Supplementary Online Material for "RNA Silencing Genes Control de Novo DNA Methylation", Simon W.-L. Chan, Daniel Zilberman, Zhixin Xie, Lisa K. Johansen, James C. Carrington and Steven E. Jacobsen

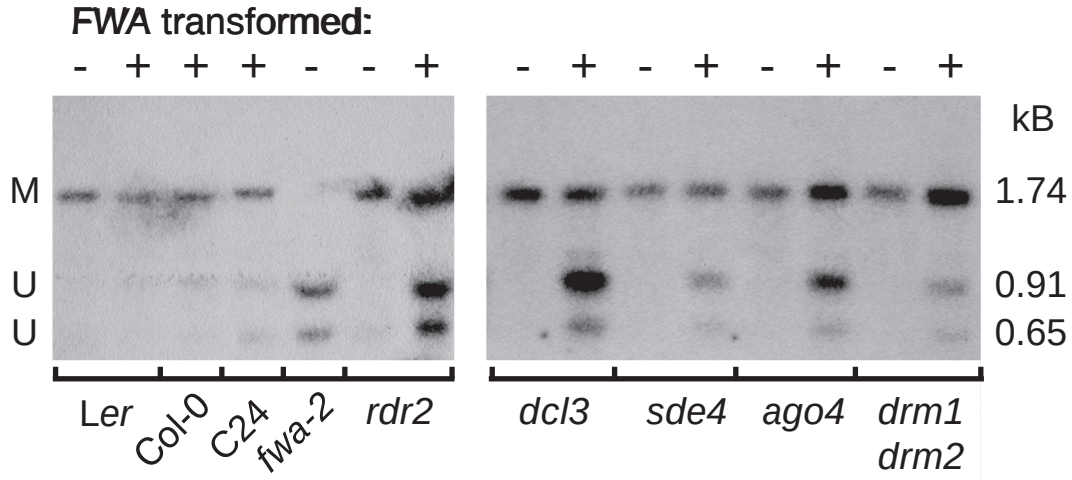

Figure S1. *FWA* Southern blot

CfoI-digested genomic DNA probed with a PCR product spanning *FWA* -1619 to 157 relative to the translational start site. *FWA* transformation is indicated with a "+" sign. "-" means untransformed. *FWA* transformation and Southern blotting were performed as described (*1*). CfoI digestion is sensitive to CG DNA methylation. Methylated band is indicated "M", unmethylated bands are labelled "U". *fwa-2* is an epigenetic, hypomethylated mutant. Untransformed *rdr2, dcl3, sde4, ago4* and *drm1 drm2* plants maintain CG methylation of the *FWA* endogene. Transformed mutants cannot silence the *FWA* transgene, and unmethylated bands are visible in addition to the endogenous *FWA* locus. The *dcl3* transformant shown flowered late with 27 leaves. *dcl3* transformants had a range of flowering times from early to very late. This intermediate late flowering phenotype may indicate redundancy with the three other Dicer homologs in *Arabidopsis.*

Table S1. Number of methylated cytosines.

FWA transgene (12 clones/Col-0; 19 clones *rdr2*).

Genotype	CG	CNG	Asymmetric
Col-0	117/240 (48.8%)	32/120 (26.7%)	84/732 (11.5%)
rdr2	14/380 (3.7%)	0/190 (0%)	2/1159 (0.1%)

FWA endogene (20 clones/genotype).

Genotype	CG	CNG	Asymmetric
L*er*	335/400 (84%)	31/200 (15.5%)	83/1220 (6.8%)
rdr2	352/400 (88%)	0/200 (0%)	6/1220 (0.5%)
dcl3	351/400 (88%)	6/200 (3%)	20/1220 (1.6%)
sde4	347/400 (87%)	7/200 (3.5%)	10/1220 (0.8%)
ago4	347/400 (87%)	5/200 (2.5%)	8/1220 (0.7%)

MEA-ISR (18 clones/L*er* and *ago4*; 22 clones *rdr2, dcl3, sde4*).

Genotype	CG	CNG	Asymmetric
L*er*	137/144 (95%)	21/36 (58.3%)	69/270 (25.6%)
rdr2	149/176 (85%)	0/22 (0%)	0/352 (0%)
dcl3	141/176 (80%)	2/22 (9.1%)	8/352 (2.3%)
sde4	145/176 (82%)	0/22 (0%)	1/352 (0.3 %)
ago4	116/144 (80.6%)	0/36 (0%)	2/270 (0.7%)

* CGG sites are counted as CG sites, not as CNG. Asymmetric is defined by cytosines within the context CHH, where H = A, T, or C. Each fraction represents the number of cytosines methylated out of the total number of sites analyzed.

Bisulfite genomic sequencing was performed as described *(1, 2)*. The L*er*-derived *FWA* transgene is distinguished from the Col-0 endogene by a polymorphism C at –703 (Col-0 = G). Data for the *drm1 drm2* mutant shown in Figure1 is reproduced from (*3*).

We previously reported that *ago4-1* did not reduce non-CG methylation of *FWA (2)*. However in three independent experiments we obtained results similar to those reported here. Our previous conclusion was based on Southern blot analysis with the methylation sensitive enzyme BglII, which cuts once within *FWA*. We have subsequently discovered that the ten CNG sites within *FWA* are stochastically methylated in wild type plants. The aggregate amount of CNG methylation in wild type plants is reproducible, but the percentage of methylation at any given site can vary tremendously from plant to plant. Thus, the BglII Southern blot assay appears to be an inadequate assay for *FWA* non-CG methylation. Furthermore, we previously performed a limited amount of bisulfite sequencing, but we were misled by a small sample size.

Mutants used in this study:

Arabidopsis locus numbers for RNA silencing mutants: *RDR2* = At4g11130 and *DCL3* = At3g43920. Both mutants are T-DNA insertions and are fully described in Xie et al., (*4*). The *rdr2* mutant is from the Syngenta collection (Garlic_1277H08) and the *dcl3* mutant from the Salk collection (SALK_005512). The nomenclature for *Arabidopsis DCL1-DCL4* was first described in (*5*). *kyp*-2, *cmt3-7* and *ago4-1* have been previously described *(2, 6, 7)*. The *sde4-1* mutant phenotype has been described (*8*).

Supplementary online references:

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