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Transcription factor regulation of ribosomal RNA in hematopoiesis

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Abstract

Purpose of review—Ribosomal RNAs (rRNAs) are transcribed within nucleoli from rDNA repeats by RNA Polymerase I (Pol I). There is variation in rRNA transcription rates across the hematopoietic tree, and leukemic blast cells have prominent nucleoli, indicating abundant ribosome biogenesis. The mechanisms underlying these variations are poorly understood. The purpose of this review is to summarize findings of rDNA binding and Pol I regulation by hematopoietic transcription factors.

Recent findings—Our group recently used custom genome assemblies optimized for human and mouse rDNA mapping to map nearly 2200 ChIP-Seq datasets for nearly 250 factors to rDNA, allowing us to identify conserved occupancy patterns for multiple transcription factors. We confirmed known rDNA occupancy of MYC and RUNX factors, and identified new binding sites for CEBP factors, IRF factors, and SPI1 at canonical motif sequences. We also showed that CEBPA degradation rapidly leads to reduced Pol I occupancy and nascent rRNA in mouse myeloid cells.

Summary—We propose that a number of hematopoietic transcription factors bind rDNA and potentially regulate rRNA transcription. Our model has implications for normal and malignant hematopoiesis. This review summarizes the literature, and outlines experimental considerations to bear in mind while dissecting transcription factor roles on rDNA.

Keywords

CEBPA; hematopoiesis; leukemia; ribosome; rRNA; transcription factor

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

The hematopoietic system, like any major organ system, is composed of cells of varying sizes, functions, and proliferation rates [1,2■■,3■]. Each cell type has a tightly regulated number of ribosomes, and it is theorized that ribosome concentration is a crucial determinant of how a cell's complex transcriptome, comprising mRNAs with a range of translation initiation efficiencies, is translated into its appropriate proteome [4]. Ribosomes are ribonucleoprotein machines composed of four ribosomal RNAs (rRNAs) and nearly 80 ribosomal proteins; altered ribosome abundance can result from altered levels of rRNAs, ribosomal proteins, or over 200 additional proteins that are required for appropriate ribosomal folding, packaging, and quality control [5]. The relevance of ribosomal proteins to hematopoietic biology and disease is widely appreciated; Diamond-Blackfan anemia is caused by germline haploinsufficiency of over 20 ribosomal protein proteins, and ribosomal protein somatic deletions or mutations are observed in myeloid and lymphoid malignancies [6]. Less well appreciated is the relevance of rRNAs and the regulation of their transcription, despite the fact that rRNAs form over half of the mass of the ribosome and account for over 80% of total cellular RNA.

The four rRNAs, in descending size, are 28S, 18S, 5.8S, and 5S rRNA, the first three of which are transcribed as a single 13.4-kb nascent 47S pre-rRNA by RNA Polymerase I (Pol I), while 5S is transcribed by RNA Pol III [1]. 47S pre-rRNA is transcribed from specialized repeats known as rDNA genes, which are present in 200–600 copies in tandem arrays across 5 chromosomes (Fig. 1). These copies are near-identical, each nearly 45 kb in length, and each containing promoters, a 13.4-kb transcribed region and an nearly 30-kb intergenic spacer (IGS). This short review will focus only on recent advances in our understanding of 47S transcription in hematopoiesis, and not on processing or modification of rRNAs, the role of ribosomal proteins, or factors controlling the translational roles of mature subunits, all of which merit their own detailed consideration beyond our current scope.

Mammalian rDNA transcription machinery

Each rDNA repeat has two promoters spaced nearly 800nt apart in humans and nearly 2 kb apart in mouse [3■,7] (Fig. 1). The downstream promoter (47S promoter) is the one from which 47S pre-rRNA transcription begins, and the upstream promoter (Spacer promoter), though conserved, has unknown functions. In any given cell, a subset of rDNA repeats is activated by occupancy of the promoters and the 13.4-kb transcribed region by the HMG box protein UBTF (aka UBF), accompanied by depletion of nucleosomes across the stretch [1,8]. UBTF recruits the TATA-binding protein complex SL-1 (five subunits) to both promoters. Pol I, a 13-subunit complex, can occupy rDNA only when bound to its initiation factor RRN3. Once the Pol I-RRN3 complex is loaded, and transcription of 47S pre-rRNA has begun, RRN3 detaches and Pol I elongates along the rDNA gene. A notable difference between Pol I and Pol II is that while Pol II has a well established pause-release mechanism, it is currently controversial whether Pol I pauses, and whether its pausing could be a potential node for regulation [1,9]. TTF1 (Transcription Termination Factor 1) binds to the promoters of rDNA as well as to the 3′ end of the transcribed region [10], is required for termination of rRNA transcription, and is believed to produce a looped structure between

