explains the decrease in total MR in the past 20 years, in which strike-slip events have been active, producing moments that are smaller (1/10 to 1/20) than those in periods of accelerated subduction zone activity. The large contribution of aseismic energy release in strike-slip systems provides an explanation for the discrepancy in MR for the two types of earthquakes. Mogi (*17*) proposed that different parts of plate boundaries go through alternating periods of high and low activity, on a time scale of tens of years. This notion and the patterns revealed in the earthquake catalogs imply that the increase in seismic activity at large magnitudes ($M > 5$) in California since 1985 (*18*) or along the Anatolian fault in the 1940s (*19*) may be regional manifestations of a larger scale phenomenon involving stress transfer from subduction zones to zones of toroidal energy release and resulting in activation of specific parts of plate boundaries.

Although the large-scale progressions of great earthquakes around the Pacific and the Nazca plate have been noted, the implications have not been incorporated explicitly in the identification of seismic potentials of gaps (*20*). If the notion that portions of plate boundaries are activated in a nonrandom manner on time scales of tens of years and geographical scales of several thousands of kilometers is valid, it might be used to more accurately predict the time of occurrence of great earthquakes. The seismic gap theory has recently been challenged on the basis of statistical analysis of event occurrence in the past 20 years, primarily in subduction zone areas (*21*). According to my study, the resolution of this question may have to wait until the next period of increased activity in great subduction zone decoupling earthquakes.

This study may provide the basis for more quantitative testing of several hypotheses concerning the stress transfer from the underlying convective mantle to the tectonic plates. Data accumulated over another 10 or 20 years may be sufficient to sort out the causal relationships involving the Earth's rotation, internal processes, and earthquake energy release. In particular, we may be able to monitor deformation related to plate tectonics, not only as averaged over geological time scales but over several decades—the scale of the Global Change Program. More precise knowledge of moments and mechanisms of great earthquakes at the turn of the century would also be very valuable, if only to shed light on the possible periodicity in the alternation of great strike-slip and thrust earthquakes.

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Arabidopsis thaliana DNA Methylation Mutants

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Three DNA hypomethylation mutants of the flowering plant *Arabidopsis thaliana* were isolated by screening mutagenized populations for plants containing centromeric repetitive DNA arrays susceptible to digestion by a restriction endonuclease that was sensitive to methylated cytosines. The mutations are recessive, and at least two are alleles of a single locus, designated *DDM1* (for decrease in DNA methylation). Amounts of 5-methylcytosine were reduced over 70 percent in *ddm1* mutants. Despite this reduction in DNA methylation levels, *ddm1* mutants developed normally and exhibited no striking morphological phenotypes. However, the *ddm1* mutations are associated with a segregation distortion phenotype. The *ddm1* mutations were used to demonstrate that de novo DNA methylation in vivo is slow.

DNA methylation has been implicated in the control of a number of cellular processes in eukaryotes, including transcription (*1*), imprinting (*2*), transposition (*3*), DNA repair (*4*), chromatin organization (*5, 6*), and a variety of epigenetic phenomena (*7*), although the function of DNA methylation in eukaryotes remains the subject of continued debate. Using DNA methylation mutants to study the functions of DNA modification in eukaryotes is an attractive approach that avoids some of the limitations associated with correlative and methylation inhibitor studies. Li *et al.* (*8*) recently reported that mouse DNA methyltransferase mutants, generated by gene disruption, are unable to complete embryogenesis, although homozygous mutant cell lines are viable and apparently normal. We report here the isolation and characterization of DNA methylation mutants in the flowering plant *Arabidopsis thaliana*.

We identified *A. thaliana* DNA methylation mutants by screening directly for plants that contained hypomethylated genomic DNA using Southern (DNA) blot analysis. Filters containing Hpa II–digested genomic DNA prepared from leaves of mutagenized *A. thaliana* plants were hybridized with a cloned 180-bp repeat found in long tandem arrays at all five *A. thaliana* centromeres (*9*). Centromeric repeat arrays isolated from wild-type plants are resistant to Hpa II (CCGG) digestion (*10, 11*) because of extensive methylation of cytosines at the CpG and CpNpG sites, which is characteristic of plants (*12*) [^{5m}CCGG and C^{5m}CGG are not digested by Hpa II (*13*)]. DNA methylation mutants, on the other hand, were predicted to contain hypomethylated centromeric arrays susceptible to Hpa II digestion. We examined Southern filters containing DNA from 79 pools representing approximately 2000 plants from ethyl methanesulfonate–mutagenized M_2 populations (ecotype Columbia) to look for centromere repeat multimers of low molecular weight that were clipped from the long arrays by Hpa II. Individual plants from candidate pools were

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then screened to identify DNA hypomethylation mutants. Three independent mutant lines were isolated, and the mutations carried by these lines were designated *ddm* to refer to their decreased DNA methylation phenotype.

