Uniting the paths to gene silencing

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A new study of ribosomal RNA gene silencing in the mouse provides clues as to how repressive chromatin states become established. At center stage is a chromatin remodeling complex that recruits DNA methyltransferase and histone deacetylase activities to the gene promoter, initiating a process that includes *de novo* DNA methylation, methylation of histone H3 on Lys9 and heterochromatin protein binding.

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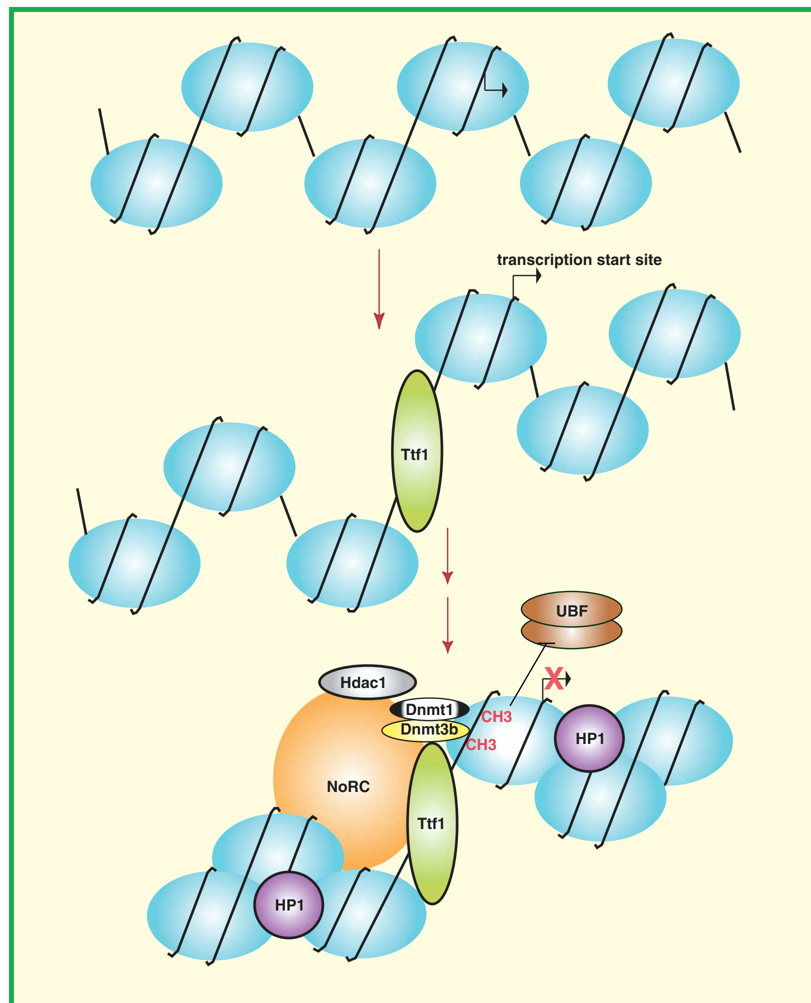
Who's on first?

Chromatin remodeling, histone modification and DNA methylation regulate gene activity in many eukaryotes[1]. Methylation of cytosines can block binding of transcription factors and create binding sites for complexes that modify chromatin, including histone deacetylases. The latter enzymes facilitate subsequent modifications, including methylation of histone H3 on Lys9, which, in turn, is needed to maintain cytosine methylation patterns[2]. This vicious cycle of DNA methylation and histone modification ensures that a silenced gene stays silenced.

How do methylation patterns first become established in this self-reinforcing cycle? Do histone modifications come first, causing changes in DNA methylation as a consequence of gene silencing? Or is it *de novo* DNA methylation that recruits repressive histone modifying complexes? The accompanying paper by Raffaella Santoro and colleagues[3] suggests that the answer may be both. By investigating how several hundred of the ribosomal RNA genes in the mouse are silenced, these authors provide evidence that a sequence-specific DNA binding protein recruits a repressive chromatin remodeling complex to the promoter. At least two DNA methyltransferases and one histone deacetylase physically interact with this chromatin remodeling complex such that *de novo* promoter methylation and histone modifications ensue. These results suggest that *de novo* DNA methylation and histone deacetylation might proceed as parallel pathways to gene silencing.