the promoter and termination site of each rDNA repeat [11]. All core machinery components (UBTF, SL-1, Pol I, RRN3, TTF1) have multiple regulatory PTMs (mostly phosphorylation) that are controlled by mTOR, AKT, MAPK, and other signaling kinases. See recent reviews for a more detailed overview of rRNA transcription [1,8,9].

Variations in rRNA transcription in the hematopoietic tree

It has long been known that terminal erythropoiesis involves shutdown of rRNA transcription and degradation of mature ribosomes [12,13]. Beyond erythropoiesis, only a handful of studies have quantified rRNA transcription in hematopoiesis. Hayashi *et al.* [14] and Jarzebowski *et al.* [15] have reported that mouse hematopoietic stem cells (HSCs) and multiple mature cell types have lower rRNA transcription compared to myeloid progenitor populations, with many-fold variation that is not explained by differences in proliferation. We recently published a protocol for FISH-Flow of nascent and mature rRNA [16■], and our group and others are currently in the process of assembling a detailed profile of rRNA dynamics in normal and disordered hematopoiesis.

Challenges in rDNA studies

Bioinformatic challenges—The repetitive nature of rDNA poses nontrivial challenges in high-throughput mapping. Standard genome assemblies historically did not contain intact rDNA sequences, and though reference sequences are available for human/mouse rDNA [17–19], they are typically not used by most investigators for routine mapping. Consequently, though ATAC-Seq, ChIP-Seq, RNA-Seq datasets and others contain rDNA-mapping reads, these reads are discarded or improperly mapped by standard pipelines. Inspired by past approaches [20,21], our group recently generated custom human and mouse genome assemblies optimized for rDNA mapping - these assemblies contain a single reference sequence inserted as an extra “chromosome R” [3■]; high-throughput datasets can be mapped to it using standard tools, enabling rDNA signal to be easily visualized (Fig. 1). We have made these custom genomes, annotation files, and positive and negative control datasets available on GitHub, hoping to make rDNA mapping more accessible to non-aficionados (https://github.com/vikramparalkar/rDNA-Mapping-Genomes). We recently used this approach to map nearly 2200 human and mouse ChIP-Seq datasets for nearly 250 transcription factors and chromatin proteins of relevance to normal and malignant hematopoiesis, and identified previously unknown rDNA-occupancy patterns for several important factors [2■■], some of which we discuss in this review.

Experimental challenges—A primary challenge in rDNA studies is determining whether a factor in question regulates transcription directly through rDNA binding, or indirectly through its Pol II mediated effects on the coding transcriptome. This question often arises in studies of gene regulation more broadly; it is increasingly appreciated that TFs (including oncogenic transcription factor fusions) regulate the transcription of only a minority of the genomic loci to which they bind [22–24]. Dissecting the direct targets of a transcription factor has historically been performed using a combination of approaches: (i) Inducible expression or degron studies to rapidly manipulate transcription factor levels and assay early effects on nascent transcription, (ii) Chromatin-tethering to selectively restore transcription factor binding only to a specific site, (iii) Editing a transcription factor motif to specifically

abolish binding to a particular locus, and (iv) Reporter assays assessing a transcription factor's ability to boost expression of a reporter transgene. Approaches (ii) and (iii) have been challenging at rDNA arrays due to their repetitive nature, and, to our knowledge, have not yet been reported in the literature. Approach (iv) has the weakness of being divorced from the native chromatin context of the locus in question, a weakness that is particularly salient for rDNA, given its specialized epigenetic features. With advancements in CRISPR base editing technology, we expect some of these challenges to soon be overcome.

Transcription factors that bind rDNA

Multiple transcription factors bind and potentially regulate rRNA transcription, but there have historically been only a small number of studies experimentally structured to uncouple direct transcription factor effects from indirect ones. We summarize below data for factors of hematopoietic relevance (Fig. 2). For factors like MYC and RUNX, we include findings from nonhematopoietic cell types that may inform future studies in hematopoietic cells. This list is not comprehensive, and our group and others have identified additional factors of interest that are worthy of investigation [2■■].

