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Structure and function of ribosomal RNA gene chromatin

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Abstract

Transcription of the major ribosomal RNAs by Pol I (RNA polymerase I) is a key determinant of ribosome biogenesis, driving cell growth and proliferation in eukaryotes. Hundreds of copies of rRNA genes are present in each cell, and there is evidence that the cellular control of Pol I transcription involves adjustments to the number of rRNA genes actively engaged in transcription, as well as to the rate of transcription from each active gene. Chromatin structure is inextricably linked to rRNA gene activity, and the present review highlights recent advances in this area.

Keywords

chromatin; chromatin remodelling; nucleosome; ribosomal DNA (rDNA); rRNA gene; RNA polymerase I; transcription

Introduction

A characteristic feature of the genes encoding the major rRNAs is their repetitive nature. Human cells have approx. 400 copies of a ribosomal DNA repeat that encode the rRNAs, distributed over the short arms of the five acrocentric chromosomes 13, 14, 15, 21 and 22. In yeast, 150–200 rDNA (ribosomal DNA) copies occur in tandem arrays on the right arm of chromosome XII. Intriguingly, only a proportion of these repeats are transcribed in actively growing cells of yeast or humans (~50% of cells in interphase). The number of repeats engaged in active transcription can influence the overall level of rRNA synthesis. So, what determines whether a particular rDNA gene is transcriptionally active or silent? Active (transcriptionally competent or transcriptionally active) and inactive (silent) rDNA genes have distinctive chromatin states, therefore chromatin context might influence the transcriptional activity of the rDNA genes. Conversely, there is evidence that the transcriptional activity of the rDNA genes can influence the chromatin state of the rDNA. In the present review, we describe the known features of rDNA chromatin, the ability of the Pol (RNA polymerase) I transcription machinery to negotiate the rDNA chromatin template and the regulatory factors involved in, and alterations accompanying, the silencing or activation of rRNA genes.

Features of rDNA chromatin

Two distinct chromatin states: active and inactive

Regularly spaced nucleosomes are detectable at the rDNA repeats, by MNase (micrococcal nuclease) digestion analysis of bulk chromatin [1]. However, the chromatin structures of individual rDNA repeats are not distinguishable by this method. Heterogeneity in the

transcriptional state of rDNA repeats was revealed by electron microscopy analyses [2], with some repeats transcribed and others transcriptionally silent. The observed high density of loading of RNA polymerases on the actively transcribed rRNA genes might preclude a 'normal' nucleosomal arrangement.

Two distinct coexisting chromatin states correlating with transcriptional activity were defined by psoralen photo-cross-linking analysis of the rDNA repeats. rDNA chromatin that is relatively refractory to psoralen cross-linking, representing the inactive rDNA repeats, was organized in regular nucleosomal arrays similar to those observed for bulk chromatin [3]. Other rDNA repeats (~50% of the total) were found in a psoralen-accessible ('open') chromatin state lacking regular nucleosomal arrays [3] and were associated with nascent rRNA, indicating that they were actively transcribed [4]. Active and inactive repeats are interspersed in yeast, arguing against control of rDNA gene activation via organization into specific 'on' and 'off' chromosomal domains and in favour of the independent regulation of each rDNA repeat [5,6], notwithstanding the global forces which operate to achieve nucleolar dominance in hybrids [7,8].

Recently, ChEC (chromatin endogenous cleavage) with MNase-fusion proteins, which allows for the precise localization of chromatin-associated factors on genomic DNA [9], was combined with psoralen photo-cross-linking analyses to demonstrate that, in contrast with the inactive rDNA repeats, the active repeats have few histones, hence few nucleosomes, associated with the transcribed regions [10]. That at least some nucleosomes are associated with the actively transcribed rDNA is also suggested by the following. The rapid replication-independent exchange of histone H3/H4 tetramers at the transcribed regions of the rRNA gene loci in the slime mould *Physarum polycephalum* suggests a dynamic nucleosomal arrangement at the active rDNA [11]. A dynamic chromatin structure of unphased nucleosomes has also been inferred from studies of the rDNA chromatin in a particular yeast mutant strain in which all rDNA repeats were active following a reduction in rDNA copy number [5], and histones H3 and H2B were present at the active rDNA repeats, albeit at a reduced level [12]. Factors Spt4p and Spt5p, which positively affect Pol II transcription elongation through chromatin, influence Pol I transcription elongation in yeast [13], and chromatin-remodelling activities such as Chd1p, Isw1p and Isw2p have been found associated with the active rDNA repeats [12]. In mammalian rDNA, nucleosomes are present at the rDNA promoter regions of both active and inactive repeats, and, significantly, the active and silent rDNA promoters are distinguishable by differential nucleosome positioning. At the active sites, the promoter-bound nucleosome covers nucleotides from -157 to -2, whereas, at silent loci, the nucleosome is 25 nt further downstream, at a position potentially incompatible with productive pre-initiation complex formation [14,15]. Inactive mammalian rDNA repeats are maintained in a silent state via association of NoRC (nucleolar-remodelling complex) with the rDNA promoter, as described below.

