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Control of Ribosomal RNA Transcription by Nutrients

Yuji Tanaka and Makoto Tsuneoka

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Abstract

The ribosome is a unique machine for protein synthesis in organisms. The construction of ribosomes is exceedingly complex and consumes the majority of the cell materials and energy. The materials for ribosome production are supplied by nutrients. Therefore, the production of ribosomes is restricted by environmental nutrients, and cells need mechanisms to control ribosome production in order to reconcile demands for cell activities with available resources. Transcription of ribosomal RNA is an essential step in ribosome biogenesis. It strongly affects the total amount of ribosome production, and thus rapidly growing cells have an elevated level of ribosomal RNA transcription. Ribosomal RNA transcription is controlled by many mechanisms, including the efficiency of preinitiation complex formation for RNA polymerase I (Pol I) and epigenetic marks in ribosomal RNA genes. These are affected by cell cycle progression, signal transduction pathways, cell-damaging stresses, nutrients such as glucose, and the metabolites. Recent studies also suggest that the epigenetic marks, acetylation and methylation, may be not only controlled by nutrients but also function as reservoirs for biological resources in chromatin. Further studies would provide information about the mechanisms cells use to adjust production of cellular components to available resources and clues for developing novel anti-cancer treatments.

Keywords: ribosomal RNA (rRNA), transcription, nutrients, glucose, epigenetic

1. Introduction

The ribosome is a unique machine for synthesizing protein in organisms. Protein synthesis is essential for all biological events, and the quantity of ribosomes substantially affects all biological activities. Rapidly growing cancer cells require synthesis of much protein and thus many ribosomes. In vertebrates, a ribosome consists of about 80 proteins and 4 structural ribosomal RNAs (rRNAs): 5S rRNA, 5.8S rRNA, 18S rRNA, and 28S rRNA [1, 2]. The construction processes are exceedingly complex and include rRNA transcription, rRNA processing,

Figure 1. Processes of ribosome construction. Ribosomal RNA transcription, processing, and association of ribosomal proteins occur in the nucleolus. Mature ribosome functions in the cytoplasm. The process is outlined in the yellow box on the right side of this figure. Ribosomes contain four structural ribosomal RNAs (rRNAs): 5S rRNA, 5.8S rRNA, 18S rRNA, and 28S rRNA. The first step of ribosome construction is transcription of ribosomal RNA (rRNA) from the ribosome RNA gene (rDNA) repeating units in the nucleolus. RNA polymerase I (Pol I) transcribes pre-rRNA, which is processed to three structured rRNAs (18S, 28S and 5.8S rRNA). 5S rRNA is synthesized by RNA polymerase III. In the mature ribosome, 18S rRNA is contained in the 40S ribosome (small subunit of ribosome), and 28S, 5.8S, and 5S rRNAs are contained in the 60S ribosome (large subunit of ribosome). Ribosomal protein assembly, rRNA processing, and maturation occur in the nucleolus, and ribosomes are exported to the cytoplasm and perform the translation activity.

synthesis of ribosome proteins and regulatory proteins, assembly of rRNAs and ribosome proteins, and maturation of the ribosome (**Figure 1**). The entire process consumes up to 80% of the cell's materials [3, 4], and 80% of the energy to proliferate cells [4, 5]. The materials for ribosome production are supplied by nutrients that are taken up from the environment. Therefore, the production of ribosomes is restricted by environmental conditions, and cells

should have control of the mechanisms of ribosome production in order to reconcile demands for cell activities with the available biological resources.

Three of the four structured rRNAs (18S, 28S, and 5.8S rRNA) constituting ribosomes are produced by processing of a precursor transcript, pre-ribosomal RNA (pre-rRNA). The pre-rRNA is coded by rRNA genes (rDNA) and specifically transcribed by RNA polymerase I (Pol I) in the nucleolus. Because a single copy of rDNA is not sufficient to supply the number of rRNA molecules required, there are 100–300 copies of tandemly repeated rDNAs per haploid genome in mammals. Paradoxically, only half the copies of rDNA are in transcriptionally active forms and the rest are silent, which may provide a control step for rRNA transcription [6–9]. The transcription of rRNA is an essential step in ribosome biogenesis and affects the total number of ribosomes produced. It was suggested that 75% of total RNAs constitute rRNAs in Hela cells [10], and the rRNA transcription represents about 35% of all transcripts in proliferating cells [6], showing that rRNA synthesis uses a lot of materials. Therefore, the control of rRNA transcription plays a role in maintaining homeostasis in biological resources. In this review, we describe the control of rRNA transcription by various factors such as the cell cycle regulators, signal transduction pathways, growth factors, tumor-related proteins, and cell-damaging stresses. Then, we will discuss the control mechanisms of rRNA transcription in response to nutrients.

Figure 2. Pre-initiation complex for RNA polymerase I and rRNA processing. The basic composition of the pre-initiation complex (PIC) for RNA polymerase I is illustrated. PIC is assembled on the rDNA promoter by synergistic action of the upstream binding factor (UBF), which is bound at the upstream control element (UCE), selective factor 1 (SL1), which is bound to the core promoter through TATA-box binding protein (TBP), transcription initiation factor IA (TIF-IA), and RNA polymerase I (Pol I). SL1 contains TBP and Pol I-specific TBP-associated factors (TAF_Is: TAF_I110, TAF_I68, TAF_I48, TAF_I41, and TAF_I12). SL1 on the core promoter recruits RNA polymerase I through TIF-IA, which associates with both components of Pol I and SL1. After the completion of PIC formation, Pol I is released from the promoter by regulation of TIF-IA and starts to transcribe pre-rRNA. This release is the initiation step of rRNA transcription. Pre-rRNA is processed to structured rRNA, 18S, 5.8S, and 28S rRNA to construct ribosomes.

2. Formation of preinitiation complex (PIC) on rDNA promoter

The first step of rRNA transcription is the formation of the preinitiation complex (PIC) on the rDNA promoter. The upstream binding factor (UBF), the promoter selective factor 1 (SL1), transcription initiation factor IA (TIF-IA), and RNA polymerase I (Pol I) synergistically assemble at the rDNA promoter to form PIC (**Figure 2**). While the name SL1 is used for human proteins, that for mice is transcription initiation factor 1B (TIF-IB). UBF and SL1 bind to an upstream control element (UCE) and the core promoter region of rDNA, respectively. SL1 recruits Pol I through TIF-IA, and UBF stabilizes the binding of SL1 to Pol I at the rDNA promoter [6, 11].

SL1 is a protein complex consisting of TATA-box binding protein (TBP), Pol I-specific TBP-associated factors (TAF_{IS}), including TAF_I110, TAF_I68, and TAF_I48, which were originally identified with TBP as essential transcriptional factors by an *in vitro* transcription assay [12]. TBP is not only a Pol I-specific factor, but also used for transcription by RNA polymerase II and III. Later, TAF_I41 and TAF_I12 were identified as members of the TAF_{IS}. TAF_I12 was originally reported as a transcription factor for RNA polymerase II [13]. One activity of TBP is binding to the TATA box in the core promoter to recruit SL1 complex. TIF-IA binds to the RPA43 subunit of Pol I complex and TAF_I110, TAF_I68, and TAF_I41 of the SL1 complex. These binding activities are essential for TIF-IA to recruit Pol I to the promoter bound by SL1 to facilitate PIC formation on the rDNA promoter. The formation of PIC is controlled by various factors (**Figure 3**). When Pol I starts the rRNA transcription, the interactions forming PIC are disrupted. This disruption is the initiation step of transcription, one of the control mechanisms.

Figure 3. Factors regulating PIC formation. The PIC for Pol I transcription is controlled by various factors, including cell cycle signals, signal transduction pathways, stress signals, oncoprotein/tumor suppressors, and others. The classes of regulatory factors are expressed with specific color boxes as indicated in the box on the right. UBF is activated by cdk family proteins, ribosomal protein S6 kinase (S6K), casein kinase II (CK2), and CBP, and is repressed by the Rb/HDAC complex. SL1 is activated by c-Myc, PCAF, and cdc14B, and repressed by p53, SIRT1, cdc2/cyclin B, CK2, PTEN, and GSK3β. TIF-IA is activated by ERK1/2, RSK, mTOR, and CK2 (activated by Akt), and repressed by JNK2 and AMPK. RNA polymerase I (Pol I) is activated by SIRT7 and CBP.

3. Control of rRNA transcription during cell cycle progression

The activities of all classes of RNA polymerases are controlled during the cell cycle progression [14, 15]. The cell cycle regulator cyclin/cdk complexes control the level of rRNA transcription (**Figure 3**). In the M phase, SL1 is inactivated by cdk1/cyclin B (cdc2/cyclin B) through phosphorylation of TAF₁₁₀ to silence rRNA transcription [16, 17]. On exiting mitosis, the phosphorylation in TAF₁₁₀ is removed by cell division cycle 14B (Cdc14B) [18]. Additionally, mitotic repression of rRNA transcription correlates with the hypo-acetylation of TAF₁₆₈ caused by Sirtuin 1 (SIRT1). The hypo-acetylation makes SL1 instable on binding to the rDNA promoter [18]. It was also reported that the site of deacetylation of TAF₁₆₈ by SIRT1 is acetylated by p300/CBP-associated factor (PCAF), which is correlated with the activation of rRNA transcription [18].