Universal transcription factors—TBP (TATA-binding protein), part of the SL-1 complex mentioned earlier, binds to the Spacer promoter and 47S promoter of rDNA, and is essential to recruit Pol I [25]. CTCF binds immediately upstream of the Spacer promoter, and its knockout leads to reduced rRNA transcription and altered nucleolar number [26], Notably, the entire cohesin complex shows rDNA occupancy immediately upstream to the CTCF peak [2■■,7], but it is unknown whether CTCF/cohesin regulate rDNA topology.

MYC—MYC is widely considered the master regulator of ribosome biogenesis, and is believed to coordinate transcription of rRNA by Pol I, transcription of genes coding for ribosomal proteins and other ribosome biogenesis factors by Pol II, and transcription of 5S rRNA by Pol III [27]. MYC directly binds human and mouse rDNA repeats, but our mapping of ChIP-Seq datasets for MYC and its dimerization partner MAX unexpectedly found that its rDNA binding sites fell in different regions of rDNA in the two species: human MYC binds within the transcribed region of rDNA, while mouse MYC shows multiple peaks in the IGS [2■■]. The strongest evidence of direct rRNA regulation by MYC comes from studies in human fibroblasts expressing MYC-ER fusion protein, in which activation of MYC by tamoxifen treatment led to increased nascent rRNA levels within 3–4 h, pointing to direct control [28,29]. However, it has also been reported that MYC promotes transcription of genes encoding UBTF and Pol I complex proteins, pointing to potential indirect mechanisms in addition to direct ones [30,31]. In contrast to our relatively detailed understanding of MYC effects on Pol II [32], there is limited mechanistic understanding of how MYC controls Pol I.

CEBP family—The transcription factor family that we found in our recent mapping to show the most striking and consistent binding to rDNA was the CEBP (CCAAT/enhancer-binding protein) family, with 19 human and 13 mouse ChIP-seq tracks showing a sharp conserved peak for four of six members of the CEBP family (CEBPA, CEBPB, CEBPG, CEBPD) [2■■]. The peak was at a canonical motif sequence nearly 5-kb downstream of

the 47S transcription start site, within the region that is transcribed into 18S rRNA. CEBP factors are a family of leucine zipper TFs that bind DNA as homo or heterodimers [33], and CEBPA is a master myeloid TF whose hematopoietic deletion is known to cause loss of the myeloid lineage [34,35]. Using an FKBP degron system in a mouse myeloid cell line, we found that CEBPA degradation led to reduced Pol I occupancy on rDNA and reduced 47S rRNA levels within 4 h, without any change in the cellular abundance of core rDNA machinery [2■■]. Occupancy of RRN3 was also proportionately reduced, while occupancy of upstream complexes was not, indicating that CEBPA controls the loading of the Pol I-RRN3 complex on rDNA. CEBPA is a single exonic gene, translated into a p42 and p30 isoform from two start codons. An extended isoform of CEBPA with nucleolar localization was previously reported to be translated from an upstream alternate start codon [36]; however, we could not identify such an extended isoform in our cells, indicating that one or both of the “usual” CEBPA isoforms likely bind rDNA. This has implications for AML, in which N-terminal mutations in the *CEBPA* gene cause selective loss of the p42 isoform [37], and it is reasonable to speculate that p30 and p42 may play divergent roles on rDNA. Overall, our work suggests that one of the normal functions of CEBPA is to promote loading of the Pol I-RRN3 complex on rDNA in a cell-type specific manner (Fig. 3). The CEBP motif on rDNA provides an attractive site for motif editing to rigorously uncouple the Pol I vs. Pol II roles of CEBPA and other CEBP family members.

IRF family and SPI1—Another prominent set of transcription factors that we identified binding to rDNA was the IRF family (Interferon regulatory factor) and SPI1 (PU.1), which are known to combinatorially control important aspects of hematopoiesis, inflammation, and immunity [38,39]. We identified peaks for multiple IRF family members on both human and mice rDNA at a conserved IRF motif immediately upstream of the Spacer promoter [2■■]. SPI1 showed divergence in binding sites between the two species, with human binding in the IGS, and mouse binding upstream of the Spacer Promoter. To our knowledge, the roles of these factors on rDNA have not been explored, but their consistent binding across a large number of independent datasets provides compelling reason for further investigation.

