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Review

Epigenetic control of RNA polymerase I transcription in mammalian cells ☆

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ABSTRACT

rRNA synthesis is regulated by genetic and epigenetic mechanisms. Epigenetic states are metastable, changing in response to appropriate signals, thereby modulating transcription in vivo. The establishment, maintenance and reversal of epigenetic features are fundamental for the cell's ability to 'remember' past events, to adapt to environmental changes or developmental cues and to propagate this information to the progeny. As packaging into chromatin is critical for the stability and integrity of repetitive DNA, keeping a fraction of rRNA genes in a metastable heterochromatic conformation prevents aberrant exchanges between repeats, thus safeguarding nucleolar structure and rDNA stability. In this review, we will focus on the nature of the molecular signatures that characterize a given epigenetic state of rDNA in mammalian cells, including noncoding RNA, DNA methylation and histone modifications, and the mechanisms by which they are established and maintained. This article is part of a Special Issue entitled: Transcription by Odd Pols.

1. Introduction

Epigenetic control of rRNA genes plays an essential role in nucleolar activity and genomic stability. The epigenetic information resides in self-propagating molecular signatures that provide a memory of previously experienced environmental signals, such as environmental stimuli, developmental cues or internal events, without changing the genetic information. These events converge on chromatin and are converted into epigenetic signatures, leading to stable albeit reversible changes in chromatin structure, DNA methylation and specific post-translational histone modifications. The coordinate combination of these processes may lock rRNA genes in specific states that co-exist in growing and growth-arrested cells. Being the most prominent nuclear body and the largest cellular transcription factory, the nucleolus represents a paradigm for studying both the organization and regulation of gene expression. Mammalian cells contain several hundred rRNA genes (rDNA) that are clustered in tandem repeats on five acrocentric chromosomes at Nucleolar Organizer Regions (NORs) in a telomere to centromere orientation (Fig. 1).

Several experimental approaches have revealed that only a subset of rRNA genes is active at any given time. Active rRNA genes exhibit a eukaryotic 'open' chromatin structure that is permissive to transcription whereas silent ones feature a more compact heterochromatic structure that is transcriptionally refractive. Recently, the existence of a third, intermediate chromatin configuration has been brought to light that marks rRNA genes that are poised for transcription activation [1]. Evidence that different chromatin states of rRNA genes co-exist even in rapidly growing cells originally came from psoralen crosslinking experiments in yeast and mammals. These studies have revealed that the ratio of psoralen-accessible and non-accessible, i.e., active and silent rDNA repeats, is stably maintained through cell cycle progression [2–4]. Nevertheless, long-term changes in the ratio of rRNA genes in an 'open' euchromatic *versus* a 'closed' heterochromatic structure correlate with cell differentiation, senescence, disease and malignancy. Hypomethylation of rRNA genes, reflecting an increase in the number of active genes at the expense of silent ones, has been associated with uncontrolled growth and rRNA synthesis in several tumors [5,6], whereas in senescent cells the rDNA promoter was found to be hypermethylated [7,8]. Given that down-regulation of rRNA synthesis limits ribosome biogenesis and protects cells from energy deprivation-induced apoptosis, elucidation of mechanisms that promote and maintain a fraction of rDNA repeats in a facultative heterochromatic conformation is a key issue that links chromatin silencing to chromosome stability and cell metabolism. This review summarizes the current knowledge of the molecular mechanisms that establish and maintain the different epigenetic states of rRNA genes, unraveling a complex interplay of chromatin modifying enzymes that act in concert with RNA-guided mechanisms to define the transcriptional state of rDNA (Table 1).

2. rRNA genes exist in distinct epigenetic states

Ribosomal genes are the most actively transcribed genes in eukaryotes [9]. Despite its high transcriptional activity, the nucleolus is closely

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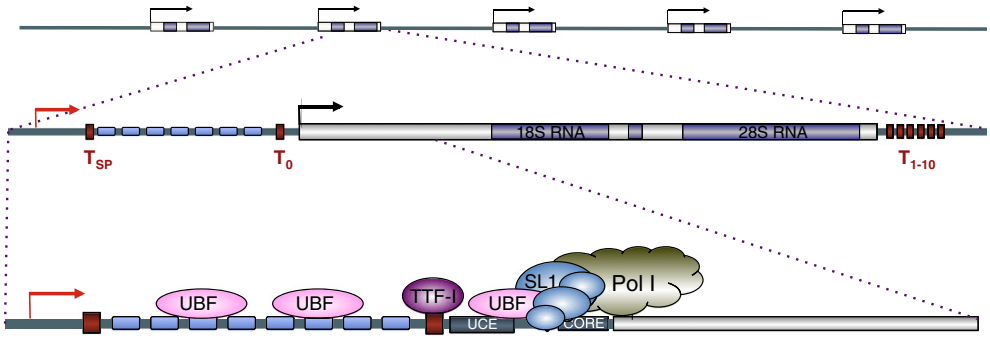

Fig. 1. Structural organization of murine rDNA clusters. Cartoon of mouse rRNA genes, derived from Genbank accession number BK0009XX. The site of transcription initiation of the 47S pre-rRNA (black arrow) and intergenic transcripts originating from a Pol I promoter 2 kb upstream of the transcription start site (red arrow) are indicated. Terminator elements located downstream of the transcription unit (T_1-T_{10}), downstream of the spacer promoter (T_{SP}) and upstream of the gene promoter (T_0) are marked (red boxes). Repetitive enhancer elements located between the spacer promoter and major gene promoter are represented by blue boxes. A magnification of the 5′-end of the rDNA transcription unit and the proteins that associate with the promoter/enhancer region is shown below.