After mitosis, rRNA transcription is re-activated by G1/S-specific cyclins (cdk4/cyclin D, cdk2/cyclin E, cdk2/cyclin A) through phosphorylation of UBF on the specific sites (cdk4/cyclin D (S484), cdk2/cyclin E (S388, S484), cdk2/cyclin A (S388): in mouse) [19, 20].

4. Signal transduction pathways control rRNA transcription

Protein synthesis is required for cell growth, and the signal transduction pathways that affect cell growth, including phosphoinositide 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) signaling and ERK (MAPK) signaling, are involved in the regulation of rRNA transcription (**Figure 3**).

The PI3K-AKT-mTOR signal pathway is stimulated by binding of insulin/Insulin-like growth factors (IGF) to their cognate receptors on the cell surface. AKT activates rRNA transcription through the phosphorylation of CK2. CK2 regulates rRNA transcription at multiple levels by affecting the formation of PIC, initiation, elongation, and reinitiation, through phosphorylation of several proteins including UBF, TAF₁₁₀ (SL1), and TIF-IA [21–26]. mTOR activates rRNA synthesis by translocating TIF-IA into the nucleolus using kinase activity [27]. The ribosomal protein S6 kinase (S6K), which is a downstream kinase of mTOR, also activates rRNA synthesis through regulation of UBF-SL1 interaction by phosphorylation of UBF. The mTOR activity also enhances the expression of UBF [28]. SNF2 histone linker PHD RING helicase (SHPRH), which was identified as a RAD5 homolog and known as E3 ubiquitin-protein ligase, binds to rDNA promoters using its PHD domain and promotes recruitment of Pol I to rDNA (**Figure 4**). This activation of rRNA transcription by SHPRH is inhibited in an mTOR-dependent manner [29]. K-demethylase 4A (KDM4A)/JMJD2A activates rRNA transcription on serum stimulation (**Figure 4**). This activation is mediated through the PI3K/serum/glucocorticoid regulated kinase 1 (SGK1) signaling cascade independent of the AKT pathway. SGK1 is one of the downstream kinases of PI3K signaling. The serum-stimulated KDM4A decreases a repressive histone H3K9me3 mark modification in rDNA to activate rRNA transcription [30]. In mouse adipocytes, polymerase I transcription and release factor (PTRF)/Cavin-1 promotes rRNA transcription, which is induced by insulin and repressed by fasting (**Figure 4**). The stimulation of rRNA transcription by PTRF is mediated by the formation of the transcription loop that

links the transcriptional start sites and termination sites. The formation may enhance transcriptional reinitiation [31].

The binding of epidermal growth factor (EGF) or its related ligands to their cognate receptors on the cell surface stimulates a signaling cascade including the GTPase Ras, the kinases Raf, MAP kinase-ERK kinase (MEK), and extracellular signal-regulated kinase (ERK). ERK activates rRNA transcription through phosphorylation of UBF in the promoter [32] and gene body regions [33] (**Figure 3**). This phosphorylation decreases the binding capacity of UBF to rDNA. In this case, it was reported that the dissociation of UBF from rDNA enhances Pol I release from the promoter, leading to activation of rRNA transcription. Additionally, ERK/90 kDa ribosomal S6 kinase (RSK) phosphorylates TIF-1A to activate rRNA transcription [34] (**Figure 3**). It is still unclear how the phosphorylation by RSK induces rRNA transcription.

Figure 4. Signal transduction pathways under growth factor controlled rRNA transcription. Growth factors including insulin, insulin-like growth factor (IGF-1), epidermal growth factor (EGF), and unidentified serum factors (Serum) control rRNA transcription through signal transduction pathways, such as PI3K/mTOR, PI3K/SGK and ERK1/2, which control PIC components (SL1, TIF-1A, and Pol I), transcription factors (PTRF/Carvin-1, c-Myc and SHPRH) or chromatin/nucleosome regulators (IncRNA, NuRD, and KDM2B).

Elevation of the concentration of calcium ions (Ca^{2+}) in the cytoplasm stimulates the signaling pathway of calcium/calmodulin-dependent protein kinase II (CaMKII). The stimulated CaMKII activates S6K, which phosphorylates UBF to activate rRNA transcription in colorectal cancer (CRC) (**Figure 3**). In CRC, the function of adenomatous polyposis coli (APC) gene is frequently lost and the level of Ca^{2+} is increased in the cells [35].

5. Control of rRNA transcription by unidentified serum factors

Serum, used to supplement the cell culturing medium, contains many factors that control rRNA transcription. Although all factors and signal cascades are not completely identified, they perform critical functions in the regulation of rRNA transcription (**Figure 4**).

Depletion of serum from a culture medium represses rRNA transcription. c-Myc plays a critical role for cell growth and proliferation in many types of cells, and is deregulated and over-expressed in tumor cells. c-Myc associates with the promoter and transcribed regions of rDNA and activates rRNA transcription in response to serum stimulation [36].

K-demethylase 2B (KDM2B)/JHDM1B is bound to rDNA to repress rRNA transcription. The repression is associated with the demethylation of trimethylated lysine 4 on histone H3 (H3K4me3) by KDM2B. Serum starvation increases the recruitment of KDM2B on rDNA, and resupply of serum decreases it. These data suggest that the activity of KDM2B in controlling rRNA transcription is regulated by serum factors [37].

The specific long non-coding RNAs (lncRNAs) are induced during periods of quiescence, such as serum starvation, and increase the level of histone H4K20me3 on the rDNA promoter in a suppressor of variegation 4-20 homolog (Suv4-20 h)-dependent manner. The elevated level of H4K20me3 leads to chromatin compaction. The lncRNAs are antisense transcripts against rDNA, are termed the promoter and pre-rRNA antisense (PAPAS), and associated with rDNA [38].

A chromatin remodeling complex, nucleosome remodeling deacetylase (NuRD), establishes the poised state of rDNA through regulation of histone modifications and nucleosome positions. The level of the state of rDNA is increased in the growth-arrested conditions induced by serum starvation and differentiation [39].

6. Oncoprotein and tumor suppressors in rDNA transcription

Tumor cells show abnormal growth that is thought to be associated with the elevation of ribosome biogenesis, and regulation of rRNA transcription by oncogenes and tumor-suppressor genes was reported.

The oncoprotein c-Myc is the product of oncogene *c-myc*, and its expression is stimulated by serum and associated with rRNA transcription [36] (**Figure 4** and Section 5). It was reported that c-Myc binds to the sites with the consensus sequences on rDNA and stimulates rRNA

transcription [40]. c-Myc is also reported to control the PIC factors such as UBF and ribosomal proteins [41–43]. Therefore, c-Myc activates ribosome biogenesis at multiple steps [3, 44, 45] .

The *rb* gene is a tumor-suppressor gene. Rb protein binds to UBF, which may be related to restriction of cell proliferation by Rb [46]. The binding of Rb to UBF inhibits the binding of UBF to rDNA [47] or inhibits the binding of UBF to SL1 [48], both of which result in the repression of rRNA transcription (**Figure 3**). Another report suggested that phosphorylated Rb (pRb) creates a complex with histone deacetylase (HDAC) and decreases the acetylation of UBF to repress rRNA transcription [49]. In this study, it was also reported that the acetylation in UBF is modified by CREB-binding protein (CBP) (**Figure 3**).

A tumor-suppressor gene, *p53*, is frequently mutated in tumors, and p53 protein represses rRNA transcription through prevention of the interaction between SL1 and UBF [50] (**Figure 3**). The phosphatase and tensin homolog deleted from chromosome 10 (PTEN) is known as a tumor suppressor. PTEN represses rRNA transcription by disrupting the SL1 complex in its lipid phosphatase activity-dependent manner [51] (**Figure 3**). It was also reported that PTEN is phosphorylated by glycogen synthase kinase (GSK) 3 β [52]. GSK3 β and PTEN are selectively enriched in the nucleoli of RAS-transformed cells and associate with the promoter and coding region of the rDNA [53]. An activated GSK3 β mutant abolishes rRNA transcription and associates with TAF₁₁₀ in the SL1 complex [53]. These results suggest a repressive function for GSK3 β on rRNA transcription that supports its role as a tumor suppressor.

7. Controls of rRNA transcription by cell-damaging stresses

A variety of stresses such as UV, ionizing radiation, heat shock, and osmotic shock attack cellular vital components like DNA, proteins, and lipid membranes. These stresses also affect rRNA transcription.

c-Jun N-terminal kinase (JNK) phosphorylates c-Jun at the NH₂-terminal Ser63 and 73 residues in response to UV irradiation and other stress stimuli [54]. JNK2 inactivates rRNA transcription through phosphorylation of TIF-IA to inhibit its function of bridging between Pol I and SL1 [55] (**Figure 3**).