RUNX family—RUNX factors, as well as RUNX1-RUNX1T1 fusion protein, have been reported to bind rDNA, with persistent binding during mitosis that was visualized as striking co-localization with UBTF on rDNA arrays of mitotic chromosomes in a number of human and mouse cell types [40–42]. In our ChIP-Seq mapping, we found broad occupancy of RUNX factors to promoters as well as to the entire 13-kb transcribed region of rDNA, without a discrete peak that would be expected if binding were driven by a TF motif [2■■]. Such a pattern of ChIP-Seq signal indicates that RUNX rDNA occupancy is likely mediated through interactions with core machinery, potentially UBTF. In nonhematopoietic cell types, RUNX2 and RUNX3 have been reported to repress rRNA transcription [40,41], while conditional hematopoietic deletion of RUNX1 leads to HSCs with smaller size, reduced ribosome content, and reduced expression of several ribosomal protein genes [43]. This effect is restricted to HSCs, indicating that RUNX1 may promote rRNA transcription and associated gene networks in a context-specific manner. Further studies will be required to illuminate the extent to which RUNX effects on rDNA/rRNA are mediated via its occupancy on rDNA chromatin.

Considerations when testing transcription factor-rDNA regulation

Since rRNA transcription is highly sensitive to a variety of cellular stresses and stimuli, it is critical to take into account multiple alternate explanations when trying to draw a direct mechanistic link between the manipulation of a TF and a change in rRNA transcription (Fig. 4). These alternate explanations may range from indirect effects on cell cycle or apoptosis, to effects on ribonucleotide availability, to changes in abundance of the core rDNA machinery. We urge investigators dissecting TF-rDNA regulation to bear these considerations in mind in the course of their studies.

CONCLUSION

We propose that the rate of rRNA transcription is fine-tuned in each hematopoietic cell type by multiple mechanisms, one of which is through direct rDNA binding and control by some of the same master transcription factors that control the cell's coding transcriptome. This model has implications for normal and malignant hematopoiesis, given the diversity of transcription factors that control cellular identity and fate, and whose mutations or dysregulation lead to cancer. We hope that recent tools and approaches will make systematic interrogation of rRNA transcription more accessible in hematopoietic biology and beyond.

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■■ of outstanding interest

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KEY POINTS

- Ribosomal RNAs are transcribed from hundreds of rDNA repeats, and their transcription rate varies in different hematopoietic cell types.
- Recent mapping pipelines have made rDNA more accessible for high-throughput mapping.
- Several hematopoietic transcription factors, including MYC, CEBP, RUNX family, and others, bind rDNA, and varying levels of evidence are available to support their role in regulating rRNA transcription.
- We provide a list of experimental considerations for future studies of TF-rDNA regulation, including potential indirect mechanisms that should be taken into account and ruled out.
- Overall, we propose a model in which the transcription of rRNA, just like the coding transcriptome, is regulated in a cell-type specific manner in normal and malignant hematopoietic cells by master transcription factors.

