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Review

Coordinated Control of rRNA Processing by RNA Polymerase I

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Ribosomal RNA (rRNA) is co- and post-transcriptionally processed into active ribosomes. This process is dynamically regulated by direct covalent modifications of the polymerase that synthesizes the rRNA, RNA polymerase I (Pol I), and by interactions with cofactors that influence initiation, elongation, and termination activities of Pol I. The rate of transcription elongation by Pol I directly influences processing of nascent rRNA, and changes in Pol I transcription rate result in alternative rRNA processing events that lead to cellular signaling alterations and stress. It is clear that in divergent species, there exists robust organization of nascent rRNA processing events during transcription elongation. This review evaluates the current state of our understanding of the complex relationship between transcription elongation and rRNA processing.

Highlights

Transcription elongation rate by RNA polymerase I dynamically controls ribosome biogenesis directly.

Conservation of transcription factors and processing pathways suggests that dynamic regulation of Pol I is conserved from lower to higher eukaryotes.

Dysregulation of transcription by Pol I and pre-rRNA processing is observed in several diseases, resulting in altered cell growth and signaling pathways.

Emerging technologies both *in vivo* and *in vitro* will allow for a better understanding of the dynamic nature of ribosome biogenesis.

The Discovery of Cotranscriptional Processing of the Ribosomal RNA (rRNA)

In all living organisms, and in some viruses, DNA (the genetic material) is transcribed by a DNA-dependent RNA polymerase to produce RNA. This RNA transcript may then be modified to become a 'message' (mRNA), a 'ribosomal' RNA (rRNA), 'transfer' RNA (tRNA), or one of many types of small regulatory/guide RNAs (snRNA, snoRNA, miRNA, etc.). In prokaryotes, all RNA molecules are synthesized by a single RNA polymerase, referred to here as RNAP. Eukaryotic cells deploy at least three nuclear RNA polymerases (referred to as numbered Pols) and these enzymes have evolved specialized sets of gene targets, primarily aligned with the major classes of RNAs (Figure 1). Interestingly, plants have evolved additional RNA polymerases that are thought to primarily synthesize regulatory RNAs [1–5]. Furthermore, eukaryotic mitochondria and chloroplasts carry one or more RNA polymerases that synthesize unique subsets of RNA, including rRNA [6]. This review, however, will focus on the major eukaryotic nuclear RNA polymerases and model bacterial enzymes.

The ribosome is a large ribonucleoprotein complex that is responsible for catalyzing protein synthesis in all living cells. RNA provides the majority of the mass of the ribosome and is responsible for catalyzing peptide bond formation [7–10]. It has long been appreciated that the rRNA is intricately and stably assembled into the ribosome, as many positions within the rRNA are covalently modified, and the structures adopted by the highly conserved rRNA species are crucial to function [11]. However, it was initially unclear as to whether modifications and RNA processing events occur cotranscriptionally (during RNA synthesis) or entirely post-transcriptionally, as fully active ribosomes can be reconstituted from isolated components with 50–100% efficiency under specific conditions [11,12]. Thus, evolutionary pressure for the coordination of transcription and rRNA processing was uncertain [13]. Interestingly, an early study on the sequential nature of rRNA processing hinted at the process occurring cotranscriptionally [14]. In that study, it was shown that changes in the rate of prokaryotic RNAP transcription alter the efficiency of ribosome biogenesis. It was later demonstrated that at least some RNA modifications occur cotranscriptionally [15]. In these studies, a strain of *Escherichia coli* was engineered such that the rRNA (*rrn*) operons were under the control of a T7 phage promoter instead of the endogenous promoters for RNAP. The transcription elongation rate of T7 RNA polymerase is greater than that of RNAP, and the switch

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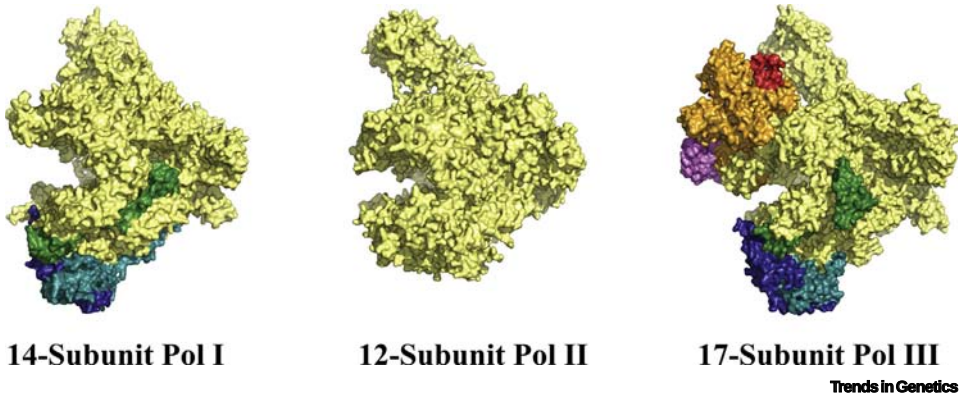