A variety of genomic DNA sequences are hypomethylated in the *ddm* mutants, including highly reiterated and low copy number sequences. Most of the cytosine methylation in the *A. thaliana* genome is found in the highly repeated DNA fraction (*14*), a large portion of which is accounted for by the 180-bp centromere repeat family and the ribosomal RNA genes. All three homozygous mutants contain hypomethylated centromeric repetitive arrays that are more susceptible to Hpa II digestion than are arrays isolated from wild-type plants (Fig. 1A). Two of the mutants, identified provisionally as A and B (*ddm1-1/ddm1-1* and *ddm1-2/ddm1-2*, respectively), also contain severely hypomethylated 18S, 5.8S, and 25S ribosomal genes (rDNA) (Fig. 1B). Methylation of other genomic sequences, including the 5S ribosomal RNA genes and single-copy and moderately reiterated telomere-associated sequences, is also reduced in mutants A and B (Table 1). Not all genomic sequences are hypomethylated in the *ddm* mutants, however. Two rare examples of methylated single-copy sequences in *A. thaliana* were unaffected in the mutants. In mutant A, methylation of the centromere repeat arrays is reduced to comparable amounts in leaves, stems, flower buds, and roots, which suggests that the DNA hypomethylation caused by the *ddm* mutations is not tissue specific (*15*).

Because plant genomes contain 5-methylcytosine (5mC) at both CpG and CpNpG sites, it was of interest to determine if the mutations affect methylation at both sites. Centromeric arrays isolated from the mutants are more completely digested than wild-type arrays by Msp I, an isoschizomer of Hpa II that cleaves C^{5m}CGG but does not digest ^{5m}CCGG (*13*) (Fig. 1A). Similarly, Msp I digestion of the rDNA from *ddm* mutants A and B is more complete than the digestion of wild-type rDNA (Fig. 1B). Moreover, the methylation-sensitive endonuclease Eco RII, which cleaves CC(A/T)GG but not C^{5m}C(A/T)GG, digests rDNA purified from these mutants more completely than rDNA from wild-type plants (*15*). These data suggest that the mutants are reduced in DNA methylation at both CpG and CpNpG sites.

The cytosine methylation amounts in *ddm* mutants A and B are reduced to 25 to 30% of the amount in wild-type plants (Fig. 2). Mutant C exhibits a modest reduction to 83% of the wild-type amount, consistent with the less severe hypomethylation identified by Southern analysis for this mutant (Fig. 1 and Table 1). The two most severe hypomethylation mutations do not complement each other (Table 2), and we have designated the alleles *ddm1-1* and *ddm1-2* (*16*).

Homozygous *ddm* mutants do not exhibit striking changes in plant vigor, fertility, color, flower morphology, or flowering time when grown under standard laboratory conditions. The normal development and morphology of the *A. thaliana ddm* mutants stand in marked contrast to the embryonic lethal phenotype of the engineered mouse DNA methyltransferase mutants (*8*). Although we do not yet know if the *A. thaliana ddm* mutations are directly comparable with the engineered DNA methyltransferase lesions, the amounts of genomic 5mC in both the mouse mutants and the more severe *ddm* mutants are equal (~30% of wild-type amounts). The contrasting phenotypes of the mouse and *A. thaliana* mutants may reflect fundamental differences in the functions of DNA methylation in plants and mammals.