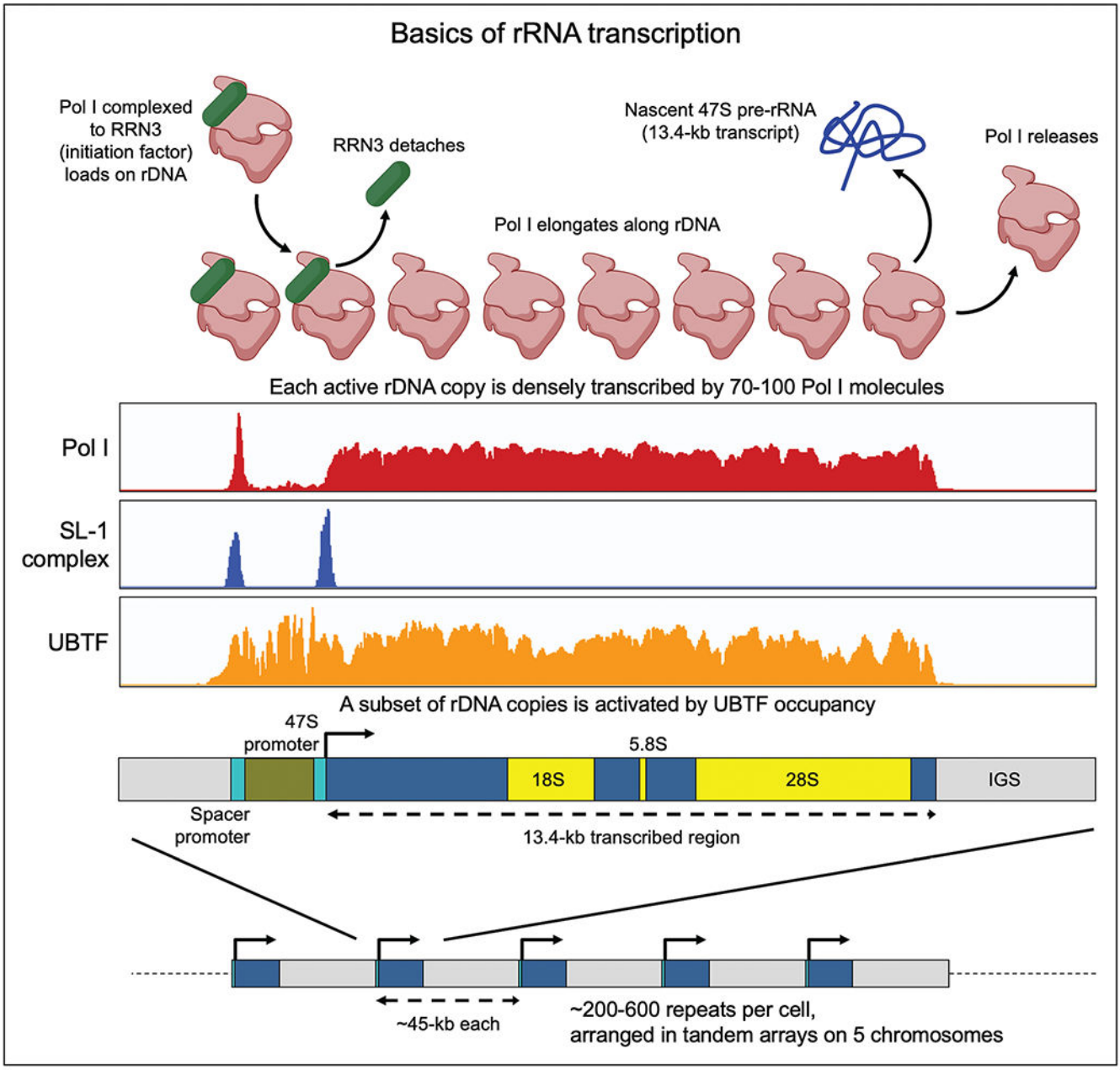

FIGURE 1.

Basics of rRNA transcription. rDNA repeats are present in hundreds of near-identical copies per cell in tandem arrays. A subset of repeats is activated and transcribed in any given cell. Note: Pol I and SL-1 occupancy is seen both at the Spacer promoter and the 47S promoter, but their functions at the former are unknown. Adapted from [3■].