The DNA damage caused by ionizing radiation also induces the repression of rRNA transcription through other pathways, which involves Nijmegen breakage syndrome protein 1 (NBS1)-treacle, Ataxia Telangiectasia Mutated (ATM), and breast cancer susceptibility gene I (BRCA1). In the presence of double strand breaks induced by ionizing radiation, NBS1 translocates and accumulates in nucleoli in a treacle-dependent manner to silence rRNA transcription [56]. The *treacle* gene was found to be mutated in Treacher Collins syndrome, which is characterized by deformation of bones and other tissues in the face. ATM-dependent signaling was shown to shut-down rRNA transcription in response to chromosome breaks [57]. BRCA1, known as a tumor suppressor, was reported to interact with UBF, SL1, and Pol I. In response to DNA damage, BRCA1 bound to rDNA is dissociated, and induces instability of Pol I on rDNA to repress rRNA transcription [58].

Heat shock at 42°C represses rRNA transcription through the inactivation of TIF-IA by inhibition of CK2-dependent phosphorylation of TIF-IA and the lncRNAs PAPAS-dependent nucleosome regulation by NuRD complex [59]. Hypotonic stress represses rRNA transcription through upregulation of PAPAS to trigger nucleosome repositioning by NuRD [60]. In these conditions, Suv420h2 was neddylated and the levels of Suv420h2 and H4K20me3 marks were increased. However, the relationship between PAPAS and Suv420h2 was not clear. Cytoskeletal stress, which is related to cell shape, represses rRNA transcription through Rho-associated protein kinase (ROCK). ROCK is one of the kinases of myosin and induces recruitment of HDAC on rDNA, resulting in deacetylation of histone acetylated lysine 9 and 14 on histone H3 (H3K9/14) [61].

8. Control of rRNA transcription by nutrients

Cells obtain biological resources for cellular activities from their environment. The sensing of environmental nutrients is important for efficient usage of nutrients and maintaining cells. In murine intestinal epithelium, apical transcripts are more efficiently translated, because ribosomes were more abundant on the apical sides. Refeeding of fasted mice induces a basal to apical shift of mRNAs encoding ribosomal proteins, which is associated with an increase in their translation and increased protein production. These mechanisms allow efficient nutrient absorption in response to the rich conditions, although the molecular mechanisms are not clear [62]. It was shown that mTOR senses the levels of amino acids, especially leucine, in cells, and controls the translation activity through regulation of the eukaryotic translation initiation factor 4E binding protein (4E-BP)-eukaryotic translation initiation factor 4E (eIF4E) axis and p70 S6K-S6 axis [4, 63, 64]. Recently, increasing evidence shows the presence of specific mechanisms to control rRNA transcription in response to nutrients (Figure 5).

8.1. Amino acids

The starvation of amino acids affects the frequency of initiation of nucleolar RNA polymerase, which was later established to be an rRNA transcription by Pol I [65]. The starvation of amino acids decreases the interaction of TIF-IA with SL1 and Pol I [66]. As described above in Section 4, mTOR controls rRNA transcription, and is important for regulation of rRNA transcription in response to amino acid levels. Amino acid starvation inhibits the activity of mTOR and its downstream kinase S6K. mTOR and S6K control TIF-IA and UBF, respectively, to regulate rRNA transcription [27, 67].

c-MYC is also involved in regulation of rDNA transcription in response to starvation of amino acids. Although translation of c-Myc is reported to be controlled by mTOR signaling [68, 69], the stabilization of c-Myc in response to amino acid starvation is controlled by an mTOR-independent pathway [70].

8.2. Guanosine triphosphate (GTP)

It was reported that the sizes of ATP and GTP intracellular pools affect the level of nucleolar RNA synthesis (rRNA transcription) [71]. Recently, the consensus sequences for GTP binding

Figure 5. Glucose and amino acids control rRNA transcription. Glucose, amino acids, and GTP control rRNA transcription through several pathways, such as ERK, mTOR/S6K, and AMPK, which control PIC components (UBF, SL1, and TIF-IA), transcription factors (c-Myc), or chromatin regulators (KDM2A, PIH1, and SIRT1/NML). High glucose activates ERK and mTOR/S6K pathways to control UBF. High glucose also activates PIH1 to control chromatin. Glucose depletion or low energy conditions activate SIRT1/NML and the AMPK pathway. Activated AMPK controls the activities of TIF-IA and KDM2A. Activated KDM2A controls rDNA chromatin to inhibit rRNA transcription. Amino acid depletion represses the mTOR pathway, resulting in the repression of TIF-IA, and decreases the expression of c-Myc. Guanosine triphosphate (GTP) is bound to TIF-IA, and the binding is required to control rRNA transcription.

were identified in TIF-IA [72], and the binding of TIF-IA to GTP is required for the interaction of TIF-IA with ErbB3-binding protein (Ebp1). Ebp1 controls ribosomal biogenesis when located in the nucleolus [73]. Therefore, the level of GTP appears to be sensed by TIF-IA to affect rRNA transcription (**Figure 5**).

8.3. Glucose

The major energy source for cells is glucose. Glucose is used to synthesize ATP. ATP is essential for most biological activities, including ribosome biogenesis. Several studies demonstrated that the levels of glucose and ATP production affect rRNA transcription.

Ribosomal biogenesis including rRNA transcription was reported to be induced by high glucose treatment or diabetes. A high level of glucose activates UBF through ERK1/2 and mTOR in kidney glomerular epithelial cells of mice [74].

The PIH1 domain-containing protein 1 (PIH1)/Nop17 is reported to enhance rRNA transcription through the recruitment of SNF5-Brg1 complex on the rRNA promoter [75] (**Figure 5**). The complex increases acetylation of several histones, except histone H4K16Ac, on rDNA in high glucose conditions. Until now, the acetylation marks of histone in rDNA, excluding the acetylation at K16 in histone H4 (H4K16Ac), are linked to activation of transcription. The acetylated histones function as active marks in transcription in many cases because the acetylation of histone weakens the interaction of histone octamers with DNA, and the acetylated histones are recognized by several transcription-activating factors. On the other hand, the H4K16Ac mark is reported to be recognized by nucleolar remodeling complex (NoRC) in rDNA, which induces chromatin-silencing status [76]. Glucose starvation dissociates PIH1 and the SNF5-Brg1 complex from rDNA and increases histone H4K16Ac marks, which repress rRNA transcription [75]. Another report suggested that PIH1 interacts with mTORC1 to stabilize it, resulting in enhancement of rRNA transcription [77].

8.4. AMPK is activated by glucose starvation

Glucose starvation decreases ATP production and activates AMPK (**Figure 5**). The AMP-activated kinase (AMPK) is known as an energy sensor, which recognizes the ratios of AMP, ADP, and ATP and regulates many phenomena in cells to maintain energy homeostasis.

Additionally, a recent study showed the existence of an AMP/ADP-independent mechanism that triggers AMPK activation (**Figure 6**). Glycolysis is a determined sequence of 10 enzyme-catalyzed reactions. In the fourth step, the hexose ring of fructose 1, 6-bisphosphate (FBP) is split by aldolase into two triose sugars: dihydroxyacetone phosphate (a ketose) and glyceraldehyde 3-phosphate (an aldose). When extracellular glucose is decreased, intracellular FBP is decreased, and aldolase unoccupied by FBP promotes the formation of a lysosomal complex containing v-ATPase axin, liver kinase B1 (LKB1), and AMPK, which regulates AMPK activity [78]. These results suggest that the decreased level of the metabolite in glycolysis controls AMPK before the reduction of ATP production just after changing environmental conditions, emphasizing that AMPK is a highly sensitive monitor of energy conditions.

AMPK induces phosphorylation of TIF-IA (**Figure 5**). The phosphorylation of TIF-IA by AMPK reduces interaction of TIF-IA with SL1, decreases the TIF-IA amount on the rDNA promoter, and interrupts PIC assembly, which results in the reduction of rRNA transcription [79].

KDM2A, identified as mono- and di-methylated lysine 36 on histone H3 (H3K36me1/2) demethylase [80], is accumulated in the nucleolus and binds to rDNA [81]. The repression of rRNA transcription by KDM2A is induced in response to serum and glucose starvation (**Figure 5**). The repression requires the demethylase activity of KDM2A on the rDNA promoter [82]. The KDM2A-dependent regulation affects the levels of protein synthesis [81]. The demethylase activity of KDM2A proceeds with a co-reaction in which α -ketoglutarate (α -KG)

Figure 6. Control of methylation by energy status and methyl marks as a reservoir for biological resources. Chromatin components are methylated and demethylated by specific enzymes influenced by metabolites in energy production. Formaldehyde is produced as a demethylation byproduct, directly generates one carbon unit, fuels the folate cycle through alcohol dehydrogenase 5 (ADH5) activity, and can be used as a source for production of SAM, which is used for methylation and nucleotides. AMPK, AMP-activated protein kinase; DHF, dihydrofolate; FBP, fructose 1, 6-bisphosphate; 3PG, 3-phosphoglycerate; SAM, S-adenosylmethionine; THF, tetrahydrofolate.

is converted to succinate, both of which are organic acids constituting the TCA cycle. Interestingly, the enzyme activity of KDM2A is controlled by cell-permeable succinate (dimethyl succinate; DMS), suggesting that metabolites in the TCA cycle affect KDM2A activity. Recently, it was found that glucose starvation in the presence of serum induces the repression of rRNA transcription by KDM2A, in which activated AMPK induces KDM2A activity [83] (**Figure 5**). Interestingly, treatment with a low concentration of the glycolysis inhibitor 2-deoxy-D-glucose (2DG) induces KDM2A-dependent repression of rRNA transcription associated with histone demethylation on the rDNA promoter, although it does not dissociate TIF-1A from the rDNA promoter. Treatment with a high concentration of 2DG induces both the dissociation of TIF-1A from the rDNA promoter and KDM2A-dependent demethylation of the rDNA promoter. These results suggest that the repression of rRNA transcription in response to glucose starvation is performed by two different mechanisms: epigenetic regulation by KDM2A and TIF-1A regulation, depending on the glucose starvation level.