Certain modifications of the DNA and associated histones also distinguish the active and silent rDNA genes. The promoters of actively transcribed mouse rRNA genes are hypomethylated [16,17], and the associated histones are highly acetylated, with the opposite being true of inactive gene promoters [16]. Furthermore, the silent copies of the rDNA promoters have methylated histone H3K9 (histone H3 Lys⁹) and are associated with HP1 (heterochromatin protein 1). Active rDNA repeats have additional distinguishing features. Yeast Hmo1, an HMG (high mobility group)-box-containing protein, shown previously by chromatin immunoprecipitation to localize to the entire rDNA repeat [18,19], is specifically associated with the active rDNA chromatin. Hmo1 is not essential for establishment of the active chromatin state, however, and a role for Hmo1 has yet to be defined [10]. The mammalian Pol I transcription factor UBF (upstream binding factor), which has multiple HMG boxes, activates promoter-specific Pol I transcription [20] through the stimulation of

promoter escape by Pol I [21] and, perhaps, by increasing the local concentration of Pol I and transcription initiation factor SL1 (selectivity factor 1) at the rDNA [22,23]. At the promoter, UBF dimerizes and, in binding DNA, has the ability to induce formation of an 'enhancesome', in which ~ 140 bp of DNA is organized in a 360° turn as a result of six in-phase bends generated by three of the six HMG boxes in each UBF monomer [24]. The methylation of a single CpG dinucleotide at -133 in the mouse promoter is sufficient to prevent UBF from binding the nucleosomal rDNA promoter and to abolish rDNA chromatin transcription [25]. The requirement for CpG methylation at specific positions appears to be echoed in the human system, where transcriptional silencing might be a consequence of the binding to these sites of MBD2 (methyl-CpG-binding protein 2), which could recruit HDAC (histone deacetylase) co-repressor complexes [26].

UBF and rDNA chromatin

UBF binding is not restricted to the promoter region and is found throughout the rDNA repeat [23,27], but, unlike yeast Hmo1, UBF is present at transcriptionally active and inactive loci [28]. Large arrays of heterologous UBF-binding sequences integrated at ectopic sites in human chromosomes efficiently recruit UBF and form novel secondary constrictions, termed pseudo-NORs (nucleolar organizer regions) due to their morphological similarity to the decondensed rDNA repeat regions (NORs) on the acrocentric chromosomes [23]. Furthermore, large-scale chromatin decondensation was induced when UBF was targeted, via a *lac*-repressor fusion protein, to a heterochromatic amplified chromosome region, which contains *lac*-operator repeats [22]. These results suggest that UBF can dramatically influence chromatin structure [29], perhaps promoting steps to convert rDNA chromatin into a transcriptionally competent form. Interestingly, however, UBF does not appear to be required to maintain transcriptional competence, since adenoviral infection of cells, which leads to a sequestration of the majority of UBF away from the nucleolus, does not detectably affect rRNA synthesis by Pol I [30]. Perhaps the chromatin alterations induced by UBF are associated with the events following DNA replication, involving the re-establishment of a nucleosomal structure on the newly replicated rDNA coding regions [31]. UBF molecules bound to the transcribed regions of the rDNA impede elongation of transcription by Pol I [21,32], and this is modulated by the growth-factor regulated ERK (extracellular-signal-regulated kinase) phosphorylation of UBF [32,33]. Challenges for the future will be to understand how UBF remodels chromatin, the consequences for rDNA transcription and whether the role of UBF is related to the high affinity of UBF for nucleosomal DNA and/or to its ability to displace histone H1 in reconstituted chromatin [34].

Transcription through chromatin

The rate of rRNA synthesis, and the linked processing events [35], could depend upon the efficiency by which Pol I transcribes through nucleosome-containing regions of the active rDNA genes. Biochemical analyses have established that Pol III and SP6 RNA polymerase have the intrinsic ability to transcribe through a nucleosome, and this involves the translocation of the nucleosome to a position upstream of its original site during polymerase passage, without the disruption of the majority of octamer-DNA contacts [36,37]. Nucleosomes impede Pol II transcription, and chromatin transcription is facilitated by histone chaperones, such as the FACT (facilitates chromatin transcription) complex, which induce transient release of an H2A-H2B dimer and so destabilize the nucleosome to facilitate Pol II passage [38-40], consistent with the observed rapid exchange of these dimers in cells [41]. Chromatin transcription by mammalian Pol I also appears to require histone chaperone activities provided, for example, by nucleolin [42,43], nucleophosmin (B23) [44,45] and/or FACT (J.L. Birch and J.C.B.M. Zomerdijs, unpublished work).

