Stop-and-Go: Dynamics of Nucleolar Transcription During the Cell Cycle

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ABSTRACT: Entry into mitosis correlates with nucleolar disassembly and shutdown of ribosomal RNA (rRNA) gene (rDNA) transcription. In telophase, nucleoli reform and transcription is reactivated. The molecular mechanisms underlying the dynamics of nucleolar transcription during the cell cycle are manifold. Although mitotic inactivation of the RNA polymerase I (Pol I) transcription machinery by posttranslational modifications has been extensively studied, little is known about the structure of rDNA chromatin during progression through mitosis. Methylation of histone H2A at glutamine 104 (H2AQ104me), a dedicated nucleolar histone modification, is lost in prometaphase, leading to chromatin compaction, which enforces mitotic repression of rRNA genes. At telophase, restoration of H2AQ104me is required for the activation of transcription. H2AQ104 methylation and chromatin dynamics are regulated by fibrillarin (FBL) and the NAD^+-dependent nucleolar deacetylase sirtuin 7 (SIRT7). Deacetylation of FBL is required for the methylation of H2AQ104 and high levels of rDNA transcription during interphase. At the entry into mitosis, nucleoli disassemble and FBL is hyperacetylated, leading to loss of H2AQ104me, chromatin compaction, and shutdown of Pol I transcription. These results reveal that reversible acetylation of FBL regulates methylation of nucleolar H2AQ104, thereby reinforcing oscillation of Pol I transcription during the cell cycle.

KEYWORDS: SIRT7, fibrillarin, acetylation, H2AQ104 methylation, rDNA, transcription, cell cycle, RNA polymerase I

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Introduction

The biogenesis of ribosomes is a coordinated multistep process that takes place in the nucleolus, where rRNA is synthesized, processed, modified, and assembled into ribosomal subunits. As rRNA synthesis is rate limiting for ribosome biogenesis, transcription of pre-rRNA is the convergence point that collects and integrates information from cellular signaling cascades to regulate ribosome production in response to external signals, such as nutrient availability, growth factors, and cellular stress.

In addition to up- or down-regulation in response to environmental signals, rRNA synthesis oscillates during the cell cycle. Transcription of rRNA genes (rDNA) is maximal during S- and G_2-phase. At the entry into mitosis, nucleoli undergo extensive morphological changes and rDNA transcription ceases almost completely. The inactive RNA polymerase I (Pol I) transcription machinery remains associated with the nucleolar organizer regions (NORs), thus bookmarking active rDNA repeats.[1] Mitotic repression of transcription is caused by posttranslational modification of several components of the basal transcription machinery. In addition, a recent study has uncovered a chromatin-based mechanism that reinforces transcriptional repression at the onset of mitosis and reactivates nucleolar transcription at the entry into G_1-phase.[2]

Cell Cycle-Dependent Regulation of Basal Pol I Transcription Factors

Pol I transcription fluctuates during the cell cycle. Transcription is maximal during S- and G_2-phase, is repressed during mitosis, and slowly recovers during G_1-phase. During G_1-phase, the DNA-binding basal factor UBF is phosphorylated at Ser484 by CDK4/cyclin D and CDK2/cyclin E, which is important for the activation of rDNA transcription at the cell cycle restriction point. During S-phase, a further increase in UBF activity is achieved by phosphorylation at Ser388 by CDK2/cyclin E&A, which strengthens the interaction of UBF with Pol I.[3]

UBF binds to the rDNA promoter cooperatively with SL1/TIF-IB, a multiprotein complex comprising the TATA-box-binding protein (TBP) and five TBP-associated factors (TAF_Is). At the onset of mitosis, SL1/TIF-IB is inactivated by phosphorylation of TAF_I110 at a threonine residue (Thr852) by CDK1/cyclin B. Phosphorylation of TAF_I110 impairs the interaction of SL1/TIF-IB with UBF, which is required for the assembly of a productive pre-initiation complex at the rDNA promoter.[4,5] At the end of mitosis, Thr852 is dephosphorylated by hCdc14B, a phosphatase that is sequestered in the nucleolus during interphase and is released from rDNA during mitosis. Dephosphorylation of TAF_I110 by hCdc14B activates SL1/TIF-IB and facilitates rDNA transcription at the entry into the G_1-phase.[6]

Superimposed on this regulation is acetylation of TAF_I68, another subunit of SL1/TIF-IB. Acetylation of TAF_I68 by the lysine acetyltransferase p300/CBP-associated factor (PCAF) stimulates the interaction of TAF_I68 with the rDNA promoter, hence augmenting transcription initiation.[7] The PCAF-dependent acetylation of TAF_I68 is counteracted by sirtuin 1 (SIRT1), the founding member of a family of conserved NAD^+-dependent lysine deacetylases, termed sirtuins. SIRT 1-dependent deacetylation of TAF_I68 leads to transcriptional

repression.[6] In contrast, another member of the sirtuin family, SIRT7, is associated with active rDNA repeats and is required for the activation of Pol I transcription at the exit from mitosis.[2,8] Thus, different members of the sirtuin family play divergent roles in the regulation of rDNA transcription, SIRT1 repressing and SIRT7 stimulating Pol I transcription.