AMPK phosphorylates dozens of proteins, but until now KDM2A has not been detected as a substrate of AMPK kinase activity. AMPK also controls the activity of mTOR [84], and mTOR

is a candidate kinase for control of the states of histone methylation in the rDNA promoter, but currently there is no evidence connecting mTOR and KDM2A. Further studies are required to determine how the KDM2A activity in the rDNA promoter is induced by AMPK. H3K36me2 on the rDNA promoter which is demethylated by KDM2A on starvation is quickly restored by refeeding glucose and serum [82]. The data suggest that the control of H3K36me2 levels on rDNA promoters is reversible by changes in nutrient status, although which enzyme induces methylation of H3K36me2 on the rDNA promoter in response to refeeding of glucose and serum remains unknown. The control mechanism of rRNA transcription through epigenetic regulation by KDM2A may be a fine tuning device that quickly reflects nutrient states around cells.

8.5. Sirtuins

Sirtuins target a wide range of cellular proteins in the nucleus, cytoplasm, and mitochondria for post-translational modification by acetylation (SirT1, 2, 3, and 5) or ADP-ribosylation (SirT4 and 6). The deacetylase activity of sirtuins is controlled by the cellular NAD^+/NADH ratio, where NAD^+ works as an activator, while nicotinamide and NADH act as inhibitors (**Figure 7**). The acetylation regulates a wide variety of cellular functions. Sirtuins participate in various cellular processes, deacetylating both chromatin and non-histone proteins, and their roles in aging have been extensively studied. Sirtuins may also play a critical role in tumor initiation and progression as well as drug resistance. Reduced compounds such as glucose and fatty acids are oxidized, thereby releasing energy. This energy is transferred to NAD^+ by reduction to NADH, as part of glycolysis, the citric acid cycle, and β -oxidation. The mitochondrial

Figure 7. Control of acetylation by energy status and acetyl marks as a reservoir for biological resources. Chromatin components are acetylated and deacetylated by specific enzymes whose activities are influenced by metabolites in energy production. During deacetylation, the acetyl group from the substrate is accepted by the ribose to produce O-acetyl-ADP-ribose (OAADPr). A cytoplasmic esterase, which was suggested to be ADP-ribosyl hydrolase 3 (ARH3), hydrolyzes OAADPr to acetate and ADPr. Acetate that is generated from the deacetylation may be changed to acetyl-CoA, and used as resource for acetyl marks and lipogenesis. ACECSI, acetyl-CoA synthetase 1; ACL, ATP-citrate lyase; OAADPr, O-acetyl ADP-ribose.

NADH is then oxidized in turn by the electron transport chain, which generates ATP through oxidative phosphorylation.

SIRT1 was reported to be required for the recruitment of nucleomethylin (NML) on rDNA. In response to glucose starvation, rRNA transcription is repressed through NML-induced chromatin regulation [85]. Although it is not clear that SIRT1 shows deacetylase activity on starvation, SIRT1 induces the deacetylation of p53, and this deacetylation activity is required for the repression of rRNA transcription. Further, the NAD⁺ synthesis enzyme nicotinamide mononucleotide adenylyltransferase (NMNAT1) modulates the repression of rRNA transcription [86]. As described above, SIRT1 is required for mitotic repression of rRNA transcription through deacetylation of TAF₆₈ (**Figure 3** and section 3). TAF₆₈ is acetylated by PCAF to restart transcription in the mitotic exit phase [18].

On the other hand, SIRT7, another SIRT family member, was reported to activate rRNA transcription depending on the deacetylation activity, through regulation of PAF53, which is an important component of Pol I complex [87–91].

8.6. Epigenetic marks may play a role in conserving biological resources

Acetyl-CoA is used as an acetyl group donor on acetylation of histone and other proteins (**Figure 7**). Acetyl-CoA is produced from pyruvate, acetate, or fatty acid oxidation in multiple metabolisms. The amount of acetyl-CoA affects the activity of histone acetyltransferases (HATs). S-adenosylmethionine (SAM) is known to be used as a methyl donor in DNA/histone (protein) methylation (**Figure 6**). SAM is produced through the condensation of methionine and ATP by methionine adenosyltransferase (MAT). The production of SAM is mediated through the folate and methionine cycles. The amount of SAM is thought to affect the activity of methylase. The production of the two epigenetic marks is clearly affected by energy production processes, suggesting that intracellular energy conditions affect the modifications of epigenetic marks [92–94].

Further, enzymes detaching the marks are also affected by metabolites in energy production. NAD⁺ activates sirtuin deacetylase, while nicotinamide and NADH inhibit the activity (**Figure 7**). The demethylase activity of KDM2A is controlled by the amounts of intracellular ATP through AMPK (**Figure 6**), and probably more directly by succinate. In another report, chromatin-associated fumarase generating fumarate inhibits the demethylation activity of KDM2A on the promoter region of the RNA polymerase II gene [95]. Additionally, 2-hydroxy-glutarate (2-HG), which was produced from α -KG by a mutant type isocitrate dehydrogenase (IDH) also modulates several jmjC-type enzymes including the lysine-specific demethylases (KDMs) such as KDM2A and Tet methyl-cytosine dioxygenases (TETs) [96–98]. Therefore, metabolites reflecting intracellular energy conditions can control the enzymes for detaching the epigenetic marks as well as adding them.

Interestingly, during deacetylation, the glycosidic bond of the nicotinamide ribose is cleaved to yield nicotinamide, and the ribose accepts the acetyl group from the substrate to produce O-acetyl-ADP-ribose (OAADPr). *In vitro*, a cytoplasmic esterase from humans and yeast, which was suggested to be ADP-ribosyl hydrolase 3, hydrolyzes OAADPr to acetate and ADPr [99]. Therefore, acetyl marks of proteins that are deacetylated by sirtuin may also be

used as a source of acetate for acetyl marks and lipogenesis (**Figure 7**). KDMs catalyze histone lysine demethylation through an oxidative reaction. The catalytic reaction begins with the coordination of molecular oxygen (O_2) by Fe(II) and the conversion of α -KG to succinate and CO_2 with the concomitant hydroxylation of the methyl group of the peptide substrate. The resulting carbinolamine is unstable and degrades spontaneously to an unmethylated peptide and the cytotoxic molecule formaldehyde (**Figure 6**). Recently, it was shown that formaldehyde reacts spontaneously with glutathione (GSH) to yield S-hydroxymethylglutathione (HMGS), and subsequently HMGS is oxidized by alcohol dehydrogenase 5 with NAD(P)⁺ to create S-formylglutathione. This biochemical route provides a cell with formaldehyde detoxification as well as utilizable one-carbon units, which contribute to nucleotide synthesis. Therefore, cells reserve materials for one-carbon metabolism as methyl marks [100], which are released by KDM2A on starvation. These two examples of acetylation and methylation suggest that modifications of the epigenetic marks are not only controlled by intracellular energy conditions, but also function as reservoirs in chromatin for biological resources.

9. Applications for therapy

Enlarged nucleoli have been recognized as a hallmark of cancer cells [101, 102]. Elevated levels of rRNA transcription and protein synthesis are often observed in cancer cells. These observations suggest the possibility that the control of rRNA transcription could regulate the proliferation of cancer cells. Actually, the anti-cancer effects of some compounds are associated with down-regulation of rRNA transcription. Cisplatin [103], mitomycin C [104], and 5-fluorouracil [105], well-known anti-cancer drugs, are reported to inhibit rRNA transcription [11]. However, it is not clear whether the reduction of rRNA transcription in cancer cells is causal or only as a consequence of inhibition of cell growth.

On the other hand, there are drugs that appear to reduce rRNA transcription and then repress cancer growth. Actinomycin D (Dactinomycin) specifically represses rRNA synthesis at low concentrations through inactivation of transcriptional elongation by Pol I by interaction with GC-rich regions of rDNA, and thus inhibits growth of cancer cells. Actinomycin D is used as a chemotherapy medication to treat a number of types of cancer, including gestational trophoblastic neoplasia [106], Wilms tumor [107], rhabdomyosarcoma [108], Ewing's sarcoma [109], and malignant hydatidiform mole [110].

CX-5461 was identified by screening for selective inhibitors of Pol I but not Pol II transcription. CX-5461 specifically inhibits ribosomal RNA transcription by impairment of SL1 binding to the rDNA promoter [111] and thus exhibits anti-cancer activity [83, 111]. The inhibition of rRNA synthesis by CX-5461 leads to senescence and autophagy in a p53-independent manner in a tumor cell line [111], to activation of p53-dependent apoptotic signaling in Myc-overexpressing B-lymphoma cells (E μ -Myc lymphoma cells) [112], and to activation of the ATM/Ataxia Telangiectasia and Rad3-related protein (ATR) pathway in acute lymphoblastic leukemia to induce G2 arrest and apoptosis [113]. The potential therapeutic effect of CX-5461 was demonstrated in xenograft models using human pancreatic carcinoma (MIA PaCa-2), melanoma (A375) [111], biphenotypic B myelomonocytic leukemia (MV 4;11) [112] and breast cancer susceptibility gene II (BRCA2) deficient colon cancer (HCT116) [114], and in mice

models transplanted with p53 wild-type E μ -Myc lymphoma [112]. The treatment of CX-5461 in these experiments hardly affected on the health and body weights of mice [111, 112].