Histone chaperones are important in the reassembly of nucleosomes in the wake of passing polymerases, and so could prevent spurious transcription initiation from otherwise exposed cryptic start sites in the transcribed region [46,47].

Alterations in rDNA chromatin: silencing and activation

Epigenetic inheritance of chromatin modifications in mammalian cells maintains the active and silent chromatin states of the rDNA repeats in the daughter cells. In mammalian cells, the inheritance of specific cytosine methylations is important [48]. Chromatin modification in yeast, however, does not involve methylation of the rDNA, since yeast have no DNA methylase activities. In yeast, the active or silent state of the rDNA repeats are also re-established following DNA replication [31,49], but it is unclear how yeast cells select an rDNA repeat for activation or repression; it appears to be stochastic, although the ratio of active to inactive genes does not change drastically. Chromatin structures in yeast and mammalian systems are dynamically altered by the (combined) actions of ATP-dependent chromatinremodelling activities and activities that covalently modify histones [50]. The open chromatin structure of yeast rDNA requires transcribing Pol I complexes [6], suggesting that Pol I transcription might remodel chromatin.

Growth-factor-induced and nutrient-regulated signalling pathways have been shown to regulate several chromatinremodelling complexes in mammalian and yeast cells [51,52] and, consequently, could affect rRNA gene promoter accessibility [53]. For example, stationary-phase yeast cells repress rRNA transcription in part through reducing the number of active rDNA repeats. Furthermore, the targeting of the Rpd3–Sin3 HDAC co-repressor complex to the rDNA can lead to deacetylation of histone H4 and inactivation of individual rRNA genes, and this is controlled by the TOR (target of rapamycin) signalling pathway [53,54].

TTF-I (transcription termination factor I), which can bind to the mammalian rDNA promoter proximal terminator element (T_0) in addition to the terminators downstream of the rRNA genes, is key in the recruitment of chromatinremodelling complexes that either co-activate [55] or co-repress transcription from the mammalian rDNA chromatin [16,56].

Silencing of rDNA chromatin

In silencing the rDNA promoter, TTF-I recruits NoRC, a complex of TIP5 (TTF-I-interaction protein 5) and SNF2h, which induces sliding in an ATP- and histone H4 tail-dependent manner of the promoter-bound nucleosome, and this contributes to the repression of Pol I transcription [15,57,58]. The binding of the bromodomain of TIP5 to acetylated Lys¹⁶ of histone H4 is a prerequisite to NoRC function [59]. NoRC interacting with TTF-I near the promoter recruits SNF2h, involved in nucleosome sliding, HDAC1 and DNMTs (DNA methyltransferases) 1 and 3 through the bromodomain and PHD (plant homeodomain) finger of TIP5 [59]. Although the consequent deacetylation of H4 is not sufficient for silencing, this deacetylation is a prerequisite to the CpG methylation of the rDNA promoter, which contributes to the establishment and maintenance of a silent rDNA chromatin state [16,60–63]. Additionally, the establishment and maintenance of a specific heterochromatic configuration at the rDNA promoter, as reflected in histone H3K9 and H4K20 methylation and HP1 recruitment, requires rDNA intergenic spacer transcripts to interact with TIP5 of NoRC [64]. In nucleolar dominance (the NORs of one parental species are dominant over the other in interspecies hybrids), the mechanisms of rDNA silencing also involve the co-ordinated and interdependent action of chromatin remodellers, histone and DNA modifiers [65–68].

The phenomenon of transcriptional silencing of Pol II-transcribed genes by rDNA chromatin (commonly referred to as rDNA silencing) is not to be confused with silent rDNA chromatin

(i.e. not active for Pol I transcription). SIR (silent information regulator) 2, which is associated with yeast rDNA chromatin, is an NAD-dependent histone deacetylase [69] that represses recombination between the tandemly repeated ribosomal RNA genes [70] and is involved in Pol II transcriptional silencing by influencing rDNA chromatin accessibility [71–75]. A mammalian Sir2 homologue, SIRT7 (sirtuin 7), is associated with active rRNA genes, and with Pol I and histones, and functions as a positive regulator of Pol I transcription [76].

