Mini Review

iMedPub Journals
http://www.imedpub.com

Biochemistry & Molecular Biology Journal
ISSN 2471-8084

2016
Vol. 2 No. 1: 4

DOI: 10.21767/2471-8084.100013

Management of rRNA Transcription Activity in a Human Genome

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Citation: Kupriyanova NS, Ryskov AP. Management of rRNA Transcription Activity in a Human Genome. Biochem Mol Biol J. 2016, 2:1.

Abstract

Protein synthesis is a fundamental cell process, performing by ribosomes. Ribosome biogenesis involves transcriptional and many post-transcriptional steps [1,2]. The control of cell growth is tightly connected with rRNA synthesis. In the human genome, rDNA clusters that comprise the so-called nucleolar organizers (NORs) span acrocentric chromosomes 13, 14, 15, 21, and 22. Moreover, many rDNA-similar segments can be detected on (NOR)-chromosomes. It is appreciated now days that the nucleolus plays a fundamental role in the regulation of molecular networks. RDNA consists of the zone coding functionally active rRNA promoting by RNA polymerase I (Pol I), and ribosomal intergenic spacer (rIGS). Today it is clear that rIGS comprises highly intricate structure consisting of a variety of functionally specific segments which have the property of activating under specific conditions, and can be easily transported to different genomic regions.

Although similarity is very high in structure and function of rDNA repeating units in all vertebrates, a number of specific features are inherent to humans and higher primates [3,4]. The current model for transcriptional regulation of rRNA proposes two overlapping mechanisms. For short-term regulation, the transcription rate at active rDNA is altered by reversible modification of Pol I transcription factors, whereas long-term regulation is mediated by epigenetic mechanisms when specific chromatin modifications alter the ratio of active to silent copies of rRNA genes. Transcription of rRNA genes and maturation of rRNA play a central role in the complex network that controls cell growth and proliferation. A body of evidence argues that changes in nucleolar organizer (rDNA) activity can be not a result of tumorigenesis, but a cause of it. In this review we try to assess varied factors affecting rRNA and non-coding RNA (ncRNA) transcription activity, and correlate these data with surprisingly high content in the genome (high mobility) of the short segments surrounding ncRNA regions in the rIGS.

Keywords: rRNA; Transcription; Genome; Protein synthesis; Tumorigenesis

Abbreviations: rRNA: Ribosomal RNA; rDNA: Ribosomal DNA, NORs: Nucleolar Organizers; (NOR)-Chromosomes: Non-Acrocentric Chromosomes; Pol I: RNA Polymerase I; rIGS: Ribosomal Intergenic Spacer; ncRNA: Non-Coding RNA; UBF: Upstream Binding Factor; SL1: Promoter Selectivity Factor; NoDS: Nucleolar Detention Signals

Received: March 16, 2016; **Accepted:** April 05, 2016; **Published:** April 10, 2016

rDNA Promoter Complex

Initiation of rDNA transcription requires assembly of a specific multiprotein complex at the rDNA promoter containing Pol I and a number of ancillary proteins. In humans, the joint action of two Pol I-specific factors that bind to the rDNA promoter, the upstream binding factor (UBF) [5], and the promoter selectivity factor, termed SL1leads to the assembly of the preinitiation complex. UBF activates rRNA gene transcription by recruiting Pol I to the rDNA promoter, by stabilizing binding of TIF-IB/SL1, and by displacing the histone H1 [6,7]. UBF can also regulate Pol I promoter escape [8] and transcription elongation [9].

In many cases, the proliferation rate of cancer cells is proportional to the level of UBF [10,11].

Once more group of proteins (TAFI) should be mentioned here. These proteins perform important tasks in transcription complex assembly, mediating specific interactions between the rDNA promoter and Pol I.