Figure 1. The RNA Polymerases Have Structurally Diverged to Suit Their Cellular Roles. Structural studies of the eukaryotic nuclear RNA polymerases [Pol I Protein Data Bank (PDB): 4C2M; Pol II PDB: 1WCM; and Pol III PDB: 5FJ9] have highlighted differences among the Pols. Yellow subunits are conserved among the Pols. The green Pol I A12.2 and Pol III C11 subunit is conserved between Pols I and III and the N-terminal domain of A12.2 and C11 is homologous to the Rpb9 subunit of Pol II. Teal Pol I subunit A34 and Pol III subunit C25 and purple Pol I subunit A49 and Pol III subunit C37 are conserved between Pols I and III. Pol III has three additional subunits as compared to Pol I: red subunit C31, pink subunit C34, and orange subunit C82.

resulted in substantial rRNA processing defects. Importantly, these findings not only suggested that RNA processing occurs cotranscriptionally in prokaryotes, it was the first evidence suggesting the rate at which genomic transcription occurs could be a driving factor in the cotranscriptional processing of RNA. Further work confirmed that the sequential nature of ribosome assembly is needed to overcome entropic barriers of ribosome assembly *in vivo* [16].

In eukaryotes, ribosome assembly requires the activity of all three nuclear RNA polymerases. Pol I is responsible for synthesizing the three largest rRNAs, Pol III synthesizes the smallest rRNA (5S rRNA), and Pol II is responsible for synthesizing the mRNAs that encode ribosomal proteins (RPs) [8,17]. Coordination of the three Pols is critical for efficient ribosome biogenesis. Pioneering work visualized synthesis of the rRNA in *Xenopus* and in yeast using electron microscopy (EM) [18–21]. These Miller chromatin spreads or 'Christmas trees' (so named due to the lengthening RNA 'limbs' that resembled evergreen trees) provided the first direct physical observation of the eukaryotic ribosomal DNA (rDNA) repeats. They revealed that the rRNA is cotranscriptionally folded into 'terminal knobs' and highlighted the incredible density of polymerases on the rDNA. This technique was further expanded in a number of studies which collectively illustrated that the rRNA is not only cotranscriptionally folded, but also cotranscriptionally cleaved and processed by snoRNAs and other ribosome biogenesis factors [22–25]. These and many other elegant studies established the complex organization of pre-rRNA processing on the growing nascent transcript [22,23,26]. Observing this organization immediately raised the question: how do transcription kinetics influence RNA processing and vice versa?

Early Evidence for the Coordination between the Rate of Ribosome Biogenesis and Processing

While pioneering EM studies revealed that rRNA is cotranscriptionally folded and initially processed, it was not fully appreciated until 2007 that the rate at which transcription occurs can directly influence the processing and folding events of the rRNA [27]. Using a genetic screen, a series of mutations were identified in the genes encoding the second largest subunit of Pol I, the Rpa135 subunit, which reduced the enzyme's transcription elongation rate. Interestingly, it was found that in cells carrying these point mutations, processing of the large and small pre-rRNAs

was perturbed and overall ribosome biosynthesis was dysregulated (Figure 2, Key Figure). Since it is known that rRNA folds cotranscriptionally, the simplest mechanistic explanation for this observation is that nascent RNA adopts alternative structures depending on the rate of synthesis. These alternative structures recruit rRNA binding and processing factors differentially, resulting in perturbed RNA processing [28,29]. Since a point mutation in Pol I, which decreases the Pol I elongation rate, directly influenced the efficiency of rRNA processing, these data suggest that transcription elongation rate and rRNA processing are functionally coupled [27]. Consistent with this model, elegant studies using a rapid harvesting and analysis technique demonstrated that nascent rRNA transcripts are cotranscriptionally methylated and processed [30]. It was concluded that the cotranscriptional nature of processing is critical for the correct architecture of the mature rRNAs and, ultimately, mature ribosomes. Together, these studies display a clear mechanistic rationale for the careful regulation of the rate of transcription elongation by Pol I. If eukaryotes rely on a balance between transcription elongation and rRNA processing, it logically follows that the rate of transcription elongation could be a target for dynamic control of rRNA synthesis.