Activation of rDNA chromatin

Little is known of the selective TTF-I mediated recruitment of remodelling activities that activate rDNA genes, other than that it is independent of histone H4 tails [57]. However, chromatin-remodelling complexes specific for active rDNA repeats have been identified and include WICH, a chromatinremodelling complex containing WSTF (William's syndrome transcription factor) and SNF2h [77], and CSB (Cockayne syndrome group B) protein, a member of the SWI/SNF family of ATP-dependent chromatin-remodelling activities. CSB is required for rDNA transcription and is part of a protein complex that contains Pol I, TFIIF (transcription factor IIF), and basal Pol I transcription initiation factors [78]. CSB is found at active rDNA associated with histone methyltransferase G9a, which methylates histone H3 on Lys⁹ in the pre-rRNA coding region and this appears to be important for transcription elongation through chromatin [79] (note that this modification at the promoter is associated with inactive rDNA).

Concluding remarks

Collectively, the data suggest that rDNA has a complex and dynamic chromatin structure and that actively transcribed rDNA chromatin is distinct both from that of silent rDNA genes and from that of other (Pol II-) transcribed loci. Future genetic analysis in combination with biochemical analysis of isolated active rDNA chromatin will provide additional clues to the constituents and structure of active rDNA repeats, define proteins required in the establishment and/or maintenance of active rDNA chromatin, and identify factors that facilitate chromatin transcription elongation by Pol I.

Ribosomal RNA synthesis by Pol I is crucially dependent upon nutrient availability and drives ribosome biogenesis, a process important for cell growth and proliferation [80–82]. Deregulation of Pol I transcription perturbs normal cell growth, leading to cell death or uncontrolled cell growth and proliferation, potentially resulting in developmental defects or cancer in mammals [83–86]. It is therefore important to further our understanding of the regulation of rDNA chromatin organization and its influence on rRNA gene transcription by Pol I.

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Abbreviations used

CSB	Cockayne syndrome group B
FACT	facilitates chromatin transcription
H3K9 (etc.)	histone H3 Lys ⁹ (etc.)

HDAC	histone deacetylase
HMG	high mobility group
HP1	hetreochromatin protein 1
MNase	micrococcal nuclease
NOR	nucleolar organizer region
NoRC	nucleolar-remodelling complex
Pol	RNA polymerase
rDNA	ribosomal DNA
TTF-I	transcription termination factor I
TIP5	TTF-I-interaction protein 5
UBF	upstream binding factor

References

1. Lohr D. Chromatin structure differs between coding and upstream flanking sequences of the yeast 35S ribosomal genes. *Biochemistry*. 1983; 22:927–934. [PubMed: 6301531]
2. Miller OL Jr, Beatty BR. Visualization of nucleolar genes. *Science*. 1969; 164:955–957. [PubMed: 5813982]
3. Conconi A, Widmer RM, Koller T, Sogo JM. Two different chromatin structures coexist in ribosomal RNA genes throughout the cell cycle. *Cell*. 1989; 57:753–761. [PubMed: 2720786]
4. Dammann R, Lucchini R, Koller T, Sogo JM. Chromatin structures and transcription of rDNA in yeast *Saccharomyces cerevisiae*. *Nucleic Acids Res*. 1993; 21:2331–2338. [PubMed: 8506130]
5. French SL, Osheim YN, Cioci F, Nomura M, Beyer AL. In exponentially growing *Saccharomyces cerevisiae* cells, rRNA synthesis is determined by the summed RNA polymerase I loading rate rather than by the number of active genes. *Mol. Cell. Biol*. 2003; 23:1558–1568. [PubMed: 12588976]
6. Dammann R, Lucchini R, Koller T, Sogo JM. Transcription in the yeast rRNA gene locus: distribution of the active gene copies and chromatin structure of their flanking regulatory sequences. *Mol. Cell. Biol*. 1995; 15:5294–5303. [PubMed: 7565678]
7. Lewis MS, Pikaard DJ, Nasrallah M, Doelling JH, Pikaard CS. Locus-specific ribosomal RNA gene silencing in nucleolar dominance. *PLoS ONE*. 2007; 2:e815. [PubMed: 17726545]
8. Preuss S, Pikaard CS. rRNA gene silencing and nucleolar dominance: insights into a chromosome-scale epigenetic on/off switch. *Biochim. Biophys. Acta*. 2007; 1769:383–392. [PubMed: 17439825]
9. Schmid M, Durussel T, Laemmli UK. ChIC and ChEC; genomic mapping of chromatin proteins. *Mol. Cell*. 2004; 16:147–157. [PubMed: 15469830]
10. Merz K, Hondele M, Goetze H, Gmelch K, Stoeckl U, Griesenbeck J. Actively transcribed rRNA genes in *S. cerevisiae* are organized in a specialized chromatin associated with the high-mobility group protein Hmo1 and are largely devoid of histone molecules. *Genes Dev*. 2008; 22:1190–1204. [PubMed: 18451108]
11. Thiriet C, Hayes JJ. Replication-independent core histone dynamics at transcriptionally active loci *in vivo*. *Genes Dev*. 2005; 19:677–682. [PubMed: 15769942]
12. Jones HS, Kawauchi J, Braglia P, Alen CM, Kent NA, Proudfoot NJ. RNA polymerase I in yeast transcribes dynamic nucleosomal rDNA. *Nat. Struct. Mol. Biol*. 2007; 14:123–130. [PubMed: 17259992]
13. Schneider DA, French SL, Osheim YN, Bailey AO, Vu L, Dodd J, Yates JR, Beyer AL, Nomura M. RNA polymerase II elongation factors Spt4p and Spt5p play roles in transcription elongation by RNA polymerase I and rRNA processing. *Proc. Natl. Acad. Sci. U.S.A.* 2006; 103:12707–12712. [PubMed: 16908835]

