

Reduced DNA Methylation is Associated with Developmental Abnormalities. Morphological abnormalities in both vegetative and reproductive structures were observed in plants from the T_2 and subsequent generations. The abnormal plants had decreased stature, smaller rounded leaves, leaves with margins curled toward the upper leaf surface, decreased fertility, and reduced apical dominance resulting in a bushy appearance. Antisense plants had shorter roots (5.83 ± 0.21 cm, antisense; 7.3 ± 0.25 cm, wild type) with more branching at the root crown (1.91 ± 0.133, antisense; 1.34 ± 0.07, wild type). Phenotypes were variable with individual plants displaying some or all of these characteristics (Fig. 3 *A* and *B*). The severity of the abnormal phenotype correlated with the extent of demethylation. In families 4 and 22–6, which had a smaller reduction in methylation (Table 1), phenotypes were similar, but less severe (Fig. 3*B*), than those in family #10 (Fig. 3*A*).

Arabidopsis flowers have four different organs arranged in concentric rings or whorls; there are four sepals in the outer whorl, four petals in the second whorl, six stamens (the male reproductive organs) in the third whorl, and, in whorl four, a single, female reproductive organ consisting of two fused carpels. Floral homeotic genes specify organ identity and their function is restricted to defined domains on the floral bud that are coincident with the organ whorls (for reviews, see refs. 8 and 9). Some flowers on the antisense transgenic plants showed homeotic transformations of floral organs (Fig. 3 *C–I*), with the flowers resembling those described on plants of floral homeotic mutants (9). We observed flowers with an increased number of stamens and reduced carpel tissue (Fig. 3 *C* and *I*), as in the *superman* (*sup*) mutant (31, 32); however, when carpels developed, they contained ovules of normal morphology. When organs in the inner two whorls were transformed into petals or staminoid petals (Fig. 3*E*), organ number increased (like *superman agamous* mutants; refs. 31 and 32). In other flowers, where sepals were replaced by carpelloid tissue, the number of organs in whorls two and three decreased (Fig. 3 *F* and *H*) as in *apetala2* mutants (33). Sometimes extra flowers developed in place of a floral organ or in the internode between floral organs (Fig. 3*G*), comparable to mutation in *apetala1* (34).

Flowers on a single plant showed a spectrum of these phenotypic abnormalities and flowers formed later in development were more severely affected (Fig. 3*D*). Floral abnormalities were most common and diverse in family 10, the family with the lowest level of DNA methylation. Abnormal flowers were observed in all antisense families, with reduced methylation, and similar phenotypes were observed on plants with equivalent methylation levels (compare Fig. 3 *F* and *H*). This, together with the observation that, in family 10, some antisense-null plants with 40–65% of normal methylation produced abnormal flowers (Fig. 3*F*), suggests that the floral abnormalities arose as a result of decreased DNA methylation rather than by mutation at the site of the T-DNA insertion.

The hemizygous T_1 10 plant, which had reduced apical dominance and fertility, was phenotypically different from the T_1 plants in the other families used in this study. Plant and

FIG. 3.   Phenotypic abnormalities in plants and flowers from methyltransferase antisense plants with reduced levels of DNA methylation. (*A*) Flowering T_2 plants from family 10, both of which carry the antisense construct. The plant on the left has a relatively normal phenotype whereas the plant on the right has decreased apical dominance (is branched), smaller leaves and is greatly reduced in size (reproduced from ref. 30). (*B*) Sibling T_2 plants from family 22–6, both of which carry the methyltransferase antisense. The plant on the left is smaller and bushier than normal, while the plant on the right has leaves which curl to the upper surface. (*C*) A flower from a hemizygous T_3 plant, line 10.1. The organs in the outer two whorls are normal but there are 12 stamens and the carpels of gynoecium (female reproductive organ) are unfused. (*D*) An inflorescence from a homozygous T_4 plant, line 10.5. The early flowers which have extra stamens were followed by flowers with four sepals then petals or staminoid petals in all internal whorls. (*E*) A flower from a hemizygous T_3 plant, line 10.2. This flower has an increased number of organs with four normal sepals then petals or staminoid petals in all the inner whorls. (*F*) A flower from an antisense-null T_4 plant, line 10.2. This flower has no petals and a reduced number of stamens in the third whorl. Two sepals are carpelloid with stigmatic papillae and has ovules on the margin (indicated by an arrow). (*G*) A flower from a homozygous T_4 plant, line 10.5. This flower has an increased number of whorls (note the dried petals, extending down the stem), all organs internal to sepals are petals or staminoid petals, and there are secondary flowers within this flower. (*H*) A flower from a T_3 plant, line 4.1. This flower has a carpelloid sepal with ovules on the margin (indicated by an arrow) and a reduction in the number of organs in whorls two and three. (*I*) A flower from a T_2 plant, line 39.25. This flower has normal organs in the outer two whorls but it has 12 stamens and the carpels of the gynoecium are not fused.