SIRT7 Regulates Fibrillarin Acetylation and H2AQ104 Methylation

Fibrillarin (FBL) is an abundant nucleolar protein with a dual function. FBL mediates 2′-O methylation of pre-rRNA and methylation of nucleolar histone H2A at glutamine 104 (H2AQ104me). H2AQ104me is a specific nucleolar chromatin mark that correlates with Pol I transcription.[9,10] FBL is acetylated at several lysine residues by the acetyltransferase CREB-binding protein (CBP) and deacetylated by the NAD^+-dependent deacetylase SIRT7.[2] Acetylation weakens the association of FBL with its histone substrate and attenuates H2AQ104 methylation. Deacetylation by SIRT7, on the contrary, promotes H2AQ104 methylation and boosts Pol I transcription. In support of SIRT7-mediated deacetylation of FBL promoting H2AQ104 methylation, depletion of SIRT7 or double-knockdown of SIRT7 and FBL led to similar reduction in H2AQ104me. Overexpression of wild-type SIRT7, but not a catalytically inactive SIRT7 mutant, rescued H2AQ104me and pre-rRNA synthesis in SIRT7-depleted cells, validating that the enzymatic activity of SIRT7 is required for H2AQ104me. Accordingly, hyperacetylation of FBL by the inhibition of SIRT7 using nicotinamide or overexpression of CBP led to the attenuation of the H2AQ104me signal, emphasizing that hyperacetylation of FBL inhibits H2AQ104 methylation.

FBL-dependent regulation of H2AQ104me levels by reversible acetylation is exemplified during cell cycle progression. Like rDNA transcription, H2AQ104 methylation fluctuates during the cell cycle. At the onset of mitosis, FBL is released from the NORs, while SIRT7 and several components of the transcription machinery remain associated with the NORs.[8,11] This topological separation of SIRT7 and FBL results in hyperacetylation of FBL, which in turn leads to loss of H2AQ104me and establishment of a transcription-refractive chromatin structure. At the exit from mitosis, deacetylation of FBL augments the restoration of H2AQ104me and resumption of rDNA transcription. Overexpression of wild-type FBL or an acetylation-deficient FBL mutant (4KR-FBL) restored H2AQ104me and nucleolar transcription in late telophase. Overexpression of an acetylation-mimetic mutant (4KQ-FBL), on the contrary, did not promote H2AQ104 methylation or pre-rRNA synthesis. Moreover, antibodies against FBL blocked the reactivation of Pol I transcription and reformation of nucleoli at the exit from mitosis, supporting that hyperacetylation of FBL compromises H2AQ104 methylation and rDNA transcription at the entry into mitosis.[12]

Mitotic loss of H2AQ104me is accompanied by reduced chromatin accessibility, which coincides with nucleolar disassembly and transcriptional shutdown. In telophase, FBL is deacetylated by SIRT7, which is required for methylation of H2AQ104 and transcription activation. Thus, acetylation-mediated regulation of FBL activity is another mechanism that mediates fluctuations of Pol I transcription by regulating H2AQ104me levels to establish a transcription-permissive or -refractive chromatin architecture.

Concluding Remarks

Multiple mechanisms regulate cell cycle-dependent fluctuations of rDNA transcription to ensure the controlled biogenesis of ribosomes. In addition to changes in the activity and localization of components of the Pol I transcription machinery, the nucleolar chromatin undergoes dramatic alterations in distinct phases of the cell cycle (Figure 1). Significantly, the acetylation state of FBL regulates methylation of histone H2A at Q104, a specific epigenetic mark that demarcates active rDNA copies and is dedicated to nucleolar transcription. Methylation of H2A at glutamine 104 contributes to opening of rDNA chromatin and/or recruitment of factors that are required for Pol I transcription. Nucleoli in SIRT7 or FBL-depleted cells are small and fragmented, indicating that nucleolar assembly is compromised upon hyperacetylation of FBL.[2] It is still not clear how H2AQ104me promotes Pol I transcription. The part of histone H2A comprising Q104 has been shown to interact with facilitator of chromatin transcription (FACT). FACT is a conserved histone chaperone that comprises structure-specific recognition protein 1 (SSRP1) and suppressor of Ty 16 (SPT16) and destabilizes H2A/H2B dimers.[10] FACT-dependent destabilization of H2A/H2B dimers promotes transcription elongation through chromatin and facilitates re-deposition of nucleosomes in the wake of elongating Pol I.[13] One possible mechanism by which H2AQ104me is removed could be that glutamine methylation is regulated by exchange of histones. In this case, the newly incorporated histone H2A requires FBL-mediated methylation after each round of mitosis. This is in accord with studies showing that the chromatin state of rDNA is re-established during each cell cycle to facilitate transcription and replication.[14] Thus, during mitosis, H2AQ104me-insensitive histone chaperones, such as NAP1,[10] may be responsible for the exchange of Q104-methylated histone H2A, which is reinforced by inhibition of de novo H2AQ104 methylation due to hyperacetylation of FBL.