14. Langst G, Becker PB, Grummt I. TTF-I determines the chromatin architecture of the active rDNA promoter. *EMBO J.* 1998; 17:3135–3145. [PubMed: 9606195]
15. Li J, Langst G, Grummt I. NoRC-dependent nucleosome positioning silences rRNA genes. *EMBO J.* 2006; 25:5735–5741. [PubMed: 17139253]
16. Santoro R, Li J, Grummt I. The nucleolar remodeling complex NoRC mediates heterochromatin formation and silencing of ribosomal gene transcription. *Nat. Genet.* 2002; 32:393–396. [PubMed: 12368916]
17. Nemeth A, Guibert S, Tiwari VK, Ohlsson R, Langst G. Epigenetic regulation of TTF-I-mediated promoter–terminator interactions of rRNA genes. *EMBO J.* 2008; 27:1255–1265. [PubMed: 18354495]
18. Hall DB, Wade JT, Struhl K. An HMG protein, Hmo1, associates with promoters of many ribosomal protein genes and throughout the rRNA gene locus in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 2006; 26:3672–3679. [PubMed: 16612005]
19. Kasahara K, Ohtsuki K, Ki S, Aoyama K, Takahashi H, Kobayashi T, Shirahige K, Kokubo T. Assembly of regulatory factors on rRNA and ribosomal protein genes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 2007; 27:6686–6705. [PubMed: 17646381]
20. Jantzen HM, Admon A, Bell SP, Tjian R. Nucleolar transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. *Nature.* 1990; 344:830–836. [PubMed: 2330041]
21. Panov KI, Friedrich JK, Russell J, Zomerdijs JC. UBF activates RNA polymerase I transcription by stimulating promoter escape. *EMBO J.* 2006; 25:3310–3322. [PubMed: 16858408]
22. Chen D, Belmont AS, Huang S. Upstream binding factor association induces large-scale chromatin decondensation. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101:15106–15111. [PubMed: 15477594]
23. Mais C, Wright JE, Prieto JL, Raggett SL, McStay B. UBF-binding site arrays form pseudo-NORs and sequester the RNA polymerase I transcription machinery. *Genes Dev.* 2005; 19:50–64. [PubMed: 15598984]
24. Bazett Jones DP, Leblanc B, Herfort M, Moss T. Short-range DNA looping by the *Xenopus* HMG-box transcription factor, xUBF. *Science.* 1994; 264:1134–1137. [PubMed: 8178172]
25. Santoro R, Grummt I. Molecular mechanisms mediating methylation-dependent silencing of ribosomal gene transcription. *Mol. Cell.* 2001; 8:719–725. [PubMed: 11583633]
26. Ghoshal K, Majumder S, Datta J, Motiwala T, Bai S, Sharma SM, Frankel W, Jacob ST. Role of human ribosomal RNA (rRNA) promoter methylation and of methyl-CpG-binding protein MBD2 in the suppression of rRNA gene expression. *J. Biol. Chem.* 2004; 279:6783–6793. [PubMed: 14610093]
27. O'Sullivan AC, Sullivan GJ, McStay B. UBF binding *in vivo* is not restricted to regulatory sequences within the vertebrate ribosomal DNA repeat. *Mol. Cell. Biol.* 2002; 22:657–668. [PubMed: 11756560]
28. Sullivan GJ, Bridger JM, Cuthbert AP, Newbold RF, Bickmore WA, McStay B. Human acrocentric chromosomes with transcriptionally silent nucleolar organizer regions associate with nucleoli. *EMBO J.* 2001; 20:2867–2874. [PubMed: 11387219]
29. Wright JE, Mais C, Prieto J-L, McStay B. A role for upstream binding factor in organizing ribosomal gene chromatin. *Biochem. Soc. Symp.* 2006; 73:77–84. [PubMed: 16626289]
30. Lawrence FJ, McStay B, Matthews DA. Nucleolar protein upstream binding factor is sequestered into adenovirus DNA replication centres during infection without affecting RNA polymerase I location or ablating rRNA synthesis. *J. Cell Sci.* 2006; 119:2621–2631. [PubMed: 16763197]
31. Lucchini R, Sogo JM. Replication of transcriptionally active chromatin. *Nature.* 1995; 374:276–280. [PubMed: 7885449]
32. Stefanovsky V, Langlois F, Gagnon-Kugler T, Rothblum LI, Moss T. Growth factor signaling regulates elongation of RNA polymerase I transcription in mammals via UBF phosphorylation and r-chromatin remodeling. *Mol. Cell.* 2006; 21:629–639. [PubMed: 16507361]
33. Stefanovsky VY, Langlois F, Bazett-Jones D, Pelletier G, Moss T. ERK modulates DNA bending and enhancesome structure by phosphorylating HMG1-boxes 1 and 2 of the RNA polymerase I transcription factor UBF. *Biochemistry.* 2006; 45:3626–3634. [PubMed: 16533045]