floral phenotypes in family 10 became more abnormal in successive generations. The homozygous T_2 plant, 10.5, was reduced in stature but otherwise normal, whereas all its T_3 progeny had aberrant flowers that were usually sterile. The few T_4 progeny had additional floral homeotic transformations, and most were completely infertile.

A sixth family, 39, had methylation levels (10–20% of wild type) comparable to family 10. The T_1 plant in this family had reduced apical dominance, decreased fertility, and some abnormal flowers. Most flowers on its T_2 progeny were abnormal, and the floral phenotypes (Fig. 3*I*) were similar to those in family 10 (Fig. 3*C*). The types of floral abnormalities correlate with the extent of demethylation, suggesting that the homeotic transformations were caused by the same mechanism in both families.

In addition to the morphological abnormalities observed in the antisense plants the timing of the transition from the vegetative to the reproductive phase of development was altered (data not shown), indicating that DNA methylation is involved in the timing of developmental processes.

Reduced DNA Methylation Is Associated with Aberrant Gene Expression. The floral homeotic transformations that we observed in methyltransferase antisense plants suggested that the expression of floral genes may be abnormal. We found that the floral homeotic genes, *AGAMOUS* (*AG*) and *APETALA3* (*AP3*), were expressed in leaves of methyltransferase antisense plants, whereas in wild-type plants, expression of these genes is confined to restricted domains of the floral bud (8, 9). Fig. 4 shows that *AP3* transcripts were detected in leaf RNA from antisense plants from families 22–6, 10, and 39, but not in

untransformed C24. Similar results were obtained for *AG*, indicating that activation of transcription in vegetative tissue was not unique to *AP3*. Demethylation of genomic DNA resulted in ectopic expression of these genes in the antisense plants.

DISCUSSION

Methylation Patterns Were Not Restored in Each Generation. The introduction of the *METI* antisense construct resulted in reduced levels of DNA methylation in the progeny of about 60% of the primary transformants. Methylation levels varied both between and within antisense families. The variation seen within a family reflected a difference in copy number of the transgene; plants that were homozygous for the antisense showed the greatest demethylation. In some progeny that did not inherit the transgene, methylation levels remained below wild type, suggesting that normal methylation levels were not restored in a single generation. Vongs *et al.* (5) found in progeny of a cross between a *ddm1* homozygote and a wild-type plant that, just as in the antisense-null plants, *de novo* methylation was slow, if indeed it occurred at all. Repeated crossing to wild-type plants gradually restored the methylation level; this is consistent with progressive loss of hypomethylated DNA, rather than *de novo* methylation (5). These data indicate that *Arabidopsis* plants lack mechanisms to restore the normal level of methylation after passage through meiosis, suggesting that plants may not undergo the cycle of general demethylation followed by remethylation, observed in early stages of mammalian embryo development (for review, see ref. 36).

DNA Methylation Plays a Role in Plant Development. Plants with low levels of DNA methylation displayed many phenotypic abnormalities suggesting that leaf, root, inflorescence, and flower development were perturbed. Phenotypes were variable between plants of the same family and became more severe in successive generations, even though methylation levels remained unchanged. Homozygous *ddm1* plants also showed phenotypic abnormalities after several generations of selfing (6). The progressive increase in the phenotypic aberrations could result from the segregation of different demethylated sites among sexual progeny.
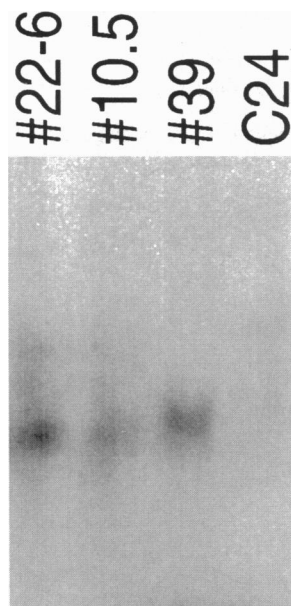

FIG. 4.   Northern analysis showing expression of *AP3* in vegetative tissue of methyltransferase antisense plants. Total RNA isolated from leaves of pooled T$_2$ or T$_3$ progeny of antisense plants, or untransformed C24 plants grown in sterile culture. The blot was probed with an *AP3* (35) antisense transcript.