The Rate of Transcription Elongation by Pol I is a Target for Dynamic Control of Ribosome Biosynthesis

Since transcription elongation rate influences the efficiency of pre-rRNA processing, in turn, factors that influence Pol I transcription elongation can have far reaching effects on ribosome synthesis. Generally, there are three mechanisms by which Pol I transcription elongation can be modulated: (i) direct modification of the enzyme, (ii) interaction with *trans*-acting transcription factors, or (iii) modification of the chromatin template. Here, we discuss examples of each type of potential regulatory event.

Key Figure

Elongation Rate of Pol I Drives rRNA Folding and Ribosome Maturation

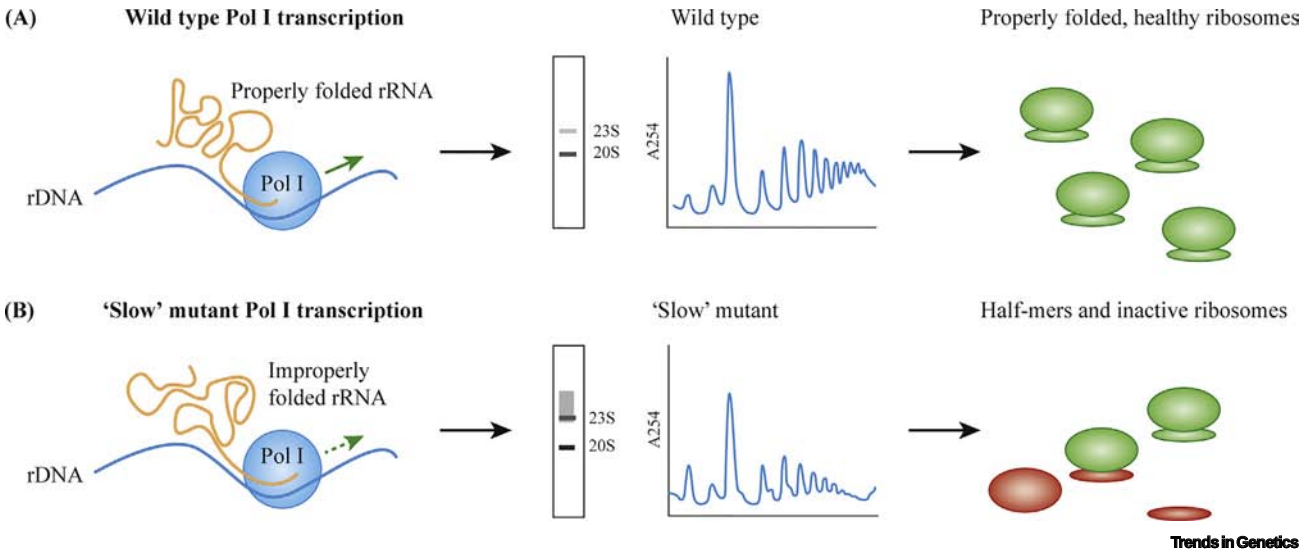

Figure 2. (A) Transcription of the rDNA (dark blue line) by wild type Pol I (light blue circle) results in properly folded rRNA (orange line), which is then properly processed into 20S rRNA and results in correct polysome profiling signifying properly folded, healthy ribosomes (green ovals). (B) Slow polymerase mutants result in improperly folded rRNA, which result in incorrectly cleaved pre-rRNAs (such as the 23S rRNA), polysome profiles with half-mers, and ultimately inactive ribosomes. Polysome profile and northern illustrations were adapted from previously published data [27].