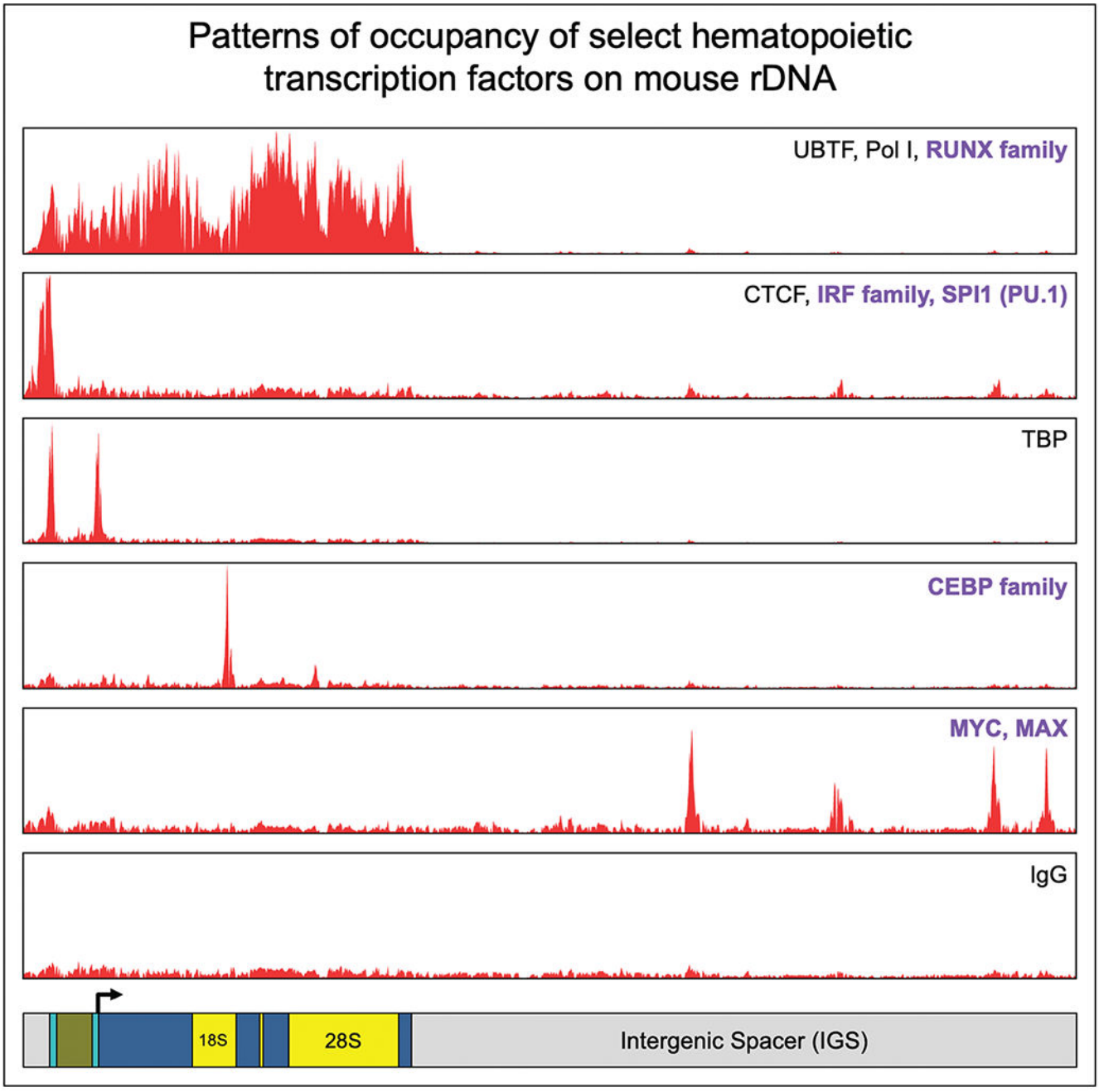

FIGURE 2.

Occupancy patterns of selected hematopoietic transcription factors on mouse rDNA. Patterns of ChIP-Seq occupancy on mouse rDNA, shown as averaged consensus track signals for multiple factors per pattern. Select transcription factors of relevance to normal and malignant hematopoiesis are listed in bold for each binding pattern. Adapted from [2■■].