Our genetic characterization of the *ddm1* mutations has led to two findings. The first concerns the rate of de novo methylation in vivo. Heterozygotes (*ddm1/+*) produced by one outcross to wild-type plants contain amounts of 5mC halfway between the amounts in the two parents (Fig. 3). The mutations are recessive, not semidominant, because repeated backcrossing of heterozygotes to wild-type parents generates plants that contain

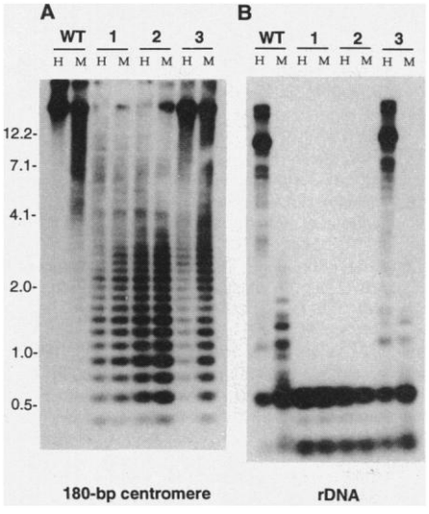

Fig. 1. Hypomethylation of repetitive DNA in *ddm* mutants. Total genomic DNA samples from wild-type *A. thaliana* Columbia plants (WT) and the DNA methylation mutants (lane 1, mutant A = *ddm1-1/ddm1-1*; lane 2, mutant B = *ddm1-2/ddm1-2*; lane 3, mutant C = homozygote of the third *ddm* mutation) were digested with Hpa II (H) or Msp I (M), electrophoresed in an 0.8% agarose gel, and transferred to a nylon membrane. The filter was first hybridized with a cloned *A. thaliana* 180-bp centromere repeat (*10, 11*) (**A**) and subsequently rehybridized with a probe from the *A. thaliana* rDNA locus (*19*) (**B**). Size markers are on the left in kilobase pairs.

Table 1. Genomic DNA sequences affected by the *ddm* mutations. Qualitative assessments are given for the methylation levels of various genomic sequences in the *A. thaliana* hypomethylation mutants (A = *ddm1-1/ddm1-1*, B = *ddm1-2/ddm1-2*, C = homozygote of the third *ddm* mutation). The assessments are based on Southern blot analysis as in Fig. 1 [see (*19*) for description of probes]. The symbol + indicates that no differences between the methylation status of wild-type and mutant DNAs could be discerned, +/− signifies that some hypomethylation is evident, and − denotes a severe or marked reduction in methylation.

Genomic sequence	*ddm* mutant		
	A	B	C
Repeated			
180-bp centromere	−	−	+/−
rDNA	−	−	+
5*S* RNA	−	−	+
Telomere-associated (YpAtT7)	−	−	+
Single-copy			
Telomere-associated (YpAtT1)	−	−	+
Anonymous (λbAt105)	+	+	+
Anonymous (λbAt118)	+	+	+

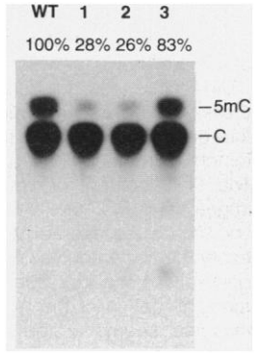

Fig. 2. Amounts of 5mC are reduced in the *ddm* mutants. Estimations of total 5mC amounts in the *ddm* mutants were determined by sampling cytosine methylation at Taq I sites (TCGA). Genomic DNAs prepared from either wild-type *A. thaliana* Columbia (WT) or hypomethylation mutant plants (lane 1, mutant A = *ddm1-1/ddm1-1*; lane 2, mutant B = *ddm1-2/ddm1-2*; lane 3, mutant C = homozygote of the third *ddm* mutation) were digested with Taq I, which cleaves TCGA regardless of cytosine methylation. The methylation status of the terminal cytosines was determined as described by Cedar *et al.* (*20*). 5-Methyl-deoxycytidine and deoxycytidine were separated by one-dimensional thin-layer chromatography with solvent 1 (*20*). We quantitated amounts of 5mC using a Fujix BAS2000 Bio-Imaging Analyzer (Fuji Medical Systems, Stamford. Connecticut). Percentages at top indicate 5mC content (5mC/5mC + C) normalized to WT (100%); C, deoxycytidine.

Table 2. Segregation of *ddm1* alleles. Mutants are overrepresented in the progeny that resulted from self pollination of *ddm1*/+ heterozygotes. Progeny plants were phenotyped by Southern blot analysis with Hpa II and an *A. thaliana* rDNA probe (*19*) that unambiguously distinguishes *ddm1/ddm1* homozygotes from *ddm1*/+ and wild-type plants. Heterozygotes in crosses 1, 2, and 3 were constructed by one outcross to wild-type *A. thaliana* Landsberg erecta plants. The progeny scored from crosses 1 and 2 arose from several sibling parents, whereas all the progeny scored from cross 4 were from a single family. The *ddm1-2*/+ heterozygote used in cross 4 was constructed by four backcrosses to wild-type *A. thaliana* Columbia plants to eliminate concern that we incorrectly scored *Ddm1* plants that had inherited unmethylated rDNA loci as mutants. *P* values, calculated by the χ^2 test, refer to the probability that the data would be observed given the expected segregation of a monogenic recessive marker (3:1::methylated:hypomethylated). No *P* value was calculated for cross 2 because of the small number of progeny phenotyped.