34. Kermekchiev M, Workman JL, Pikaard CS. Nucleosome binding by the polymerase I transactivator upstream binding factor displaces linker histone H1. *Mol. Cell. Biol.* 1997; 17:5833–5842. [PubMed: 9315641]
35. Schneider DA, Michel A, Sikes ML, Vu L, Dodd JA, Salgia S, Osheim YN, Beyer AL, Nomura M. Transcription elongation by RNA polymerase I is linked to efficient rRNA processing and ribosome assembly. *Mol. Cell.* 2007; 26:217–229. [PubMed: 17466624]
36. Studitsky VM, Kassavetis GA, Geiduschek EP, Felsenfeld G. Mechanism of transcription through the nucleosome by eukaryotic RNA polymerase. *Science.* 1997; 278:1960–1963. [PubMed: 9395401]
37. Bednar J, Studitsky VM, Grigoryev SA, Felsenfeld G, Woodcock CL. The nature of the nucleosomal barrier to transcription: direct observation of paused intermediates by electron cryomicroscopy. *Mol. Cell.* 1999; 4:377–386. [PubMed: 10518218]
38. Kireeva ML, Walter W, Tchernajenko V, Bondarenko V, Kashlev M, Studitsky VM. Nucleosome remodeling induced by RNA polymerase II: loss of the H2A/H2B dimer during transcription. *Mol. Cell.* 2002; 9:541–552. [PubMed: 11931762]
39. Orphanides G, Wu WH, Lane WS, Hampsey M, Reinberg D. The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. *Nature.* 1999; 400:284–288. [PubMed: 10421373]
40. Belotserkovskaya R, Oh S, Bondarenko VA, Orphanides G, Studitsky VM, Reinberg D. FACT facilitates transcription-dependent nucleosome alteration. *Science.* 2003; 301:1090–1093. [PubMed: 12934006]
41. Kimura H, Cook PR. Kinetics of core histones in living human cells: little exchange of H3 and H4 and some rapid exchange of H2B. *J. Cell Biol.* 2001; 153:1341–1353. [PubMed: 11425866]
42. Angelov D, Bondarenko VA, Almagro S, Menoni H, Mongelard F, Hans F, Mietton F, Studitsky VM, Hamiche A, Dimitrov S, Bouvet P. Nucleolin is a histone chaperone with FACT-like activity and assists remodeling of nucleosomes. *EMBO J.* 2006; 25:1669–1679. [PubMed: 16601700]
43. Rickards B, Flint SJ, Cole MD, Leroy G. Nucleolin is required for RNA polymerase I transcription *in vivo*. *Mol. Cell. Biol.* 2007; 27:937–948. [PubMed: 17130237]
44. Okuwaki M, Matsumoto K, Tsujimoto M, Nagata K. Function of nucleophosmin/B23, a nucleolar acidic protein, as a histone chaperone. *FEBS Lett.* 2001; 506:272–276. [PubMed: 11602260]
45. Murano K, Okuwaki M, Hisaoka M, Nagata K. Transcription regulation of the rRNA gene by a multifunctional nucleolar protein, B23/nucleophosmin, through its histone chaperone activity. *Mol. Cell. Biol.* 2008; 28:3114–3126. [PubMed: 18332108]
46. Mason PB, Struhl K. The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation *in vivo*. *Mol. Cell. Biol.* 2003; 23:8323–8333. [PubMed: 14585989]
47. Kaplan CD, Laprade L, Winston F. Transcription elongation factors repress transcription initiation from cryptic sites. *Science.* 2003; 301:1096–1099. [PubMed: 12934008]
48. Santoro R. The silence of the ribosomal RNA genes. *Cell. Mol. Life Sci.* 2005; 62:2067–2079. [PubMed: 16041568]
49. Lucchini R, Sogo JM. Chromatin structure and transcriptional activity around the replication forks arrested at the 3' end of the yeast rRNA genes. *Mol. Cell. Biol.* 1994; 14:318–326. [PubMed: 8264598]
50. Grummt I, Pikaard CS. Epigenetic silencing of RNA polymerase I transcription. *Nat. Rev. Mol. Cell Biol.* 2003; 4:641–649. [PubMed: 12923526]
51. Cheung P, Tanner KG, Cheung WL, Sassone-Corsi P, Denu JM, Allis CD. Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. *Mol. Cell.* 2000; 5:905–915. [PubMed: 10911985]
52. Damelin M, Simon I, Moy TI, Wilson B, Komili S, Tempst P, Roth FP, Young RA, Cairns BR, Silver PA. The genome-wide localization of Rsc9, a component of the RSC chromatin-remodeling complex, changes in response to stress. *Mol. Cell.* 2002; 9:563–573. [PubMed: 11931764]
53. Tsang CK, Bertram PG, Ai W, Drenan R, Zheng XF. Chromatin-mediated regulation of nucleolar structure and RNA Pol I localization by TOR. *EMBO J.* 2003; 22:6045–6056. [PubMed: 14609951]

