



Suppl.Fig.3 Daxinger et al.

Supplementary Figure 3: Possible way to distinguish primary and secondary siRNAs of endogenous 24-nt siRNA populations.

Nearly all endogenous 24-nt 'heterochromatic' siRNAs depend on NRPD1 (the largest subunit of Pol IV), NRPE2a (the shared second largest subunit of Pol IV and Pol V), RDR2 and DCL3 for their biogenesis (Kasschau et al., 2007; Zhang et al., 2007; Mosher et al., 2008). This is evidenced by the uniform disappearance of endogenous 24-nt siRNAs in *nRPD1*, *nRPE2* and *rdr2* mutants (examples of endogenous siRNAs shown here: *Tag2*, *SAT5*, siRNA1003, siRNA02 and solo LTR siRNAs).

By contrast, endogenous 24-nt siRNAs show variable reductions in mutants defective in NRPE1 (the largest subunit of Pol V) and DRD1, a SNF2-like factor that is thought to act together with Pol IVb to facilitate *de novo* methylation of DNA at the siRNA targeted site (Matzke et al., 2007). For example, the siRNAs from soloLTR and siRNA02 are present at wild type levels in *nRPE1* and *drd1* mutants, whereas those from siRNA1003 and SAT5 repeats are reduced but still detectable in *nRPE1* and *drd1* mutants. As a final variation, the 24-nt siRNAs detected with the *Tag2* probe nearly disappear in the *nRPE1* and *drd1* mutants.

Based on our finding from the transgene system that secondary siRNA depends on pre-existing primary RdDM appears to attract the secondary siRNA-generating machinery (Pol IV, RDR2, DCL3), and on the known requirement for Pol V and DRD1 to facilitate *de novo* methylation at the small RNA-targeted site, we propose that secondary siRNAs are those that are not made in *nRPE1* and *drd1* mutants. This proposal applies to the endogenous siRNAs shown as indicated to the right of the blots.

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