A new paradigm for the regulation of the mammalian ribosomal RNA genes

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Abstract
Ribosome assembly occurs co-transcriptionally on the rRNA genes. This process requires the co-ordinated expression and assembly of many hundreds of proteins and is finely tuned to cell and organism growth. Co-ordinate regulation of the rRNA genes and the ribosomal protein genes is therefore essential for high-fidelity ribosome assembly. Recent work shows that RNA gene transcription is regulated at the level of elongation via the mitogen-activated protein kinase pathway. We argue that this may provide an explanation for the high fidelity of ribosome assembly.

Introduction
When so much emphasis is being placed on the role of protein coding genes, it is a sobering thought that a eukaryotic cell must provide on average ten ribosomes for every mRNA it synthesizes. Cytoplasmic ribosomes account for approx. 80% of total cellular RNA. Each ribosome is some 4 Md in weight and consists of four RNAs, the 18 S, 5.8 S, 28 S and 5 S rRNAs, and 80 or so proteins. Hence, ribosome biogenesis is the major limit to growth and proliferation. In the present paper, we will briefly review the mechanisms that co-ordinate production of the rRNAs with growth in mammalian cells.

The RNA genes encode the three largest RNAs of the ribosome, as part of a single precursor, in mammals called the 47 S RNA. Initial assembly of the ribosome occurs co-transcriptionally with 47 S rRNA synthesis, leading to a 90 S precursor particle, a process elegantly visualized in the ‘Miller-Spread’ electron micrographs [1]. Structural studies of the ribosome suggest that this 5’ to 3’ stepwise co-transcriptional assembly is important in establishing the complex secondary structure of the mature rRNAs and in positioning the r-proteins [2,3].

Transcription initiation on the rRNA genes
In eukaryotes, a dedicated set of proteins is used to transcribe the rRNA genes, allowing the cell to regulate these genes independently of the rest of the genome. The α-amanatin-resistant DNA-dependent RPI (RNA polymerase I) is dedicated solely to the transcription of the rRNA genes. In mammals, formation of an RPI pre-initiation complex requires SL1 (selectivity factor 1; or mTIF-IB) and an HMG1-box [DNA-binding motif homologous with those of HMG1 (high mobility group protein 1)] DNA-binding protein, UBF (upstream binding factor), see Figure 1. Originally it was believed that UBF specifically bound the upstream and/or core promoter regions, creating a situation propitious for the SL1 complex to bind and form a ‘stable’ pre-initiation complex [4]. In vitro, this complex is able to recruit RPI and, in the presence of nucleotide triphosphates, to initiate transcription. When it was discovered that the N-terminal half of UBF (core UBF) could form the ‘enhancesome’, a nucleoprotein structure that somewhat resembles the nucleosome, the finding led to a speculative explanation for the co-operativity between UBF and SL1 [5–8]. In this model, UBF juxtaposes the two promoter domains, presenting the SL1-binding sites on the same side of a DNA superhelix, see Figure 1. However, it was later found that UBF does not restrict itself to the RPI promoter but is found throughout the rDNA repeat [9]. This suggests that UBF defines a specialized rRNA gene chromatin and is consistent with its indiscriminate DNA-binding properties [7].

Growth factor regulation of the rRNA genes
Unlike protein coding genes, expression of the rRNA genes can only be regulated at four distinct levels: gene activation, transcription initiation, transcription elongation and rRNA degradation. Since the rRNA genes exist in tandem arrays of several hundred copies, the number of active genes could in principle vary. However, in mammalian cell culture, several-fold changes in rRNA synthesis can be observed without a detectable change in active gene number [10]. Long-term serum withdrawal and cycloheximide and glucocorticoid treatments have demonstrated that the ability of RPI to initiate transcription, as measured in vitro, is regulated via phosphorylation of mammalian Rrn3/TIF-IA, a protein essential for promoter-directed RPI initiation [11–14], see Figure 1. These results led to claims that rRNA gene regulation occurred exclusively at the level of transcription initiation via regulation of Rrn3/TIF-IA [15].

Despite intense study of the regulation of RPI and Rrn3 activities, the first molecular pathway linking growth

Key words: growth factor, mammalian cell, ribosomal RNA gene, RNA polymerase I, RNA transcription elongation.
Abbreviations used: CREB, cAMP-response-element-binding protein; CBP, CREB-binding protein; ERK, extracellular-signal-regulated kinase; HMG1, high mobility group protein 1; HMG1-box, DNA-binding motif homologous with those of HMG1; MAPK, mitogen-activated protein kinase; RPI, RNA polymerase I; RNP, ribonucleoprotein; SL1, selectivity factor 1; UBF, upstream binding factor.
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factor signalling to rRNA gene transcription came from a study of UBF [16]. Response to stimulation of human and mouse cells by EGF (epidermal growth factor) and by direct activation of the Raf–MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase]–ERK pathway was shown to require the phosphorylation of UBF. The two N-terminal HMG-boxes of UBF display consensus ERK phosphorylation sites on their DNA-binding surfaces. Phosphorylation of these sites was shown to be required for growth factor stimulation of rRNA gene transcription. Given that UBF binds throughout the rRNA genes, these results led us to speculate that UBF might regulate transcription elongation [13,14,16].