associated with heterochromatin and a significant fraction of rRNA genes is transcriptionally silent. The fact that even in proliferating cells with a high demand of ribosome biogenesis about half of the rRNA genes is epigenetically silent, permits insight into the mechanisms that establish the active and silent state, respectively, and allows elucidation of the functional impact of maintaining a certain ratio of active and silent rDNA repeats for cell surveillance and genomic stability. Generally, transcriptionally active genes are characterized by an 'open', euchromatic structure, are free of regularly spaced nucleosomes and are accessible to psoralen, a drug that intercalates into double-stranded DNA and generates covalent interstrand links upon UV irradiation [2]. Silent rRNA genes, on the other hand, exhibit a compact heterochromatic structure, display regularly spaced nucleosomes and are inaccessible to psoralen. Though the relative amounts of active and silent rDNA repeats are stably maintained through cell cycle progression [10], long-term changes in the ratio of genes in a euchromatic *versus* a heterochromatic structure have been observed. For example, down-regulation of rDNA transcription in terminally differentiated murine granulocytes correlates with decreased UBF occupancy without increasing promoter methylation [11]. Moreover, liver cells have more active genes than lung cells, correlating with increased rRNA synthesis [12]. Thus rRNA genes undergo dynamic changes, suggesting epigenetic mechanisms that establish down-regulation of rDNA transcription in terminally differentiated cells.

Two classes of Nucleolus Organizer Regions (NORs) exist, active and inactive ones, the former representing secondary constrictions at the short arm of acrocentric chromosomes that are associated with Pol I and transcription factors [13–15]. This indicates that the Pol I transcription machinery remains bound to mitotic NORs at genes that were active during the preceding interphase. In contrast, previously inactive genes are condensed, form no secondary constrictions and are not associated with the Pol I transcription apparatus. NORs are induced by binding of UBF to rDNA bound UBF recruiting Pol I together with multiple Pol I-associated proteins [16,17]. With regard to the epigenetic features of active and silent rRNA genes, active genes are characterized by DNA hypomethylation, acetylation of histone H4 and dimethylation of histone H3 at lysine 4 (H3K4me2), whereas silent rRNA genes are demarcated by repressive epigenetic marks, such as DNA hypermethylation, histone H4 hypoacetylation, and trimethylation of H3K9, H4K20 and H3K27 [18–22]. DNA methylation, the covalent addition of a methyl group to the 5′-position of cytosine within CpG dinucleotides, generally correlates with transcriptional silencing, and alterations in the CpG methylation pattern are hallmarks of many human diseases and cancer. Hypomethylation of rRNA genes correlates with decreased genomic stability, suggesting that silencing entails the assembly of a generally repressive chromatin structure that is less accessible to the cellular recombination machinery [23,24]. A systematic search for alterations of DNA methylation has revealed a region of rDNA that is specifically hypermethylated with age in both spermatozoa and liver of male rats, indicating that rDNA methylation is susceptive to age-dependent alteration of cellular functions [25]. Activation of a large fraction of normally silent rRNA genes by genetic inactivation of DNA methyltransferases

Table 1
Epigenetic regulators of rDNA transcription.

Regulators of DNA methylation			
Name	Activity	Nucleolar function	References
Dnmt1	DNA methyltransferase	Repressor	[18,26,29,94]
Dnmt3a	DNA methyltransferase	Repressor	[94]
Dnmt3b	DNA methyltransferase	Repressor	[18,26,67,94]
GADD45a	DNA demethylase	Activator	[100]
MeCP2	Methyl-CpG binding protein	Repressor	[5]
MBD1	Methyl-CpG binding protein	Repressor	[5]
MBD2	Methyl-CpG binding protein	Repressor	[5]
MBD3	Methyl-CpG binding protein	Activator/ Repressor	[5,29]
Histone modifying enzymes			
CBP	Acetyltransferase	Activator	[92,98]
GCN5	Acetyltransferase	n.d.	[102]
MOF	H4K16 histone deacetylase	Repressor	[71]
PCAF	Acetyltransferase	Activator	[92,97,102]
p300	Acetyltransferase	Activator	[92,102]
HDAC1	Histone deacetylase	Repressor	[19,98]
HDAC2	Histone deacetylase	Repressor	[96]
SirT1 (mSir2a)	NAD^+-dependent deacetylase	Repressor	[27,71,85,97]
SirT7	NAD^+-dependent deacetylase	Activator	[89]
KDM2A	Lysine demethylase	Repressor	[101]
KDM2B (JHDM1B, FBXL10)	Lysine demethylase	Repressor	[82]
KDM4C (JMJD2B)	Lysine demethylase	Activator	[88,90]
PHF8	Lysine demethylase	Activator	[83,104]
G9a	Histone methyltransferase	Activator	[42]
Nucleomethylin	H3K9me2 binding protein	Repressor	[85]
PRMT5	Arginine methyltransferase	Repressor	[93]
Suv39H1	H3K9 histone methyltransferase	Repressor	[85]
Chromatin remodeling factors			
ATRX	ATP-dependent helicase	Repressor	[91,95]
CHD4	ATPase/helicase	Activator/ Repressor	[1,81]
CHD7	ATP-dependent helicase	Activator	[103]
CSB	DNA-dependent ATPase	Activator	[42,72]
BRG1	ATPase of the SWI/SNF complex	n.d.	[96]
SNF2H	DNA-dependent ATPase	Activator/ Repressor	[43,52,102]
TIP5	Large subunit of NoRC	Repressor	[19,43]
WSTF	Williams syndrome transcription factor	Activator	[99,102]
NuRD	Nucleosome remodeling and deacetylation complex	Activator/ Repressor	[1,81]