Developmental Abnormalities Correlated with Altered Patterns of Gene Expression. Our data suggest that ectopic expression of the two floral homeotic genes, *AP3* and *AG*, causes some of the floral homeotic transformations on plants with reduced levels of methylation. Other experiments have shown that ectopic expression of these two genes resulted in similar homeotic transformations; constitutively expressed *AP3* caused *sup*-like phenotypes and *apetala2*-like flowers resulted from constitutive expression of *AG* (37, 38). The *sup*-like flowers on the antisense plants had extra stamens but ovules were normal, similarly ovule development was unaffected in 35S-*AP3* transgenic plants (E.J.F., unpublished data); in contrast, mutation of the *SUP* gene increased stamen number and affected ovule development (31, 32, 39). These data suggest that SUPERMAN was functional in the *sup*-like flowers on the antisense plants and that the phenotype was a result of ectopic expression of *AP3*.

The type of floral abnormality was correlated with the extent of demethylation; *ap2*-like phenotypes occurred in plants with a small reduction in methylation whereas flowers on plants with methylation <35% of the wild-type level were *sup*-like. Severity of the floral phenotype increased during development; *sup*-like flowers were replaced, later in development, by flowers similar to the double mutants, *superman agamous*, *superman apetala1*, and, rarely, *superman apetala2*, as well as the *superman agamous apetala1* triple mutant (31, 32). In floral homeotic mutants, the gradient in severity of the floral phenotype is reversed, with early flowers being more severely affected than the later flowers (31, 34). The phenotypic gradients may result from increasing activity of other, redundant floral genes partially compensating for the mutation (8); increased activity of redundant genes could enhance the effects of ectopic gene expression in the antisense plants, resulting in the gradient of increasing severity seen in these plants. The more extreme phenotypes of later generations may be a consequence of the accumulation of different demethylated sites in sexual progeny.

Reducing DNA Methylation May Alter Gene Expression by Changing Chromatin Structure. Our observations suggest that the primary effect of the methyltransferase antisense transgene is to reduce DNA methylation. The methylation status of a gene, particularly of the promoter, can influence transcription (for review, see refs. 40 and 41). Ectopic expression could result directly from demethylation of promoter elements of the floral genes or of transcription factors that regulate them, or from an alteration in the chromatin structure surrounding these genes. Alternatively, gene expression could be altered by insertional mutagenesis caused by transposition of previously methylated, inactive transposable elements (for review, see ref. 41).

The phenotype of the *Arabidopsis curly leaf* (*clf*) mutant, which includes curled leaves, early flowering, and *ap2*-like homeotic transformation in late flowers (P. Puangsomlee and J. Goodrich, personal communication), is similar to that of some methyltransferase antisense plants. In both *clf* mutants and methyltransferase antisense plants the floral homeotic genes, *AG* and *AP3*, are expressed ectopically. The *CLF* gene encodes a protein with homology to a member of the *Drosophila* polycomb-group proteins indicating that chromatin structure is important in regulating plant gene expression. The *Drosophila* polycomb (Pc-G) and trithorax (trx-G) group proteins affect higher order chromatin structure, and polycomb group proteins are involved in long-term gene repression by formation of stable chromatin complexes (42).

The similarity of phenotypic abnormalities in *clf* mutants and methyltransferase antisense plants suggests that DNA methylation may act in concert with a Pc-G/trx-G-like system to stabilize determined states of gene expression in *Arabidopsis*. A link between DNA methylation and gene regulation by trx-G proteins has been proposed (43).

The fundamental importance of DNA methylation for normal development in the mouse has already been established, and, in other mammals, methylation has been implicated in the control of gene expression and imprinting. In plants, decreased DNA methylation has been correlated with promotion of flowering in *Arabidopsis* (44), but its role in regulating gene expression during development has not been demonstrated. The developmental abnormalities and aberrant patterns of gene expression observed in the transgenic plants, described here, demonstrate a role for DNA methylation in many pathways of plant development.

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