Growth factors target RPI elongation

If rRNA gene transcription is regulated solely via changes in the transcription initiation rate, the number of polymerase molecules engaged in transcribing the rRNA genes should be directly proportional to the rate of rRNA synthesis. This led us to ask whether an increase in RPI loading on the rRNA genes did in fact occur during growth factor induction of the rRNA genes [10,17]. What we found clearly contradicted a simple model of initiation regulation. Using both nuclear run-on and chromatin immunoprecipitation approaches, we established that growth factor and MAPK activation of rRNA synthesis in human and mouse cells does not correlate with a significant increase in the total number of RPI transcription complexes [10,17]. Indeed, it was difficult to detect any direct correlation between rRNA synthesis levels and the RPI loadings on the genes, strong suppression of synthesis often showing the highest loading levels. Clearly then, rRNA synthesis is not regulated simply by modulating the rate of transcription initiation. The obvious explanation was that RPI transcription elongation rates must also be regulated. This we confirmed using a novel approach to measure in vivo RPI elongation rates under different conditions of growth stimulation [17]. We found that changes in RPI elongation rate quantitatively explain the full dynamic range of growth factor regulation.

To maintain the constant level of RPI engagement we observe over a wide range of rRNA synthesis rates, either (i) elongation and initiation must be exactly co-ordinately regulated or (ii) elongation must limit the rate of initiation of new transcripts. Of these two possibilities, the latter would appear much the simpler mechanistically, since by limiting elongation rates, initiation would be limited in exact proportion. Our observations that a block to MAPK activation gave an increase, albeit slight, in RPI loading while causing a drastic reduction in rRNA synthesis suggested that the reduced elongation rate was backing-up polymerases and preventing more rapid initiation [17]. Hence, it appeared from these results that elongation was dominant over initiation. In fact, we have now shown that the capacity of cell-free extracts to specifically initiate UBF-independent RPI transcription in vitro does not change when the cells were stimulated by growth factor or MAPK activation was blocked.
immediately prior to preparing the extracts (F. Langlois and T. Moss, unpublished work). Thus, 5–6-fold changes in rRNA synthesis can occur without detectable changes in the activities of SL1, RPI or Rrn3. Under these conditions, elongation must then be the dominant factor in growth factor regulation of the rRNA genes. We have now shown that growth rates of various human colon cancer cell lines are also quantitatively explained by changes in RPI elongation rate (T. Gagnon–Kugler and T. Moss, unpublished work), suggesting that regulation of elongation is a general phenomenon in unstressed mammalian cells.

The mechanism of growth factor regulation of transcription elongation

Some years ago, we demonstrated that UBF enhances RPI transcription in vivo dependent on a reversible ERK1/2 phosphorylation of its two N-terminal HMG1-boxes [16]. Given that UBF forms an rRNA gene chromatin through which RPI must transcribe, we suggested that UBF phosphorylation might regulate transcription elongation on these genes. We had shown that each of the three N-terminal-most HMG1-boxes of UBF is able to bend DNA and that a dimer of UBF induces a 360° loop in the DNA template [6–8], see Figure 1. The resulting enhancesome structure resembles the chromatin nucleosoma in its protein/DNA ratio, but induces only a single gyre in the DNA, see Figure 1. In a recent study, we showed that ERK phosphorylation of UBF leads to an unwinding of the enhancesome [18]. Thus it seemed reasonable to hypothesize that this remodelling of the enhancesome could be the means by which RPI elongation is regulated. To our surprise, we found that when DNA templates were fully engaged by UBF, RPI elongation was strongly inhibited. ERK phosphorylation of UBF fully released this inhibition while at the same time unfold the enhancesome [17,18].

A new paradigm for growth factor regulation

The above results allowed us to establish a new paradigm for RPI transcription regulation. Active rRNA genes are associated with UBF and, hence, are predominantly in an enhancesomal conformation, see Figure 1(C). ERK phosphorylation of UBF causes local remodelling of the enhancesome and permits RPI to transcribe through the UBF-bound DNA. We have shown that ERK binds directly to the HMG1-boxes of UBF it phosphorylates, suggesting that it may be recruited to the rRNA gene chromatin directly. The process of remodelling rRNA gene chromatin may need more than just ERK phosphorylation, since we have shown that acetylation of the rRNA gene chromatin by the histone acetyltransferase CBP [CREB (cAMP-response-element-binding protein)-binding protein] is also able to enhance RPI transcription [19]. These results are all the more tantalizing when one realizes that CBP is recruited to the same two HMG1-boxes as is ERK and that enhancement of RPI transcription by acetylation is completely dependent on ERK activity [10,19].

Further implications of the new paradigm

The demonstration that the RPI transcription elongation rate is a dominant regulator of rRNA synthesis suggests a mechanism for the co-ordinate regulation of pre-ribosome assembly. During co-transcriptional process, r-proteins and assembly factors must arrive on the nascent rRNA at exactly the right moment in order to be correctly positioned and ensure appropriate RNA folding. If RPI elongation were to also depend on correct RNP (ribonucleoprotein) assembly, this would provide a means of co-ordinating rRNA and r-protein synthesis. It would also provide an important means of ‘proof-reading’ pre-ribosome assembly. Consistent with these possibilities, studies of the processome, the earliest visible co-transcriptional RNP structure, suggest that its assembly is required for efficient rRNA synthesis in yeast [20].

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References


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