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Nucleolar Dominance and rDNA Methylation Directed by Small Interfering RNA

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In the December 5th issue of *Molecular Cell*, Preuss et al. (2008) demonstrated a link between small interfering RNA (siRNA)-directed de novo DNA methylation and rDNA silencing in nucleolar dominance.

In cells of interspecies hybrids, the rRNA genes derived from one of the parental species is sometimes inactivated in a phenomenon called nucleolar dominance. Nucleolar dominance involves a simple hierarchical relationship where one species' rRNA genes are always dominant over the other species; this is neither the result of random inactivation nor correlated with genomic imprinting. Nuclear dominance may be merely an extension of mechanisms that regulate rDNA expression or a separate event associated with the union of two different species genomes (McStay, 2006; Preuss and Pikaard, 2007). In a recent issue of *Molecular Cell*, Preuss et al. (2008) have used RNAi to identify genes involved in DNA methylation that are necessary for nucleolar dominance in the allopolyploid hybrid *Arabidopsis suecica*.

Most models for nucleolar dominance invoke DNA sequence changes in rDNA from one parental species that allow them to compete with rRNA genes from the other species for *trans*-activating factors, such as the nucleolar remodeling complex NoRC. However, these promoter-based models are hard to reconcile with silencing throughout species-specific *n*ucleolar *o*rganizing *r*egions (NORs) in plants, as some individual promoters from the nondominant species would be predicted to escape inactivation (McStay, 2006; Preuss and Pikaard, 2007). Furthermore, in *Arabidopsis suecica*, a naturally occurring hybrid of *Arabidopsis thaliana* and *Arabidopsis arenosa*, species-specific rDNA silencing is dynamic during development. Additionally, in synthetic *Arabidopsis* hybrids, (offspring of an *A. thaliana* and *A. arenosa* cross) nucleolar dominance is not established until the F2 generation and depends on the ratio of the two genomes. These results contradict models based on competition because a better-adapted rRNA gene would always be active regardless of genome fluctuations. Other clues to the mechanism of nucleolar dominance came from drug treatments of *Arabidopsis* hybrids with DNA methylation or histone deacetlyation inhibitors that result in the derepression of species-specific rRNA genes (Chen et al., 1998). Notably, RNAi knockdown of histone deacetylases in *A. suecica* has implicated *H*istone *D*eace*t*lyase *1* (HDT1) and *H*istone *Dea*cetylase *6* (HDA6) in nucleolar dominance (Earley et al., 2006).

Preuss et al. (2008) have gone on to knock down DNA methyltransferases and to investigate their role in nucleolar dominance. They discovered that *D*omains *R*earranged *M*ethyltransferase *2* (DRM2) is necessary for silencing of *A. thaliana*-derived 45S rRNA genes in *A. suecica*. DRM2 methylates cytosine residues in the asymmetric context CHH (where H is any nucleotide except G). Unlike symmetric CG methylation that is inherited by both daughters after cell division, the location of asymmetric CHH methylation requires de novo reestablishment. DRM2 is guided by the siRNA biogenesis pathway, and *D*icer *L*ike *3* (DCL3) and *R*NA *D*ependent *R*NA Polymerase *2* (RDR2) were also implicated in nucleolar dominance and DNA methylation of *A. thaliana*-derived NORs by knockdown in *A. suecica*. Moreover, Preuss et al. (2008) were able to discover 24 nucleotide siRNAs mapping to the 45S promoter and *i*nter*g*enic *s*pacer (IGS) of rRNA genes whose biogenesis is disrupted when DCL3 or RDR2 are perturbed. These results suggest a model whereby siRNAs guide DNA methylation and silencing of *A. thaliana*-derived NORs in *A. suecica* plants (Figure 1).

As a complementary approach, the researchers screened the entire family of *M*ethyl CpG *B*inding *D*omain (MBD) proteins for a role in nucleolar dominance. MBD proteins are believed to bind to methylated DNA in specific contexts to recruit different proteins that modify chromatin structure, like *H*istone *Dea*cetylases (HDACs) (Zemach et al., 2005). Additionally, human MDBs are involved in rRNA gene silencing and nucleolar dominance in mouse-human hybrid cell lines (McStay, 2006). The RNAi screen of MBD proteins showed

that MBD6 and MBD10 were necessary for A. *thaliana*-derived rRNA gene silencing in *A. suecica*. MBD6 was enriched in the condensed *A. thaliana*-derived NORs in both *A. thaliana* and *A. suecica* plants, and DRM2 was necessary for its localization. Importantly, the discovery that MBD6 and DRM2 are involved in both nucleolar dominance, and individual rRNA gene silencing suggests that the mechanisms overlap. However, there are some differences; for instance, perturbation of *D*eficient in *D*NA *M*ethylation *1* (*DDM1*), a chromatin remodeler involved in rRNA gene silencing, did not relieve silencing of *A. thaliana*-derived NORs in *A. suecica*, but seems to disrupt MBD6 localization in *A. thaliana* (Zemach et al., 2005).

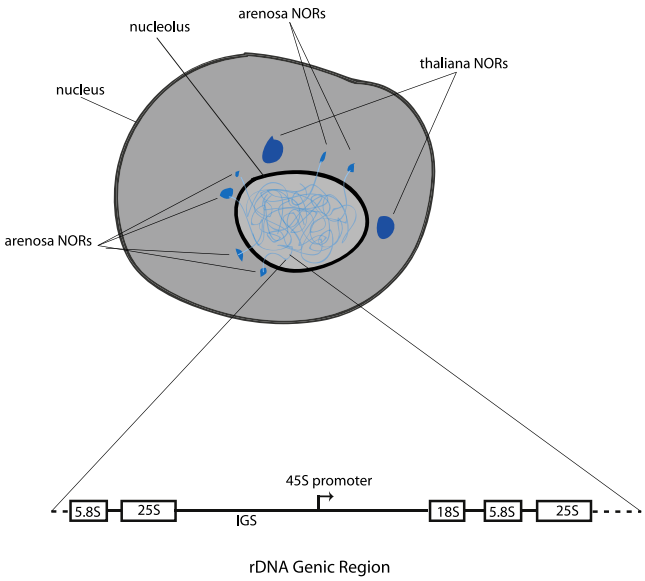

Figure 1. NORs in an *Arabidopsis suecica* Nucleus
A. thaliana and *A. arenosa* nucleolar organizing regions (NORs) differ in size in *A. suecica* plants (Pontes et al., 2003). The *A. thaliana*-derived NORs are highly condensed and overlapping, as sometimes only two NORs can be seen rather than the expected four. The *A. arenosa* rRNA genes reside partly in much smaller NORs but largely in the nucleolus, where they provide most of the rRNA.

Together, these results suggest that siRNA-guided, DRM2-directed DNA methylation recruits MBD6, followed by HDACs, to NORs to deacetylate histones and silence the rRNA genes. The transient nature of DRM2 DNA methylation (Chan et al., 2005) provides an explanation for dynamic and reversible phenotypes of nucleolar dominance in *Arabidopsis*. While this model is very intriguing, what still remains unclear is how the *A. arenosa*-derived rRNA genic regions fit into this hypothesis. A provocative idea is that small RNA might be involved, and it is likely that further profiling of *A. arenosa*-derived NORs in *A. suecica* would help to determine the mechanism of uniparental silencing.

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