RNA Silencing Genes Control de Novo DNA Methylation

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Cytosine DNA methylation silences harmful DNAs such as transposons and retroviruses (1). Maintenance DNA methyltransferases propagate pre-existing DNA methylation in the CG sequence context by methylating hemi-methylated sites after DNA replication. Much less is understood about how invasive DNAs are initially recognized and targeted to unmethylated loci to initiate gene silencing.

Transformation of Arabidopsis with the FWA gene is an assay for de novo DNA methylation and the establishment of gene silencing (2). Endogenous FWA is heritably silenced in most tissues by DNA methylation of two direct repeat promoter sequences (3). In epigenetic hypomethylated fwa alleles, ectopic FWA overexpression causes late flowering. Wild-type plants transformed with FWA efficiently silence the transgene and flower normally, whereas drm1 drm2 double mutants cannot initiate DNA methylation and silencing and therefore flower later (2). De novo DNA methylation of FWA requires a transient signal generated during Agrobacterium-mediated transformation, because an unmethylated FWA transgene initially introduced into a drm1 drm2 mutant is not methylated de novo when crossed with wild-type DRM1 DRM2. This may reflect the role of gene silencing as a defense against invasive DNA; the FWA direct repeats might mimic terminal direct repeats found in retroviruses and long terminal repeat (LTR) retrotransposons.

We used the FWA transformation assay to test de novo DNA methylation in a variety of silencing mutants (Fig. 1). Notably, hkp and cmt3, which affect gene silencing at the SUPERMAN locus, did not affect the initiation of FWA silencing. However, four mutants — RNA dependent RNA polymerase2 (rdr2, At4 g11310), dicer-like3 (dc13), silencing defective4 (sde4), and argonaute4 (ago4) — could not initiate FWA silencing and flowered late. These mutants also failed to establish DNA methylation of FWA transgenes as assayed by bisulfite genomic sequencing and Southern blotting (Fig. 1; fig. S1). However, like drm1 drm2, each mutant in the absence of the transgene flowered normally and maintained preexisting CG methylation at the endogenous FWA locus, showing that these mutants block the establishment but not the maintenance of CG methylation and gene silencing. Interestingly, RDR2, DCL3, and AGO4 are homologous to proteins involved in RNA silencing, a conserved genome defense mechanism that uses short, 21- to 25-nucleotide silencing-induced RNAs (siRNAs) to direct posttranscriptional messenger RNA (mRNA) destruction and also to cause chromatin-level gene silencing (4). siRNAs are processed from double-stranded RNA by Dicer, an RNaseIII–RNA helicase, and their synthesis often requires an RNA-dependent RNA polymerase. Argonaute proteins associate with RNA silencing effector complexes. RDR2, DCL3, SDE4, and AGO4 are required to maintain siRNAs derived from several endogenous loci (5–7). Thus our results suggest that a canonical RNA silencing pathway mediates de novo methylation of transformed direct repeats.

Non-CG methylation at some loci is thought to be maintained by persistent activity of de novo methyltransferases. For instance, drm1 drm2 eliminates non-CG methylation at direct repeats from the endogenous FWA and MEA-ISR loci (8). We found that this methylation was also strongly reduced in rdr2, dc13, sde4, and ago4 (Fig. 1).

Our data suggest that RDR2, DCL3, SDE4, and AGO4 comprise an siRNA-metabolizing pathway that guides the DRM de novo methyltransferases, both to initiate DNA methylation at direct repeats and to perpetuate non-CG DNA methylation. Previous observations have associated RNA viruses and inverted repeat transgenes with DNA methylation of homologous genomic sequences (4). RNA thus appears to be a general means of targeting de novo DNA methylation, which may indicate how sequence-specific gene silencing is established in a variety of epigenetic phenomena.

References and Notes
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