Cross	Methylated progeny (*n*)	Hypomethylated progeny (*n*)	*P*
1 (*ddm1-1*/+ × *ddm1-1*/+)	105	47	≈0.1
2 (*ddm1-1*/+ × *ddm1-2*/+)	21	13	–
3 (*ddm1-2*/+ × *ddm1-2*/+)	67	33	≈0.05
4 (*ddm1-2*/+ × *ddm1-2*/+) (backcrossed four times)	291	148	<0.001

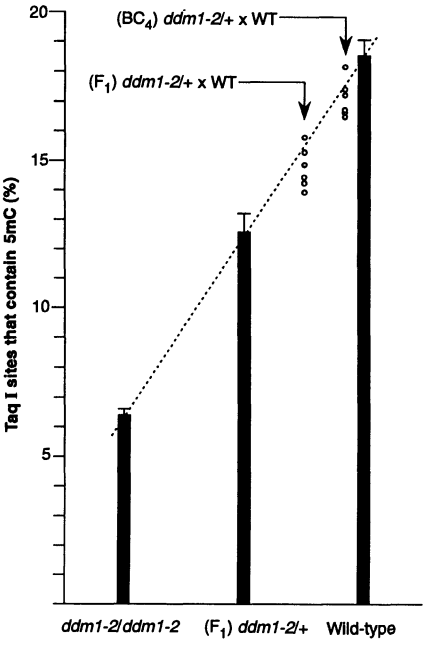

Fig. 3. De novo DNA methylation in vivo is negligible. The DNA methylation levels of plants at various stages of a backcrossing program were determined as described in Fig. 2. F_1 heterozygous *ddm1-2*/+ plants produced by the first cross exhibit DNA methylation levels at the midpoint between the parental values. An F_1 heterozygote was backcrossed to a wild-type parent to generate a segregating population [backcross generation 1 (BC_1)]; the 5mC contents of several BC_1 plants are shown for which the genotype at the *DDM1* locus was not determined. The backcrossing program continued for an additional four generations with the use of heterozygous female parents identified by progeny tests. The 5mC content of several plants from a segregating BC_5 family is plotted. All values shown are the average of three quantitation experiments. Amounts of 5mC in successive generations only increase at the rate expected for segregation of the hypomethylated chromosome segments (an approximately 50% increase per generation).

amounts of 5mC that approach the amounts found in wild-type plants in a manner consistent with the gradual loss of the hypomethylated chromosome segments by segregation (Fig. 3). The lack of appreciable remethylation of hypomethylated chromosomes transferred from *ddm1* mutants into a wild-type *Ddm1* background indicates that de novo DNA methylation is slow in vivo. This genetic result complements biochemical data that demonstrate that eukaryotic DNA methyltransferases carry out de novo methylation slowly in vitro (*17*).

The second finding of our genetic characterization is the discovery of a segregation distortion phenotype associated with the *ddm1* mutations. Progeny containing hypomethylated genomes are overrepresented in F_2 generations produced by self-pollination of *ddm1-1*/+ and *ddm1-2*/+ heterozygotes (Table 2).

The mechanisms responsible for the *ddm1* segregation distortion should yield clues about the function of DNA methylation in plants and possibly other eukaryotes. The distortion cannot be explained by loss of *Ddm1* zygotes after fertilization because we did not see a high frequency of aborted or nongerminating seeds in families that segregated *ddm1* mutations. The increased transmission of *ddm1* alleles suggests a possible function for DNA methylation in earlier processes, such as meiosis or gametogenesis. For example, the hypomethylated *ddm1* gametophytes may have a competitive advantage over *Ddm1* gametophytes during the growth and cell division of this haploid stage of the angiosperm life cycle. Alternatively, *ddm1* transmission distortion may occur earlier, during meiosis. Several meiotic drive mechanisms have been described that involve alterations in chromosome structure and behavior (*18*), processes that may be affected by the alteration of DNA methylation (6).

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