BMH-21, which was identified by cell-based screening, intercalates into GC-rich sequences, which exist at a high frequency in rDNA, and represses Pol I transcription [115]. Treatment with BMH-21 induces proteasome-dependent degradation of the largest catalytic subunit of Pol I, RPA194, resulting in a decrease of the Pol I level on rDNA. These effects were correlated to the anti-cancer activity of BHM-21. The anti-tumor activity of BMH-21 was demonstrated using human melanoma (A375) and colorectal carcinoma (HCT116) xenograft models with little effect on body weight [115]. These studies suggest that the chemicals that repress the rRNA transcription show anti-cancer activities.

Epigenetic controls of rDNA chromatin are also candidates for cancer therapy. For example, specific activation of KDM2A could reduce cancer cell proliferation. Because KDM2A activity is regulated by ATP levels through AMPK and also metabolites in energy production, control of these compound levels may regulate KDM2A activity and cell proliferation. As seen here, there are many elaborate mechanisms for control of rRNA transcription, some of which involve intracellular metabolites, which are produced from environmental nutrients. Further studies of the relationship between rRNA transcription and nutrients will provide information about the mechanisms by which cells reconcile demand and usage of biological resources, and clues for novel methods to treat cancers.

10. Conclusion

The construction of ribosomes consumes the majority of the cell's materials and energy. Because the materials for ribosome production are supplied by nutrients, the production of ribosomes is largely restricted by environmental nutrients and cells need mechanisms to control ribosome production in order to reconcile demands for cell activities with available resources. Transcription of rRNA is an essential step in ribosome biogenesis, and strongly affects the total amount of ribosome production. Ribosomal RNA transcription is controlled by many mechanisms, including the efficiency of PIC formation for Pol I and epigenetic marks in rDNA. These are affected by nutrients. Recent studies suggest that the epigenetic marks, such as acetylation and methylation, may be not only controlled by nutrients but also function as reservoirs for biological resources in chromatin. Elevated levels of rRNA transcription and protein synthesis are often observed in cancer cells, and the control of rRNA transcription can regulate their proliferation. Indeed some chemicals that repress the rRNA transcription show anti-cancer activities. Further studies of the relationship between rRNA transcription and nutrients will provide clues for novel methods to treat cancers.

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Abbreviations

AMPK	AMP-activated kinase
ATM	Ataxia telangiectasia mutated
Cdk	Cyclin dependent kinase
CK2	Casein kinase 2
ERK	Extracellular signal-regulated kinase
FBP	Fructose 1, 6-bisphosphate
GSK3 β	Glycogen synthase kinase 3 β
H3K36me2	Dimethylated lysine 36 on histone H3
H4K20me3	Trimethylated lysine 20 on histone H4
HDAC	Histone deacetylase
KDM2A	Lysine(K)-specific demethylase 2A
KDMs	Lysine(K)-specific demethylases
lncRNAs	Long non-coding RNAs
mTOR	Mammalian target of rapamycin
NuRD	Nucleosome remodeling deacetylase
PAPAS	Promoter and pre-rRNA antisense
PCAF	p300/CBP-Associated factor
PI3K	Phosphoinositide 3-kinase
PIC	Preinitiation complex
PIH1	PIH1 domain-containing protein 1
Pol I	RNA polymerase I
pre-rRNA	Pre-ribosomal RNA
PTEN	Phosphatase and tensin homolog deleted from chromosome 10
rDNA	Ribosome RNA gene
rRNA	Ribosomal RNA
RSK	ERK/90 kDa ribosomal S6 kinase
S6K	Ribosomal protein S6 kinase
SAM	S-adenosylmethionine

SIRT1	Sirtuin 1
SL1	Promoter selective factor 1
Suv420h2	Suppressor of variegation 4-20 homolog
TAF _{IS}	TBP-associated factors for RNA polymerase I
TBP	TATA-box binding protein
TIF-IA	Transcription initiation factor IA
UBF	Upstream binding factor

Author details

Yuji Tanaka and Makoto Tsuneoka*

*Address all correspondence to: tsuneoka@takasaki-u.ac.jp

Faculty of Pharmacy, Takasaki University of Health and Welfare, Takasaki, Japan

References

- [1] Khatter H, Myasnikov AG, Natchiar SK, Klaholz BP. Structure of the human 80S ribosome. *Nature*. 2015;**520**(7549):640-645. DOI: 10.1038/nature14427
- [2] Melnikov S, Ben-Shem A, Garreau de Loubresse N, Jenner L, Yusupova G, Yusupov M. One core, two shells: Bacterial and eukaryotic ribosomes. *Nature Structural & Molecular Biology*. 2012;**19**(6):560-567. DOI: 10.1038/nsmb.2313
- [3] Schmidt EV. The role of c-myc in regulation of translation initiation. *Oncogene*. 2004;**23**(18):3217-3221. DOI: 10.1038/sj.onc.1207548
- [4] Kusnadi EP, Hannan KM, Hicks RJ, Hannan RD, Pearson RB, Kang J. Regulation of rDNA transcription in response to growth factors, nutrients and energy. *Gene*. 2015;**556**(1):27-34. DOI: 10.1016/j.gene.2014.11.010
- [5] Thomas G. An encore for ribosome biogenesis in the control of cell proliferation. *Nature Cell Biology*. 2000;**2**(5):E71-E72. DOI: 10.1038/35010581
- [6] Olson MOJ. *The Nucleolus*. Vol. xxv. New York: Springer; 2011. p. 414, DOI: absent
- [7] Grummt I. Different epigenetic layers engage in complex crosstalk to define the epigenetic state of mammalian rRNA genes. *Human Molecular Genetics*. 2007;**16**(Spec No 1):R21-R27. DOI: 10.1093/hmg/ddm020

- [8] Grummt I. Life on a planet of its own: Regulation of RNA polymerase I transcription in the nucleolus. *Genes & Development*. 2003;**17**(14):1691-1702. DOI: 10.1101/gad.1098503R
- [9] Grummt I. Wisely chosen paths – regulation of rRNA synthesis: Delivered on 30 June 2010 at the 35th FEBS Congress in Gothenburg, Sweden. *The FEBS Journal*. 2010;**277**(22): 4626-4639. DOI: 10.1111/j.1742-4658.2010.07892.x
- [10] Jackson DA, Pombo A, Iborra F. The balance sheet for transcription: An analysis of nuclear RNA metabolism in mammalian cells. *The FASEB Journal* 2000;**14**(2):242-254. DOI: absent
- [11] Drygin D, Rice WG, Grummt I. The RNA polymerase I transcription machinery: An emerging target for the treatment of cancer. *Annual Review of Pharmacology and Toxicology*. 2010;**50**:131-156. DOI: 10.1146/annurev.pharmtox.010909.105844
- [12] Zomerdijk JC, Beckmann H, Comai L, Tjian R. Assembly of transcriptionally active RNA polymerase I initiation factor SL1 from recombinant subunits. *Science* 1994;**266**(5193): 2015-2018. DOI: absent
- [13] Goodfellow SJ, Zomerdijk JC. Basic mechanisms in RNA polymerase I transcription of the ribosomal RNA genes. *Sub-Cellular Biochemistry*. 2013;**61**:211-236. DOI: 10.1007/978-94-007-4525-4_10
- [14] Loyer P, Trembley JH, Katona R, Kidd VJ, Lahti JM. Role of CDK/cyclin complexes in transcription and RNA splicing. *Cellular Signalling*. 2005;**17**(9):1033-1051. DOI: 10.1016/j.cellsig.2005.02.005
- [15] Lim S, Kaldis P. Cdk, cyclins and CKIs: Roles beyond cell cycle regulation. *Development*. 2013;**140**(15):3079-3093. DOI: 10.1242/dev.091744
- [16] Heix J, Vente A, Voit R, Budde A, Michaelidis TM, Grummt I. Mitotic silencing of human rRNA synthesis: Inactivation of the promoter selectivity factor SL1 by cdc2/cyclin B-mediated phosphorylation. *The EMBO Journal*. 1998;**17**(24):7373-7381. DOI: 10.1093/emboj/17.24.7373
- [17] Kuhn A, Vente A, Doree M, Grummt I. Mitotic phosphorylation of the TBP-containing factor SL1 represses ribosomal gene transcription. *Journal of Molecular Biology*. 1998; **284**(1):1-5. DOI: 10.1006/jmbi.1998.2164
- [18] Voit R, Seiler J, Grummt I. Cooperative action of Cdk1/cyclin B and SIRT1 is required for mitotic repression of rRNA synthesis. *PLoS Genetics*. 2015;**11**(5):e1005246. DOI: 10.1371/journal.pgen.1005246
- [19] Voit R, Hoffmann M, Grummt I. Phosphorylation by G1-specific cdk-cyclin complexes activates the nucleolar transcription factor UBF. *The EMBO Journal*. 1999;**18**(7):1891-1899. DOI: 10.1093/emboj/18.7.1891
- [20] Voit R, Grummt I. Phosphorylation of UBF at serine 388 is required for interaction with RNA polymerase I and activation of rDNA transcription. *Proceedings of the National*