Short-term Regulation

Numerous proteins, including the growth-dependent transcription initiation factor TIF-IA, PAF53, protein kinase CK2, nuclear actin, and myosin (NM1), proteins involved in DNA repair and replication, such as topoisomerases I and IIα, Ku70/80, and many others were shown to be associated with Pol I. These proteins can modulate rRNA synthesis under the influence of intracellular and external conditions. Different mutations of the proteins associated with PolI in TIF-IA phosphorylation sites, (ERK for an example), modulation of TIF-IA activity under mTOR, the mammalian target of rapamycin. mTOR action can inactivate TIF-IA by decreasing phosphorylation at Ser44 and by enhancing phosphorylation at Ser199. These changes in TIF-IA phosphorylation impair transcription complex formation [12,13].

Protein Kinases Regulate rRNA Transcription

Whereas in normal cells the rate of rRNA synthesis is tightly linked to nutritional availability, tumor cells acquire self-sufficiency resulting from activation of downstream mediator kinases that is independent of extracellular signaling events. Several protein kinases, including CK2, ERK, and mTOR, have been shown to be hyperactivated during carcinogenesis. Pol I–associated CK2 phosphorylates several components of the Pol I transcription machinery, including TIF-IA, UBF, SL1/TIF-IB, and topoisomerase IIα. MAPKs were found to activate rRNA synthesis by targeting the transcription factors TIF-IA and UBF. This signaling pathway is frequently hyperactivated in cancer cells. Since inhibition of mTOR signaling by rapamycin inactivates TIF-IA, it is not surprising that mTOR inhibitors act as powerful tumor-suppressive drugs [14].

Ribosome Function (rRNA biogenesis) and Cancer

The question remains as to whether the deregulation of rRNA synthesis itself could trigger cell transformation [15,16] or whether increased rRNA synthesis plays a secondary, but necessary, part in tumorigenesis. Potential targets for anticancer therapeutic strategy are protein kinases, such as ERK/RSK, mTOR, and CK2, which are often hyperactivated in cancer cells and are known to be required for rRNA transcription. Several approved anticancer drugs have been shown to inhibit rRNA synthesis, albeit not necessarily with the required selectivity.

There is a growing list of additional factors with oncogenic and tumor suppressor activity implicated in the modulation of RNA Pol I during malignancy [17].

Although there are clearly multiple ways to inhibit Pol I transcription and pre-rRNA processing, however the vast majority described above do it in a nonselective way. Cylene Pharmaceuticals identified a small molecule that selectively inhibited Pol I transcription, CX5461 [18]. CX5461 inhibits Pol I transcription at the initiation step by interfering with SL1/rDNA promoter binding. *In vitro* characterization identified cell lines derived from hematologic malignancies with those possessing wild-type p53 being particularly susceptible to CX5461. Normal cells were found to be resistant.

Long-term Regulation

Recent studies have shown that long-term regulation is mediated not only by specific chromatin modifications which alter the ratio of active to silent copies of rRNA genes. Analysis of the IGS region 2 kb upstream of the rRNA start site identified a 150– 250 nucleotide PolI-mediated transcript, known as the promoter-associated RNA (pRNA). This molecule was shown to be involved in targeting TIP5, the large subunit of the nucleolar remodeling complex (NoRC) that ultimately inhibits rRNA synthesis [18,19].

Through the timely induction of various ribosomal IGS noncoding RNA (IGS RNA) transcripts, the cell is capable of both regulating rRNA synthesis and sequestering large numbers of proteins, thereby modulating essential molecular networks.

As is the case with most metabolically important genes, transcription of rRNA can be modulated in response to a variety of cellular and pathological stimuli including amino acid starvation, aging, viral infection or toxic lesions. The plurifunctional nature of the nucleolus is evident in its response to physiological stimuli and stress conditions accompanied by nucleolar remodeling, and a decrease or interruption in rRNA synthesis [20,21] as well as the capture of numerous seemingly unrelated factors involved in a wide array of cellular functions [22-25].