Chance favors the prepared lab

The study by Santoro *et al.*[3] derives from a longstanding interest in Ingrid Grummt's laboratory concerning a duplicated RNA polymerase I transcription termination sequence (T_0) located just upstream of vertebrate ribosomal RNA (rRNA) gene promoters. One of its functions is to protect against rogue polymerases that might plow through the promoter from upstream sequences, disrupting transcription complexes. However, T_0 also has characteristics of a promoter element[4]. A rationale for the *cis*-stimulatory effect of T_0 became apparent upon studying transcription of mouse rRNA genes assembled into chromatin. Addition of Ttf1 (transcription termination factor), which binds T_0, was essential for transcription from such chromatin templates[5]. Apparently, Ttf1 collaborates with one or more ATP-dependent chromatin remodeling activities, resulting in



NoRC and company. A model for ribosomal RNA gene silencing in the mouse begins with Ttf1 binding, which repositions nucleosomes in the vicinity of the promoter and recruits the ATP-dependent chromatin remodeling complex NoRC[3]. DNA methyltransferase (Dnmt1, Dnmt3b) and histone deacetylase (Hdac1) activities interact with NoRC, resulting in *de novo* DNA methylation (CH3) and histone deacetylation. One consequence of promoter methylation is that binding of UBF, a transcription factor for vertebrate rRNA genes, is inhibited. Methylation of histone H3 on Lys9 and subsequent binding of heterochromatin protein 1 (HP1) ultimately help to condense the nucleosomal DNA into silenced heterochromatin.

positioned nucleosomes that allow the initiation of transcription[6].

A search for Ttf1-interacting proteins in a yeast two-hybrid screen yielded Tip5 (Ttf1-interaction protein 5), which pairs with Snf2h, the mammalian ortholog of ISWI, to form an ATP-dependent chromatin remodeling machine called NoRC (nucleolar remodeling complex)[7]. Expecting NoRC to increase transcription of a transfected rRNA minigene, Santoro *et al.*[3] found instead that overexpression of Tip5 inhibited transcription. Notably, a chemical inhibitor of cytosine methylation prevented Tip5-mediated repression. A clever PCR assay that yields a product only if methylation prevents *Hpa*II cutting of the promoter (at position –143) revealed that transfected rRNA gene promoters became methylated only when Tip5 was co-transfected. Furthermore, chromatin immunoprecipitation experiments showed that Tip5 and Snf2h stably associate with promoters methylated at position –143.

Histone H3 methylated on Lys9 and heterochromatin protein 1 (HP1), two hallmarks of silenced genes[1], were also associated with methylated promoters. By contrast, antibodies that recognize hyperacetylated histone H4 or the largest subunit of RNA polymerase I—hallmarks of active genes—precipitated only unmethylated promoter DNA. Apparently, overexpression of Tip5 causes *de novo* methylation and chromatin-mediated silencing of co-transfected rRNA gene promoters. The probable mechanism by which this occurs is suggested by the finding that epitope-tagged Tip5 co-immunoprecipitates two DNA methyltransferases, Dnmt1 and Dnmt3b, as well as the Sin3 co-repressor complex[8] that includes the histone deacetylases, Hdac1 and Hdac2.

Unifying themes

The results of Santoro *et al.*[3] are timely and important for several reasons. First, the work provides strong evidence, albeit circumstantial, for a link between chromatin remodeling and *de novo* methylation in transcriptional silencing. Second, the evidence suggests that *de novo* methylation and histone deacetylation might proceed in parallel, rather than in series, given that DNA methyltransferases and histone deacetylases interact with NoRC. These results also provide interesting parallels to other recent studies. For example, Hdac1 interactions with Dnmt1 and with Dnmt3b methyltransferases have been demonstrated in protein-coding gene silencing, although in these cases the data suggest that *de novo* methylation is not essential for the DNA methyltransferases to participate in repression[9,10]. Another recent study showed recruitment of *de novo* methyltransferase activity by a DNA-bound repressor, leading to silencing of a tumor suppressor gene[11]. The Santoro *et al.*[3] study would seem to encompass these observations, suggesting that genes transcribed by RNA polymerases I and II can use the same gene silencing machinery.

There are a few missing elements in the picture painted by Santoro *et al.*[3]. The authors do not demonstrate that T_0, the Ttf1 binding site, is essential for NoRC-mediated silencing; that Ttf1 is stably associated with methylated promoters; that NoRC actually repositions nucleosomes as a prerequisite for *de novo* methylation or histone deacetylation; or that methylation of the *Hpa*II site at position –143 is accompanied by methylation of the CpG at position –133, a modification that inhibits promoter binding by the RNA polymerase I transactivator UBF[12]. Nonetheless, one can make reasonable inferences to propose a testable model (see figure).

Ribosomal RNA gene dosage control

Eukaryotic cells regulate ribosomal RNA gene transcription, in part, by controlling the number of active genes. Only a subset are accessible to nucleases and cross linking agents; the remainder appear to be tightly packaged and silenced[13]. The epigenetic phenomenon called nucleolar dominance—the complete silencing of one parental set of rRNA genes in a genetic hybrid—provides an extreme example of rRNA gene silencing[14]. The derepression of silenced genes subjected to nucleolar dominance by chemical inhibitors of cytosine methylation or histone deacetylation was early evidence that chromatin modifications silence rRNA genes[15]. It is tempting to speculate that NoRC and its affiliated Dnmt and Hdac activities may have roles in nucleolar dominance as well as in growth-regulated rRNA gene copy number control. ☐

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