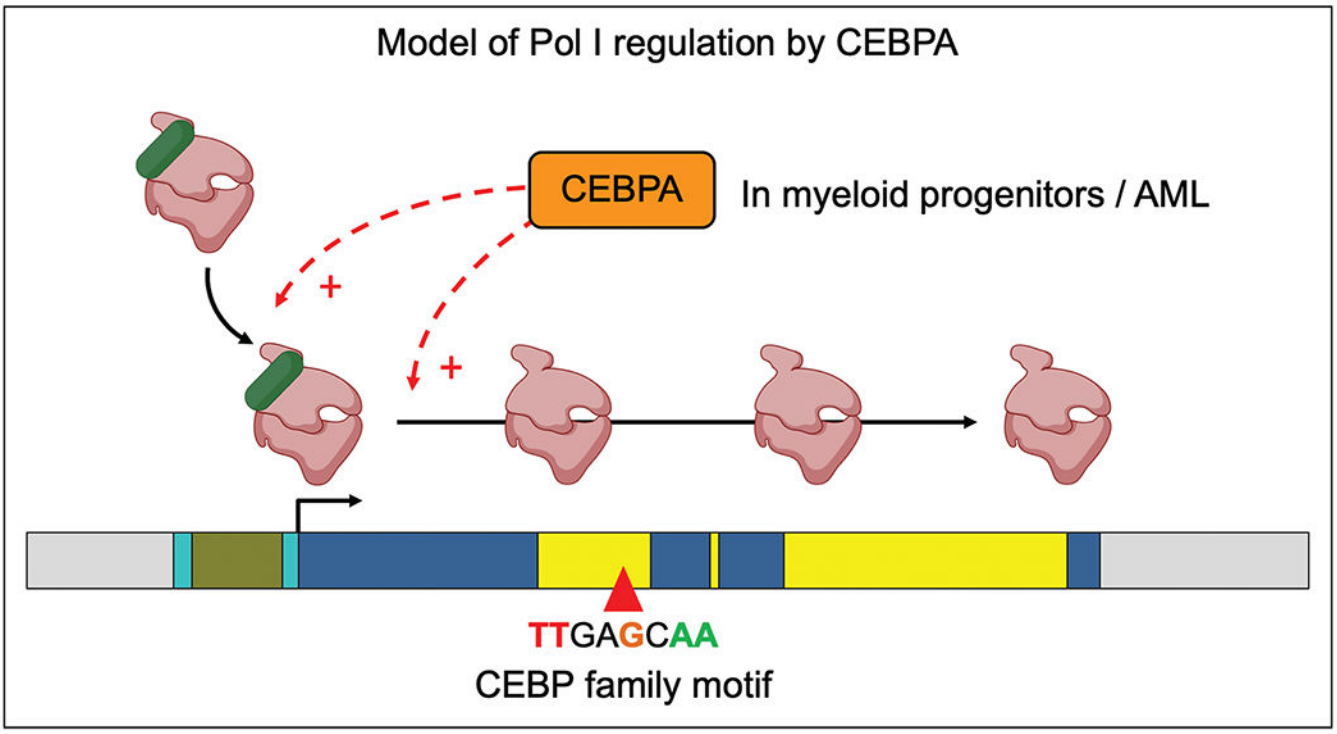

FIGURE 3.

Model of Pol I regulation by CEBPA. We propose that in myeloid progenitors and in AML, CEBPA promotes the loading of Pol I-RRN3 complex on rDNA and/or the elongation of Pol I along the transcribed region. We speculate that this mechanism may be shared by other rDNA-binding TFs.

Experimental considerations when investigating whether a transcription factor directly regulates rRNA transcription

Could my TF of interest be indirectly affecting nascent rRNA by…?	How to interrogate…
…altering cell cycle?	• Measure nascent rRNA separately in each cell cycle stage using FISH-Flow with DNA stain (see Antony et al, STAR Protocols 2023). Effects on nascent rRNA should precede any appreciable cell cycle changes.
…altering cell health?	• Measure Pol I and nascent rRNA prior to onset of apoptosis. Effects on nascent rRNA should precede any changes in cell viability.
…altering cleavage of nascent rRNA?	• Use metabolic labeling as an alternative to 47S quantification if there is suspicion that 47S levels may be confounded by changes in rate of nascent rRNA cleavage and processing.
…altering cellular supply of ribonucleotides?	• Measure cellular NTP concentrations
…altering abundance of rDNA machinery?	• Measure abundance of Pol I complex, RRN3, SL-1 complex, UBTF, TTF1.
…affecting known rDNA-regulatory TFs?	• Measure abundance of MYC, CTCF.
…affecting signaling pathways?	• Measure activity of mTOR, other signaling pathways as relevant.
…affecting unknown indirect genes or mechanisms?	• Use a degron or inducible expression system to rapidly alter TF levels and measure time course effects (within hours) on Pol I occupancy and nascent rRNA (relative to effects on Pol II and nascent mRNA). • Similarly, measure rapid time course effects on UBTF occupancy and rDNA ATAC accessibility (relative to effects on genome-wide ATAC). • Specifically interfere with ability of TF of interest to bind rDNA repeats. • Specifically tether TF of interest only to rDNA repeats.

FIGURE 4.

Considerations for TF-rDNA studies. We list several experimental considerations to be borne in mind while investigating whether a TF of interest directly regulates the transcription of rDNA into rRNA.