Academy of Sciences of the United States of America. 2001;**98**(24):13631-13636. DOI: 10.1073/pnas.231071698

- [21] Voit R, Schnapp A, Kuhn A, Rosenbauer H, Hirschmann P, Stunnenberg HG, et al. The nucleolar transcription factor mUBF is phosphorylated by casein kinase II in the C-terminal hyperacidic tail which is essential for transactivation. *The EMBO Journal* 1992;**11**(6):2211-2218. DOI: absent
- [22] Kihm AJ, Hershey JC, Haystead TA, Madsen CS, Owens GK. Phosphorylation of the rRNA transcription factor upstream binding factor promotes its association with TATA binding protein. *Proceedings of the National Academy of Sciences of the United States of America* 1998;**95**(25):14816-14820. DOI: absent
- [23] Lin CY, Navarro S, Reddy S, Comai L. CK2-mediated stimulation of Pol I transcription by stabilization of UBF-SL1 interaction. *Nucleic Acids Research*. 2006;**34**(17):4752-4766. DOI: 10.1093/nar/gkl581
- [24] Panova TB, Panov KI, Russell J, Zomerdijk JC. Casein kinase 2 associates with initiation-competent RNA polymerase I and has multiple roles in ribosomal DNA transcription. *Molecular and Cellular Biology*. 2006;**26**(16):5957-5968. DOI: 10.1128/MCB.00673-06
- [25] Bierhoff H, Dundr M, Michels AA, Grummt I. Phosphorylation by casein kinase 2 facilitates rRNA gene transcription by promoting dissociation of TIF-IA from elongating RNA polymerase I. *Molecular and Cellular Biology*. 2008;**28**(16):4988-4998. DOI: 10.1128/MCB.00492-08
- [26] Nguyen le XT, Mitchell BS. Akt activation enhances ribosomal RNA synthesis through casein kinase II and TIF-IA. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**(51):20681-20686. DOI: 10.1073/pnas.1313097110
- [27] Mayer C, Zhao J, Yuan X, Grummt I. mTOR-dependent activation of the transcription factor TIF-IA links rRNA synthesis to nutrient availability. *Genes & Development*. 2004;**18**(4):423-434. DOI: 10.1101/gad.285504
- [28] Hannan KM, Brandenburger Y, Jenkins A, Sharkey K, Cavanaugh A, Rothblum L, et al. mTOR-dependent regulation of ribosomal gene transcription requires S6K1 and is mediated by phosphorylation of the carboxy-terminal activation domain of the nucleolar transcription factor UBF. *Molecular and Cellular Biology* 2003;**23**(23):8862-8877. DOI: absent
- [29] Lee D, An J, Park YU, Liaw H, Woodgate R, Park JH, et al. SHPRH regulates rRNA transcription by recognizing the histone code in an mTOR-dependent manner. *Proceedings of the National Academy of Sciences of the United States of America*. 2017;**114**(17):E3424-E3433. DOI: 10.1073/pnas.1701978114
- [30] Salifou K, Ray S, Verrier L, Aguirrebengoa M, Trouche D, Panov KI, et al. The histone demethylase JMJD2A/KDM4A links ribosomal RNA transcription to nutrients and growth factors availability. *Nature Communications*. 2016;**7**:10174. DOI: 10.1038/ncomms10174
- [31] Liu L, Pilch PF. PTRF/Cavin-1 promotes efficient ribosomal RNA transcription in response to metabolic challenges. *eLife*. 2016;**5**:e17508. DOI: 10.7554/eLife.17508

- [32] Stefanovsky VY, Pelletier G, Hannan R, Gagnon-Kugler T, Rothblum LI, Moss T. An immediate response of ribosomal transcription to growth factor stimulation in mammals is mediated by ERK phosphorylation of UBF. *Molecular Cell*. 2001;**8**(5):1063-1073. DOI: S1097-2765(01)00384-7
- [33] 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. *Molecular Cell*. 2006;**21**(5):629-639. DOI: 10.1016/j.molcel.2006.01.023
- [34] Zhao J, Yuan X, Frodin M, Grummt I. ERK-dependent phosphorylation of the transcription initiation factor TIF-IA is required for RNA polymerase I transcription and cell growth. *Molecular Cell*. 2003;**11**(2):405-413. DOI: S1097-2765(03)00036-4
- [35] Tsoi H, Lam KC, Dong Y, Zhang X, Lee CK, Zhang J, et al. Pre-45s rRNA promotes colon cancer and is associated with poor survival of CRC patients. *Oncogene*. 2017;**36**:6109-6118. DOI: 10.1038/onc.2017.86
- [36] Arabi A, Wu S, Ridderstrale K, Bierhoff H, Shiue C, Fatyol K, et al. c-Myc associates with ribosomal DNA and activates RNA polymerase I transcription. *Nature Cell Biology*. 2005;**7**(3):303-310. DOI: 10.1038/ncb1225
- [37] Frescas D, Guardavaccaro D, Bassermann F, Koyama-Nasu R, Pagano M. JHDM1B/FBXL10 is a nucleolar protein that represses transcription of ribosomal RNA genes. *Nature*. 2007;**450**(7167):309-313. DOI: 10.1038/nature06255
- [38] Bierhoff H, Dammert MA, Brocks D, Dambacher S, Schotta G, Grummt I. Quiescence-induced LncRNAs trigger H4K20 trimethylation and transcriptional silencing. *Molecular Cell*. 2014;**54**(4):675-682. DOI: 10.1016/j.molcel.2014.03.032
- [39] Xie W, Ling T, Zhou Y, Feng W, Zhu Q, Stunnenberg HG, et al. The chromatin remodeling complex NuRD establishes the poised state of rRNA genes characterized by bivalent histone modifications and altered nucleosome positions. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;**109**(21):8161-8166. DOI: 10.1073/pnas.1201262109
- [40] Grandori C, Gomez-Roman N, Felton-Edkins ZA, Ngouenet C, Galloway DA, Eisenman RN, et al. c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. *Nature Cell Biology*. 2005;**7**(3):311-318. DOI: 10.1038/ncb1224
- [41] Poortinga G, Hannan KM, Snelling H, Walkley CR, Jenkins A, Sharkey K, et al. MAD1 and c-MYC regulate UBF and rDNA transcription during granulocyte differentiation. *The EMBO Journal*. 2004;**23**(16):3325-3335. DOI: 10.1038/sj.emboj.7600335
- [42] Dai MS, Lu H. Crosstalk between c-Myc and ribosome in ribosomal biogenesis and cancer. *Journal of Cellular Biochemistry*. 2008;**105**(3):670-677. DOI: 10.1002/jcb.21895
- [43] Poortinga G, Wall M, Sanij E, Siwicki K, Ellul J, Brown D, et al. c-MYC coordinately regulates ribosomal gene chromatin remodeling and Pol I availability during granulocyte differentiation. *Nucleic Acids Research*. 2011;**39**(8):3267-3281. DOI: 10.1093/nar/gkq1205

- [44] Schmidt EV. The role of c-myc in cellular growth control. *Oncogene*. 1999;**18**(19):2988-2996. DOI: 10.1038/sj.onc.1202751
- [45] van Riggelen J, Yetil A, Felsher DW. MYC as a regulator of ribosome biogenesis and protein synthesis. *Nature Reviews. Cancer* 2010;**10**(4):301-309. DOI: 10.1038/nrc2819
- [46] Cavanaugh AH, Hempel WM, Taylor LJ, Rogalsky V, Todorov G, Rothblum LI. Activity of RNA polymerase I transcription factor UBF blocked by Rb gene product. *Nature*. 1995;**374**(6518):177-180. DOI: 10.1038/374177a0
- [47] Voit R, Schafer K, Grummt I. Mechanism of repression of RNA polymerase I transcription by the retinoblastoma protein. *Molecular and Cellular Biology* 1997;**17**(8):4230-4237. DOI: absent
- [48] Hannan KM, Hannan RD, Smith SD, Jefferson LS, Lun M, Rothblum LI. Rb and p130 regulate RNA polymerase I transcription: Rb disrupts the interaction between UBF and SL-1. *Oncogene*. 2000;**19**(43):4988-4999. DOI: 10.1038/sj.onc.1203875
- [49] Pelletier G, Stefanovsky VY, Faubladier M, Hirschler-Laszkiewicz I, Savard J, Rothblum LI, et al. Competitive recruitment of CBP and Rb-HDAC regulates UBF acetylation and ribosomal transcription. *Molecular Cell*. 2000;**6**(5):1059-1066. DOI: S1097-2765(00)00104-0
- [50] Zhai W, Comai L. Repression of RNA polymerase I transcription by the tumor suppressor p53. *Molecular and Cellular Biology* 2000;**20**(16):5930-5938. DOI: absent
- [51] Zhang C, Comai L, Johnson DL. PTEN represses RNA polymerase I transcription by disrupting the SL1 complex. *Molecular and Cellular Biology*. 2005;**25**(16):6899-6911. DOI: 10.1128/MCB.25.16.6899-6911.2005
- [52] Al-Khouri AM, Ma Y, Togo SH, Williams S, Mustelin T. Cooperative phosphorylation of the tumor suppressor phosphatase and tensin homologue (PTEN) by casein kinases and glycogen synthase kinase 3beta. *The Journal of Biological Chemistry*. 2005;**280**(42):35195-35202. DOI: 10.1074/jbc.M503045200
- [53] Vincent T, Kukalev A, Andang M, Pettersson R, Percipalle P. The glycogen synthase kinase (GSK) 3beta represses RNA polymerase I transcription. *Oncogene*. 2008;**27**(39):5254-5259. DOI: 10.1038/onc.2008.152
- [54] Johnson GL, Nakamura K. The c-jun kinase/stress-activated pathway: Regulation, function and role in human disease. *Biochimica et Biophysica Acta*. 2007;**1773**(8):1341-1348. DOI: 10.1016/j.bbamcr.2006.12.009
- [55] Mayer C, Bierhoff H, Grummt I. The nucleolus as a stress sensor: JNK2 inactivates the transcription factor TIF-IA and down-regulates rRNA synthesis. *Genes & Development*. 2005;**19**(8):933-941. DOI: 10.1101/gad.333205
- [56] Larsen DH, Hari F, Clapperton JA, Gwerder M, Gutsche K, Altmeyer M, et al. The NBS1-treacle complex controls ribosomal RNA transcription in response to DNA damage. *Nature Cell Biology*. 2014;**16**(8):792-803. DOI: 10.1038/ncb3007