Glutamine 104 resides in the "docking domain" at the nexus between H2A/H2B and H3/H4 dimers, where methylation may cause conformational changes reminiscent of that in histone H2A.Z, which contains a glycine at this position. In fact, ChIP and FAIRE experiments have shown that the depletion of FBL or SIRT7 leads to decreased Pol I occupancy, low levels of acetylated histone H4 and rDNA chromatin compaction, supporting that H2AQ104 methylation establishes a transcription-permissive chromatin landscape.[2] The selectivity of H2AQ104me for nucleolar transcription might be necessary to generate a chromatin state that propels the high rate of rRNA synthesis.

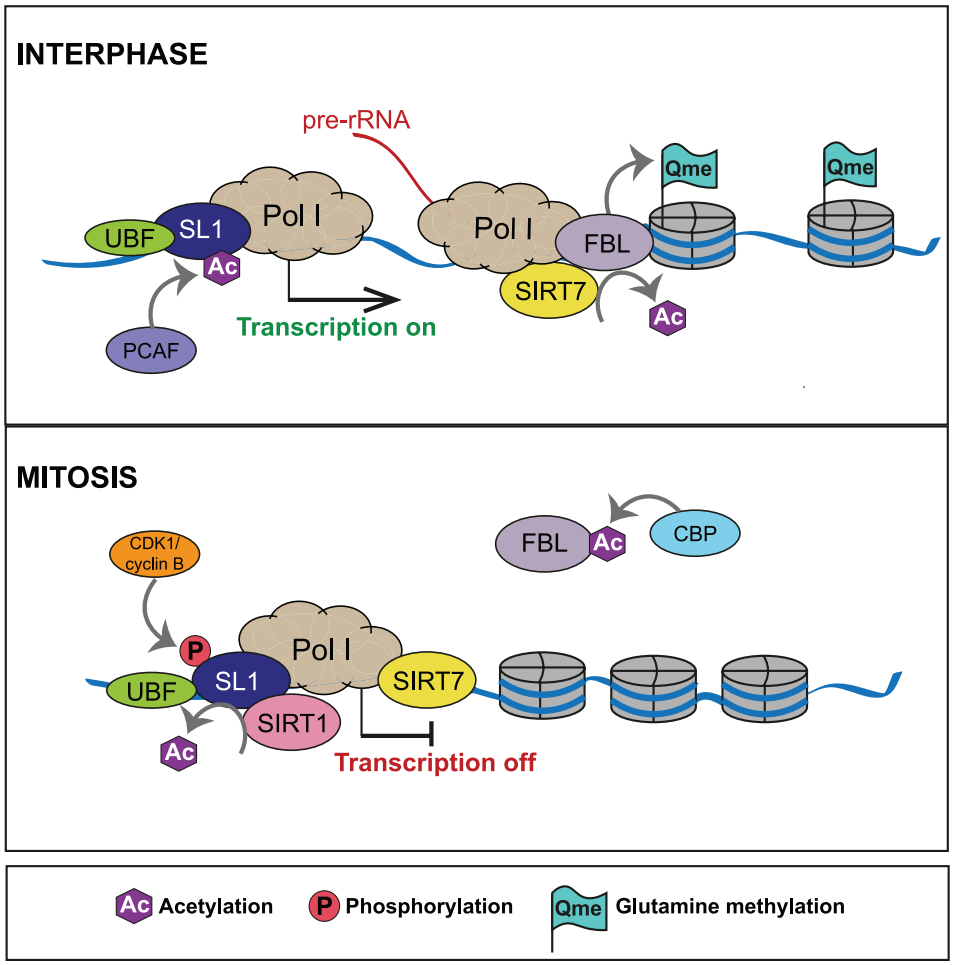

Figure 1. Posttranslational modifications of nucleolar chromatin and components of the Pol I transcription machinery during interphase and mitosis. During interphase, SL1 is acetylated and FBL is hypoacetylated. FBL mediates methylation of histone H2A at glutamine 104 (H2AQ104), which augments chromatin accessibility and Pol I transcription. At the onset of mitosis, SL1 is inactivated by CDK1/cyclin B-mediated phosphorylation and SIRT1-dependent deacetylation, while mitotic FBL is hyperacetylated. Acetylation impairs the interaction of FBL with histone H2A, which leads to loss of H2AQ104me, chromatin compaction, and shutdown of Pol I transcription.

Author contributions

A.I.-B. and I.G. wrote the manuscript.

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