Fig. 4. Chromosomal distributions of individual siRNAs in wild type (Upper) and nrpd1a/1b (Lower). miRNAs and tasiRNAs were excluded from this analysis. The remaining siRNAs were categorized by size into 21, 22, and 24 mers, and their chromosomal distribution was shown as the number of siRNAs per 100-kb window (y axis).

Fig. 5. siRNAs are depleted in genes. The 1-kb regions upstream and downstream of the transcribed regions of each known gene were divided into 20 bins (50 bp per bin); the transcribed region of each known gene was also divided into 20 bins (each bin with 5% of the gene). The number of genes overlapping with 21-, 22-, and 24-mer siRNA clusters (Top, Middle, and Bottom, respectively) in each bin was then determined (y axis).

Fig. 6. The histogram shows the fraction of 21-, 22-, and 24-mer siRNAs from wild type or nrpd1a/1b that are derived from inverted repeats, tandem repeats, and dispersed repeats. y axis, the percentage of siRNAs of a certain size in total siRNAs of the corresponding size that are derived from a given type of repeat. The table shows the percentage of each type of repeat that is associated with siRNA clusters in wild type or nrpd1a/1b. Note that although lower fractions all three types of repeats were found to be associated with siRNA clusters in nrpd1a/1b, the smallest reduction was observed for inverted repeat-associated 21-mer siRNA clusters.

Fig. 7. The overlap of 21-, 22-, and 24-mer clusters with each other in wild type.

Fig. 8. Histogram shows the fraction of 21-, 22-, and 24-mer clusters in wild type that are DNA methylated. Only clusters consisting of a single size class (i.e., those that did not overlap with another cluster of a different size) were included in this study to avoid ambiguity. The table below shows the fractions of siRNA clusters associated with MET1-dependent DNA methylation and DRM1/2 CMT3-dependent DNA methylation defined in a previous study (1). Note that ≈64% of all DNA methylation is MET1-dependent, whereas only ≈7% is DRM1/2 CMT3-dependent. Thus, the results suggest that siRNA clusters of all three sizes show higher degrees of association with DRM1/2 CMT3-dependent than with MET1-dependent methylation. One complication of this analysis is that the met1 mutant indirectly causes a loss of DRM1/2 CMT3-dependent DNA methylation at many loci (2), meaning that the fraction of siRNA clusters associated with DRM1/2 CMT3-dependent DNA methylation could be much higher than estimated here.


Fig. 9. The relative abundance of each miRNA family (A) or tasiRNA family (B) in wild type or nrpd1a/1b. y-axis: the percentage of miRNAs and tasiRNAs from a given family in total miRNAs and tasiRNAs, respectively.

Fig. 10. The secondary structure of RNAP IV-independent siRNA clusters that did not map to inverted repeats. They were likely derived from single-stranded hairpin RNA precursors, but the matched regions were too short to yield significant scores as inverted repeats.

Fig. 11. Two examples siRNAs processed by DCL1 from double-stranded, RNAP IV-dependent precursors in the dcl2 dcl3 dcl4 triple mutant.