- [57] Kruhlak M, Crouch EE, Orlov M, Montano C, Gorski SA, Nussenzweig A, et al. The ATM repair pathway inhibits RNA polymerase I transcription in response to chromosome breaks. *Nature*. 2007;**447**(7145):730-734. DOI: 10.1038/nature05842
- [58] Johnston R, D'Costa Z, Ray S, Gorski J, Harkin DP, Mullan P, et al. The identification of a novel role for BRCA1 in regulating RNA polymerase I transcription. *Oncotarget*. 2016;**7**(42):68097-68110. DOI: 10.18632/oncotarget.11770
- [59] Zhao Z, Dammert MA, Hoppe S, Bierhoff H, Grummt I. Heat shock represses rRNA synthesis by inactivation of TIF-IA and lncRNA-dependent changes in nucleosome positioning. *Nucleic Acids Research*. 2016;**44**(17):8144-8152. DOI: 10.1093/nar/gkw496
- [60] Zhao Z, Dammert MA, Grummt I, Bierhoff H. lncRNA-induced nucleosome repositioning reinforces transcriptional repression of rRNA genes upon hypotonic stress. *Cell Reports*. 2016;**14**(8):1876-1882. DOI: 10.1016/j.celrep.2016.01.073
- [61] Wu TH, Kuo YY, Lee HH, Kuo JC, Ou MH, Chang ZF. Epigenetic repression of ribosomal RNA transcription by ROCK-dependent aberrant cytoskeletal organization. *Scientific Reports*. 2016;**6**:28685. DOI: 10.1038/srep28685
- [62] Moor AE, Golan M, Massasa EE, Lemze D, Weizman T, Shenhav R, et al. Global mRNA polarization regulates translation efficiency in the intestinal epithelium. *Science*. 2017;**357**(6357):1299-1303. DOI: 10.1126/science.aan2399
- [63] Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. *Nature Reviews. Molecular Cell Biology*. 2009;**10**(5):307-318. DOI: 10.1038/nrm2672
- [64] Gonzalez A, Hall MN. Nutrient sensing and TOR signaling in yeast and mammals. *The EMBO Journal*. 2017;**36**(4):397-408. DOI: 10.15252/embj.201696010
- [65] Grummt I, Smith VA, Grummt F. Amino acid starvation affects the initiation frequency of nucleolar RNA polymerase. *Cell*. 1976;**7**(3):439-445. DOI: 0092-8674(76)90174-4
- [66] Yuan X, Zhao J, Zentgraf H, Hoffmann-Rohrer U, Grummt I. Multiple interactions between RNA polymerase I, TIF-IA and TAF(I) subunits regulate preinitiation complex assembly at the ribosomal gene promoter. *EMBO Reports*. 2002;**3**(11):1082-1087. DOI: 10.1093/embo-reports/kvf212
- [67] James MJ, Zomerdijk JC. Phosphatidylinositol 3-kinase and mTOR signaling pathways regulate RNA polymerase I transcription in response to IGF-1 and nutrients. *The Journal of Biological Chemistry*. 2004;**279**(10):8911-8918. DOI: 10.1074/jbc.M307735200
- [68] West MJ, Stoneley M, Willis AE. Translational induction of the c-myc oncogene via activation of the FRAP/TOR signalling pathway. *Oncogene*. 1998;**17**(6):769-780. DOI: 10.1038/sj.onc.1201990
- [69] Csibi A, Lee G, Yoon SO, Tong H, Ilter D, Elia I, et al. The mTORC1/S6K1 pathway regulates glutamine metabolism through the eIF4B-dependent control of c-Myc translation. *Current Biology*. 2014;**24**(19):2274-2280. DOI: 10.1016/j.cub.2014.08.007

- [70] Kang J, Kusnadi EP, Ogden AJ, Hicks RJ, Bammert L, Kutay U, et al. Amino acid-dependent signaling via S6K1 and MYC is essential for regulation of rDNA transcription. *Oncotarget*. 2016;**7**(31):48887-48904. DOI: 10.18632/oncotarget.10346
- [71] Grummt I, Grummt F. Control of nucleolar RNA synthesis by the intracellular pool sizes of ATP and GTP. *Cell*. 1976;**7**(3):447-453. DOI: 0092-8674(76)90175-6
- [72] Nguyen le XT, Lee Y, Urbani L, Utz PJ, Hamburger AW, Sunwoo JB, et al. Regulation of ribosomal RNA synthesis in T cells: Requirement for GTP and Ebp1. *Blood*. 2015;**125**(16):2519-2529. DOI: 10.1182/blood-2014-12-616433
- [73] Squatrito M, Mancino M, Donzelli M, Areces LB, Draetta GF. EBP1 is a nucleolar growth-regulating protein that is part of pre-ribosomal ribonucleoprotein complexes. *Oncogene*. 2004;**23**(25):4454-4465. DOI: 10.1038/sj.onc.1207579
- [74] Mariappan MM, D'Silva K, Lee MJ, Sataranatarajan K, Barnes JL, Choudhury GG, et al. Ribosomal biogenesis induction by high glucose requires activation of upstream binding factor in kidney glomerular epithelial cells. *American Journal of Physiology. Renal Physiology*. 2011;**300**(1):F219-F230. DOI: 10.1152/ajprenal.00207.2010
- [75] Zhai N, Zhao ZL, Cheng MB, Di YW, Yan HX, Cao CY, et al. Human PIH1 associates with histone H4 to mediate the glucose-dependent enhancement of pre-rRNA synthesis. *Journal of Molecular Cell Biology*. 2012;**4**(4):231-241. DOI: 10.1093/jmcb/mjs003
- [76] Zhou Y, Grummt I. The PHD finger/bromodomain of NoRC interacts with acetylated histone H4K16 and is sufficient for rDNA silencing. *Current Biology*. 2005;**15**(15):1434-1438. DOI: 10.1016/j.cub.2005.06.057
- [77] Kamano Y, Saeki M, Egusa H, Kakihara Y, Houry WA, Yatani H, et al. PIH1D1 interacts with mTOR complex 1 and enhances ribosome RNA transcription. *FEBS Letters*. 2013;**587**(20):3303-3308. DOI: 10.1016/j.febslet.2013.09.001
- [78] Zhang CS, Hawley SA, Zong Y, Li M, Wang Z, Gray A, et al. Fructose-1,6-bisphosphate and aldolase mediate glucose sensing by AMPK. *Nature*. 2017;**548**(7665):112-116. DOI: 10.1038/nature23275
- [79] Hoppe S, Bierhoff H, Cado I, Weber A, Tiebe M, Grummt I, et al. AMP-activated protein kinase adapts rRNA synthesis to cellular energy supply. *Proceedings of the National Academy of Sciences of the United States of America*. 2009;**106**(42):17781-17786. DOI: 10.1073/pnas.0909873106
- [80] Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, et al. Histone demethylation by a family of JmjC domain-containing proteins. *Nature*. 2006;**439**(7078):811-816. DOI: 10.1038/nature04433
- [81] Tanaka Y, Okamoto K, Teye K, Umata T, Yamagiwa N, Suto Y, et al. JmjC enzyme KDM2A is a regulator of rRNA transcription in response to starvation. *The EMBO Journal*. 2010;**29**(9):1510-1522. DOI: 10.1038/emboj.2010.56