Currently, there is an evidence of the presence of several inducible noncoding rather long nucleolus RNA molecules derived from stimuli-specific loci located within the ribosomal intergenic spacer (rIGSlncRNAs). Four stimuli-specific loci producing their own RNAs are currently denoted in the rIGS. They were termed IGS27RNA, IGS22RNA, IGS16RNA, and IGS pRNA [26-28]. Functional studies of these molecules have revealed new mechanisms for the regulation of rRNA expression, as well as a novel posttranslation regulatory pathway termed the nucleolar detention pathway. All proteins captured by rIGSlncRNAs contain the nucleolar detention signal (NoDS),i.e., a position-independent consensus sequence, which consists of at least one arginine motif (RRI/L) and a minimum of two hydrophobic triplets (LhL/v, where h represents a hydrophobic residue)[26]. These molecules have diverse functions in ubiquitination, proteasomal degradation, protein folding, and DNA replication and methylation, indicating that the NoDS may control important aspects of cellular life [27].

The correlation of the fixed 5'- and 3'-ends' number on (NOR)–chromosomes, and their distribution in the IGS27RNA, IGS22RNA, and IGS16RNA areas help to detect local nucleotides in the rIGS that are maximally susceptible to a breakage [28].

Accession numbers have been already assigned to IGS27RNA and IGS22RNA (GenBank accession nos. JN872552–JN872556, and JN872557–JN872559, respectively) [26]. All GenBank accession

numbers for IGS27RNA and for IGS22RNA are represented by practically identical sequences. The positions of the fixed 5′- and 3′-ends that are most frequent in the IGS27RNA and IGS22RNA areas are presented by 27,604–27,840 bp and 21,600–21,900 bp, respectively [28].

By fixating of the 5′ and 3′ ends' position of the rIGS – like segments on (NOR) − chromosomes, it is possible to find the regions and local nucleotides in the rIGS that are maximally often susceptible to breakage. Short rIGS segments that are not only scattered throughout (NOR) − chromosomes, but also overlap annotated genes are of more direct interest. The sequences surrounding potential break points are often represented by microsatellites, (TC)n, (TG)n, and short (3–5 bp) poly-N clusters. However, a similarity between fragments is mainly provided by extremely mutable Alu repeats, and simple sequences [29].

There is no proof that breakpoints are mandatory for Alu elements, being not specific for rIGS. On the contrary, not all Alu elements are predisposed to breaks. There are a lot of examples of Alus repeats that are almost avoided of potential break points. The most active break points are usually grouped upstream and downstream of full-sized, and shortened mutated Alu repeats insertions, which are numerous in the rIGS.

Conclusion

Although significant advances have been made toward understanding the regulation of Pol I transcription, the question remains as to whether the deregulation of rRNA synthesis itself could trigger cell transformation or whether increased rRNA synthesis plays a secondary, but necessary, part in tumorigenesis. Therefore, changes in pre-rRNA transcription and processing that accompany or precede malignant transformation are not only of great scientific interest, but offer unique possibilities to combat cancer by selectively targeting proteins. The concept of targeting key components of the machineries that produce rRNA seems quite obvious. Although the area of targeting anticancer drugs to the Pol I transcription machinery is still in its infancy, it promises to be a provocative and emerging field.

The challenge is now to develop new classes of improved targeted strategies to selectively inhibit Pol I transcription in rapidly proliferating cells and to eliminate cancer cells without harming healthy tissues or organs. Furthermore, it was found that stimuli-specific rIGS loci, and breakpoint-enriched loci are surrounded by analogous repetitive elements (microsatellites, simple sequences, and fragments of Alu elements). It should be recalled here that many repetitive DNA sequences tend to form nucleoli-associated domains in human cells [30]. These results let us to propose that when chromosomal regions saturated by repetitive DNA sequences are brought close to the nucleolus envelope, they can meet rIGS loci producing ncRNAs. Their bringing together may favor recombination events. It has also been noticed that some NORs were situated on elongated chromatin protrusions that connect nucleoli with respective chromosome territories that are spatially distanced from nucleoli [31].

Acknowledgment

This study was partly supported by RFBR (project no 16-04-00178), the RAS Program “Molecular and Cell Biology”, and the RF President’s Program (no 86-12.2016.4).

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