54. Sandmeier JJ, French S, Osheim Y, Cheung WL, Gallo CM, Beyer AL, Smith JS. RPD3 is required for the inactivation of yeast ribosomal DNA genes in stationary phase. *EMBO J.* 2002; 21:4959–4968. [PubMed: 12234935]
55. Langst G, Blank TA, Becker PB, Grummt I. RNA polymerase I transcription on nucleosomal templates: the transcription termination factor TTF-I induces chromatin remodeling and relieves transcriptional repression. *EMBO J.* 1997; 16:760–768. [PubMed: 9049305]
56. Nemeth A, Strohnner R, Grummt I, Langst G. The chromatin remodeling complex NoRC and TTF-I cooperate in the regulation of the mammalian rRNA genes *in vivo*. *Nucleic Acids Res.* 2004; 32:4091–4099. [PubMed: 15292447]
57. Strohnner R, Nemeth A, Jansa P, Hofmann-Rohrer U, Santoro R, Langst G, Grummt I. NoRC: a novel member of mammalian ISWI-containing chromatin remodeling machines. *EMBO J.* 2001; 20:4892–4900. [PubMed: 11532953]
58. Strohnner R, Nemeth A, Nightingale KP, Grummt I, Becker PB, Langst G. Recruitment of the nucleolar remodeling complex NoRC establishes ribosomal DNA silencing in chromatin. *Mol. Cell. Biol.* 2004; 24:1791–1798. [PubMed: 14749393]
59. Zhou Y, Grummt I. The PHD finger/bromodomain of NoRC interacts with acetylated histone H4K16 and is sufficient for rDNA silencing. *Curr. Biol.* 2005; 15:1434–1438. [PubMed: 16085498]
60. Zhou Y, Santoro R, Grummt I. The chromatin remodeling complex NoRC targets HDAC1 to the ribosomal gene promoter and represses RNA polymerase I transcription. *EMBO J.* 2002; 21:4632–4640. [PubMed: 12198165]
61. Santoro R, Grummt I. Epigenetic mechanism of rRNA gene silencing: temporal order of NoRC-mediated histone modification, chromatin remodeling, and DNA methylation. *Mol. Cell. Biol.* 2005; 25:2539–2546. [PubMed: 15767661]
62. Espada J, Ballestar E, Santoro R, Fraga MF, Villar-Garea A, Nemeth A, Lopez-Serra L, Ropero S, Aranda A, Orozco H, et al. Epigenetic disruption of ribosomal RNA genes and nucleolar architecture in DNA methyltransferase 1 (Dnmt1) deficient cells. *Nucleic Acids Res.* 2007; 35:2191–2198. [PubMed: 17355984]
63. Majumder S, Ghoshal K, Datta J, Smith DS, Bai S, Jacob ST. Role of DNA methyltransferases in regulation of human ribosomal RNA gene transcription. *J. Biol. Chem.* 2006; 281:22062–22072. [PubMed: 16735507]
64. Mayer C, Schmitz KM, Li J, Grummt I, Santoro R. Intergenic transcripts regulate the epigenetic state of rRNA genes. *Mol. Cell.* 2006; 22:351–361. [PubMed: 16678107]
65. Earley K, Lawrence RJ, Pontes O, Reuther R, Enciso AJ, Silva M, Neves N, Gross M, Viegas W, Pikaard CS. Erasure of histone acetylation by *Arabidopsis* HDA6 mediates large-scale gene silencing in nucleolar dominance. *Genes Dev.* 2006; 20:1283–1293. [PubMed: 16648464]
66. Probst AV, Fagard M, Proux F, Mourrain P, Boutet S, Earley K, Lawrence RJ, Pikaard CS, Murfett J, Furner I, et al. *Arabidopsis* histone deacetylase HDA6 is required for maintenance of transcriptional gene silencing and determines nuclear organization of rDNA repeats. *Plant Cell.* 2004; 16:1021–1034. [PubMed: 15037732]
67. Lawrence RJ, Earley K, Pontes O, Silva M, Chen ZJ, Neves N, Viegas W, Pikaard CS. A concerted DNA methylation/histone methylation switch regulates rRNA gene dosage control and nucleolar dominance. *Mol. Cell.* 2004; 13:599–609. [PubMed: 14992728]
68. McStay B. Nucleolar dominance: a model for rRNA gene silencing. *Genes Dev.* 2006; 20:1207–1214. [PubMed: 16702398]
69. Imai S, Armstrong CM, Kaerberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature.* 2000; 403:795–800. [PubMed: 10693811]
70. Gottlieb S, Esposito RE. A new role for a yeast transcriptional silencer gene, SIR2, in regulation of recombination in ribosomal DNA. *Cell.* 1989; 56:771–776. [PubMed: 2647300]
71. Fritze CE, Verschuere K, Strich R, Easton Esposito R. Direct evidence for SIR2 modulation of chromatin structure in yeast rDNA. *EMBO J.* 1997; 16:6495–6509. [PubMed: 9351831]
72. Smith JS, Boeke JD. An unusual form of transcriptional silencing in yeast ribosomal DNA. *Genes Dev.* 1997; 11:241–254. [PubMed: 9009206]