- [82] Tanaka Y, Umata T, Okamoto K, Obuse C, Tsuneoka M. CxxC-ZF domain is needed for KDM2A to demethylate histone in rDNA promoter in response to starvation. *Cell Structure and Function*. 2014;**39**(1):79-92. DOI: 10.1247/csf.13022
- [83] Tanaka Y, Yano H, Ogasawara S, Yoshioka S, Imamura H, Okamoto K, et al. Mild glucose starvation induces KDM2A-mediated H3K36me2 Demethylation through AMPK to reduce rRNA transcription and cell proliferation. *Molecular and Cellular Biology*. 2015;**35**(24):4170-4184. DOI: 10.1128/MCB.00579-15
- [84] Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell*. 2003;**115**(5):577-590. DOI: 10.1016/S0092-8674(03)00929-2
- [85] Yang L, Song T, Chen L, Kabra N, Zheng H, Koomen J, et al. Regulation of SirT1-nucleomethylin binding by rRNA coordinates ribosome biogenesis with nutrient availability. *Molecular and Cellular Biology*. 2013;**33**(19):3835-3848. DOI: 10.1128/MCB.00476-13
- [86] Song T, Yang L, Kabra N, Chen L, Koomen J, Haura EB, et al. The NAD⁺ synthesis enzyme nicotinamide mononucleotide adenylyltransferase (NMNAT1) regulates ribosomal RNA transcription. *The Journal of Biological Chemistry*. 2013;**288**(29):20908-20917. DOI: 10.1074/jbc.M113.470302
- [87] Chen S, Seiler J, Santiago-Reichert M, Felbel K, Grummt I, Voit R. Repression of RNA polymerase I upon stress is caused by inhibition of RNA-dependent deacetylation of PAF53 by SIRT7. *Molecular Cell*. 2013;**52**(3):303-313. DOI: 10.1016/j.molcel.2013.10.010
- [88] 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 & Development*. 2006;**20**(9):1075-1080. DOI: 10.1101/gad.1399706
- [89] Hanada K, Song CZ, Yamamoto K, Yano K, Maeda Y, Yamaguchi K, et al. RNA polymerase I associated factor 53 binds to the nucleolar transcription factor UBF and functions in specific rDNA transcription. *The EMBO Journal* 1996;**15**(9):2217-2226. DOI: absent
- [90] Meraner J, Lechner M, Loidl A, Goralik-Schramel M, Voit R, Grummt I, et al. Acetylation of UBF changes during the cell cycle and regulates the interaction of UBF with RNA polymerase I. *Nucleic Acids Research*. 2006;**34**(6):1798-1806. DOI: 10.1093/nar/gkl101
- [91] Rothblum LI, Rothblum K, Chang E. PAF53 is essential in mammalian cells: CRISPR/Cas9 fails to eliminate PAF53 expression. *Gene*. 2017;**612**:55-60. DOI: 10.1016/j.gene.2016.12.023
- [92] Wong CC, Qian Y, Yu J. Interplay between epigenetics and metabolism in oncogenesis: Mechanisms and therapeutic approaches. *Oncogene*. 2017;**36**(24):3359-3374. DOI: 10.1038/onc.2016.485
- [93] Kaelin WG, Jr., McKnight SL. Influence of metabolism on epigenetics and disease. *Cell* 2013;**153**(1):56-69. DOI: 10.1016/j.cell.2013.03.004

- [94] Krautkramer KA, Rey FE, Denu JM. Chemical signaling between gut microbiota and host chromatin: What is your gut really saying? *The Journal of Biological Chemistry*. 2017;**292**(21):8582-8593. DOI: 10.1074/jbc.R116.761577
- [95] Wang T, Yu Q, Li J, Hu B, Zhao Q, Ma C, et al. O-GlcNAcylation of fumarase maintains tumour growth under glucose deficiency. *Nature Cell Biology*. 2017;**19**(7):833-843. DOI: 10.1038/ncb3562
- [96] Losman JA, Kaelin WG, Jr. What a difference a hydroxyl makes: Mutant IDH, (R)-2-hydroxyglutarate, and cancer. *Genes & Development* 2013;**27**(8):836-852. DOI: 10.1101/gad.217406.113
- [97] Chowdhury R, Yeoh KK, Tian YM, Hillringhaus L, Bagg EA, Rose NR, et al. The onco-metabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Reports*. 2011;**12**(5):463-469. DOI: 10.1038/embor.2011.43
- [98] Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. *Cancer Cell*. 2011;**19**(1):17-30. DOI: 10.1016/j.ccr.2010.12.014
- [99] Tong L, Denu JM. Function and metabolism of sirtuin metabolite O-acetyl-ADP-ribose. *Biochimica et Biophysica Acta*. 2010;**1804**(8):1617-1625. DOI: 10.1016/j.bbapap.2010.02.007
- [100] Burgos-Barragan G, Wit N, Meiser J, Dingler FA, Pietzke M, Mulderigg L, et al. Mammals divert endogenous genotoxic formaldehyde into one-carbon metabolism. *Nature*. 2017;**548**(7669):549-554. DOI: 10.1038/nature23481
- [101] Ruggero D. Revisiting the nucleolus: From marker to dynamic integrator of cancer signaling. *Science Signaling*. 2012;**5**(241):pe38. DOI: 10.1126/scisignal.2003477
- [102] Quin JE, Devlin JR, Cameron D, Hannan KM, Pearson RB, Hannan RD. Targeting the nucleolus for cancer intervention. *Biochimica et Biophysica Acta*. 2014;**1842**(6):802-816. DOI: 10.1016/j.bbadis.2013.12.009
- [103] Jordan P, Carmo-Fonseca M. Cisplatin inhibits synthesis of ribosomal RNA in vivo. *Nucleic Acids Research*. 1998;**26**(12):2831-2836. DOI: gkb492
- [104] Rey JP, Scott R, Muller H. Induction and removal of interstrand crosslinks in the ribosomal RNA genes of lymphoblastoid cell lines from patients with Fanconi anemia. *Mutation Research*. 1993;**289**(2):171-180. DOI: 0027-5107(93)90067-P
- [105] Ghoshal K, Jacob ST. An alternative molecular mechanism of action of 5-fluorouracil, a potent anticancer drug. *Biochemical Pharmacology*. 1997;**53**(11):1569-1575. DOI: S0006-2952(97)00040-3
- [106] Turan T, Karacay O, Tulunay G, Boran N, Koc S, Bozok S, et al. Results with EMA/CO (etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine) chemotherapy in gestational trophoblastic neoplasia. *International Journal of Gynecological Cancer*. 2006;**16**(3):1432-1438. DOI: 10.1111/j.1525-1438.2006.00606.x

- [107] D'Angio GJ, Evans A, Breslow N, Beckwith B, Bishop H, Farewell V, et al. The treatment of Wilms' tumor: Results of the second National Wilms' tumor study. *Cancer* 1981;**47**(9):2302-2311. DOI: absent
- [108] Khatua S, Nair CN, Ghosh K. Immune-mediated thrombocytopenia following dactinomycin therapy in a child with alveolar rhabdomyosarcoma: The unresolved issues. *Journal of Pediatric Hematology/Oncology*. 2004;**26**(11):777-779. DOI: 00043426-200411000-00020
- [109] Jaffe N, Paed D, Traggis D, Salian S, Cassady JR. Improved outlook for Ewing's sarcoma with combination chemotherapy (vincristine, actinomycin D and cyclophosphamide) and radiation therapy. *Cancer* 1976;**38**(5):1925-1930. DOI: absent
- [110] Uberti EM, Fajardo Mdo C, Ferreira SV, Pereira MV, Seger RC, Moreira MA, et al. Reproductive outcome after discharge of patients with high-risk hydatidiform mole with or without use of one bolus dose of actinomycin D, as prophylactic chemotherapy, during the uterine evacuation of molar pregnancy. *Gynecologic Oncology*. 2009;**115**(3):476 481. DOI: 10.1016/j.ygyno.2009.09.012
- [111] Drygin D, Lin A, Bliesath J, Ho CB, O'Brien SE, Proffitt C, et al. Targeting RNA polymerase I with an oral small molecule CX-5461 inhibits ribosomal RNA synthesis and solid tumor growth. *Cancer Research*. 2011;**71**(4):1418-1430. DOI: 10.1158/0008-5472.CAN-10-1728
- [112] Bywater MJ, Poortinga G, Sanij E, Hein N, Peck A, Cullinane C, et al. Inhibition of RNA polymerase I as a therapeutic strategy to promote cancer-specific activation of p53. *Cancer Cell*. 2012;**22**(1):51-65. DOI: 10.1016/j.ccr.2012.05.019
- [113] Negi SS, Brown P. rRNA synthesis inhibitor, CX-5461, activates ATM/ATR pathway in acute lymphoblastic leukemia, arrests cells in G2 phase and induces apoptosis. *Oncotarget*. 2015;**6**(20):18094-18104. DOI: 10.18632/oncotarget.4093
- [114] Xu H, Di Antonio M, McKinney S, Mathew V, Ho B, O'Neil NJ, et al. CX-5461 is a DNA G-quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours. *Nature Communications*. 2017;**8**:14432. DOI: 10.1038/ncomms14432
- [115] Peltonen K, Colis L, Liu H, Trivedi R, Moubarek MS, Moore HM, et al. A targeting modality for destruction of RNA polymerase I that possesses anticancer activity. *Cancer Cell*. 2014;**25**(1):77-90. DOI: 10.1016/j.ccr.2013.12.009