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The chromatin remodeling complex NoRC represses transcription and controls replication timing of ribosomal genes.

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In eukaryotes, the tandem repeats rRNA genes (rDNA) exist in two distinct types of chromatin, an 'open' one that is permissive to transcription and a 'closed' one that is transcriptionally refractive. Previous results have established that the nucleolar remodeling complex (NoRC) is associated with silent rRNA genes and overexpression of TIP5, the large subunit of NoRC, silences rDNA transcription. The goal of this study was to analyse the effect of NoRC on the chromatin structure of the rDNA locus as well as the transcriptional activity and replication timing of rRNA genes.

To examine the role of NoRC in rDNA silencing, TIP5 was overexpressed in NIH3T3 cells by co-transfection of an expression vector encoding TIP5 and an artificial ribosomal minigene construct and transcription of the RNA polymerase I (Pol I) reporter gene was monitored on Northern blots or by RT-PCR. This experimental approach revealed that NoRC represses transcription of both the reporter gene and endogenous rRNA genes. NoRC-mediates transcriptional repression was accompanied by induction of heterochromatic features at the rDNA promoter, i.e., CpG methylation and histone deacetylation. If cells were treated with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (aza-dC) and /or the

histone deacetylase inhibitor trichostatin A (TSA), NoRC-mediated transcriptional repression was alleviated. These results suggest that NoRC represses Pol I transcription by establishing heterochromatic structure at the rRNA locus and that histone deacetylation and DNA methylation do not act synergistically but operate along a common mechanistic pathway. NoRC did not exert a repressive effect on a mutant rDNA in which cytosine-133 was replaced by guanine suggesting that transcriptional silencing by NoRC is brought about by 'de novo' methylation of a critical cytosine within the promoter region which in turn, impairs initiation complex formation and represses rRNA synthesis.

To investigate NoRC function in a more physiological context, a cell line was established that overexpresses Flag-tagged TIP5. Northern blots and RT-real time PCR show that pre-rRNA synthesis in NHI3T3-TIP5#6, a stable cell line that overexpresses TIP5 2-fold, was 3.6-fold reduced compared to NHI3T3 cells. Moreover, NHI3T3-TIP5#6 cells were morphologically altered and cell proliferation was retarded. The fact that 2-fold overexpression of TIP5 in NHI3T3-TIP5#6 cell has such dramatic consequences on cell physiology underscores the importance of NoRC in the regulation of cellular rRNA synthesis and ribosome biogenesis. DNA methylation and chromatin immunoprecipitation assays demonstrate that in NHI3T3-TIP5#6 cells the level of CpG methylation was enhanced, whereas histone H4 was hypoacetylated compared to parental NIH3T3 cells. Thus, moderate overexpression of TIP5 triggers the establishment of heterochromatic features at the rDNA locus.

To study whether there is a link between chromatin structure, transcriptional activity and replication timing, NIH3T3 cells were labeled with BrdU during S phase progression and nascent rDNA was analyzed by PCR. This analysis has shown that rDNA is replicated in a biphasic manner, ~60% replicating early and ~40% replicating late in S phase. In NIH3T3-TIP5#6 cells, the ratio of early to late replicating rRNA genes was altered, ~30% replicating early and ~70% replicating late. Thus, overexpression of NoRC shifts replication timing from early to late. The results reveal a clear demarcation active and inactive rRNA genes by their associated proteins and a link between rDNA transcription, chromatin structure and replication timing and demonstrate that chromatin structure is the most important determinant of replication.