73. Cioci F, Vogelauer M, Camilloni G. Acetylation and accessibility of rDNA chromatin in *Saccharomyces cerevisiae* in $\Delta top1$ and $\Delta sir2$ mutants. *J. Mol. Biol.* 2002; 322:41–52. [PubMed: 12215413]
74. Huang J, Moazed D. Association of the RENT complex with nontranscribed and coding regions of rDNA and a regional requirement for the replication fork block protein Fob1 in rDNA silencing. *Genes Dev.* 2003; 17:2162–2176. [PubMed: 12923057]
75. Buck SW, Sandmeier JJ, Smith JS. RNA polymerase I propagates unidirectional spreading of rDNA silent chromatin. *Cell.* 2002; 111:1003–1014. [PubMed: 12507427]
76. Ford E, Voit R, Liszt G, Magin C, Grummt I, Guarente L. Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. *Genes Dev.* 2006; 20:1075–1080. [PubMed: 16618798]
77. Percipalle P, Fomproix N, Cavellan E, Voit R, Reimer G, Kruger T, Thyberg J, Scheer U, Grummt I, Farrants AK. The chromatin remodelling complex WSTF–SNF2h interacts with nuclear myosin I and has a role in RNA polymerase I transcription. *EMBO Rep.* 2006; 7:525–530. [PubMed: 16514417]
78. Bradsher J, Auriol J, Proietti de Santis L, Iben S, Vonesch JL, Grummt I, Egly JM. CSB is a component of RNA pol I transcription. *Mol. Cell.* 2002; 10:819–829. [PubMed: 12419226]
79. Yuan X, Feng W, Imhof A, Grummt I, Zhou Y. Activation of RNA polymerase I transcription by cockayne syndrome group B protein and histone methyltransferase G9a. *Mol. Cell.* 2007; 27:585–595. [PubMed: 17707230]
80. Russell J, Zomerdijs JC. RNA-polymerase-I-directed rDNA transcription, life and works. *Trends Biochem. Sci.* 2005; 30:87–96. [PubMed: 15691654]
81. Grummt I. Life on a planet of its own: regulation of RNA polymerase I transcription in the nucleolus. *Genes Dev.* 2003; 17:1691–1702. [PubMed: 12865296]
82. Moss T. At the crossroads of growth control; making ribosomal RNA. *Curr. Opin. Genet. Dev.* 2004; 14:210–217. [PubMed: 15196469]
83. Ruggero D, Pandolfi PP. Does the ribosome translate cancer? *Nat. Rev. Cancer.* 2003; 3:179–192.
84. White RJ. RNA polymerases I and III, growth control and cancer. *Nat. Rev. Mol. Cell Biol.* 2005; 6:69–78. [PubMed: 15688068]
85. Valdez BC, Henning D, So RB, Dixon J, Dixon MJ. The Treacher Collins syndrome (TCOF1) gene product is involved in ribosomal DNA gene transcription by interacting with upstream binding factor. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101:10709–10714. [PubMed: 15249688]
86. Gibbons RJ, McDowell TL, Raman S, O'Rourke DM, Garrick D, Ayyub H, Higgs DR. Mutations in ATRX, encoding a SWI/SNF-like protein, cause diverse changes in the pattern of DNA methylation. *Nat. Genet.* 2000; 24:368–371. [PubMed: 10742099]