The best described mechanism by which Pol I activity is controlled is via covalent modification. In both yeast and mammalian models there is robust evidence that transcription initiation factors [upstream binding factor (UBF), Rrn3, SL1] and Pol I (A43, PAF53, PAF49) are targets for covalent modification [31–35]. Many of these studies have focused on the roles that modifications play in transcription initiation efficiency, but some data point to a role for covalent modifications governing transcription elongation [36]. Previous work identified phosphorylation sites in the A43 subunit of Pol I [36]. This core subunit is essential for Pol I activity and plays a role in transcription initiation, but in this study, the authors demonstrated that the phosphatase F-cell production 1 (Fcp1) dephosphorylates modified amino acids in A43. Further, this study showed that Fcp1 does not enhance binding of Pol I to promoter DNA, but it increased transcript abundance. These data suggest that dephosphorylation of A43 by Fcp1 directly increases Pol I elongation rate [36]. Fcp1 also removes key phosphorylation events on the C-terminal domain (CTD) of the Pol II subunit Rpb1 and these modifications play key roles in orchestrating cotranscriptional processing of mRNA (discussed later). Thus, there is potential for analogous regulatory mechanisms between Pols I and II. Later work identified sites of phosphorylation in five Pol I subunits. All of the sites were not essential for growth, but mutation of one position (serine 685 to aspartate in Rpa190) resulted in apparent defects in transcription elongation by Pol I *in vivo* [37].

In addition to covalent modification of Pol I, there exists emerging evidence that Pol I isoforms varying in subunit composition may exist and influence transcription kinetics. Yeast Pol I has 14 individual subunits. In recent years, the functional roles of individual subunits have come into focus. For example, several recent studies have focused on the roles for the A12.2 subunit in transcription by Pol I. The CTD of A12.2 is a paralogue of the transcription factor TFIIS and, like TFIIS, A12.2 directly promotes nascent RNA cleavage by Pol I when the enzyme backtracks [32,38,39]. However, recent studies reveal that A12.2 contributes more to Pol I transcription kinetics than simply by enhancing RNA cleavage. Detailed analyses of nucleotide addition kinetics by Pol I complexes with and without A12.2 demonstrated a role for A12.2 in nucleotide addition. Specifically, polymerases that lacked A12.2 added nucleotides to the growing RNA chain at a reduced rate. Thus, A12.2 activates transcription elongation [38]. Furthermore, removal of A12.2 renders the transcription elongation complex much more stable than the wild type enzyme, consistent with previously observed defects in Pol I transcription termination in *rpa12Δ* cells [40,41]. These data collectively demonstrate that Pol I exhibits different kinetic properties with and without A12.2 association.

Interestingly, recent work described heterogeneous Pol I transcription elongation complex structures where A12.2 occupies different locations in the complex [42]. These alternate conformations depend on the association of the A49/A34.5 subcomplex (analogous to Pol II TFIIF) with the enzyme [42]. It has long been known that the A49/A34.5 subcomplex can be dynamically associated with Pol I and that its association enhances Pol I transcription initiation and processivity [43,44]. Further, structural data suggests that the N-terminal portion of the A12.2 subunit may be necessary for proper coordination and stabilization of the A49/A34.5 dimer [42]. Thus, it is reasonable to suggest that reorganization of A12.2 occupancy on Pol I could have effects on the catalytic properties of the enzyme through its coordination of the A49/A34.5 subcomplex. Whether these isoforms of Pol I exist in live cells or whether their altered elongation properties influence nascent rRNA processing remains to be determined.

Over the past several years, many *trans*-acting factors have been identified to affect Pol I transcription. If a transcription factor influences Pol I transcription elongation properties, then that factor may influence the organization of pre-rRNA processing. There are two clear examples where mutations in transcription elongation factors resulted in defective rRNA synthesis and pre-rRNA

processing: the Paf1 complex (Paf1C) and Spt4/Spt5. Mutations in genes that encode Paf1C subunits were shown to cause growth defects in yeast and perturb rRNA processing [45,46]. Subsequently, it was shown that Paf1C associates with the rDNA, activates Pol I transcription elongation *in vitro*, and mutations in Paf1C subunits alter ribosome assembly [46–48]. These findings are consistent with Paf1C having a direct effect on Pol I transcription elongation, which influences rRNA processing. However, Paf1C also affects Pol II. Thus, it is also possible that mutation of Paf1C alters Pol II-dependent expression of other key ribosome assembly factors and the observed ribosome assembly effects are independent of Pol I transcription elongation. Similarly, deletion of *SPT4* or point mutations in *SPT5* give rise to defects in rRNA synthesis and processing [49,50]. Both of those factors associate with Pol I *in vitro* and localize to the rDNA *in vivo* [49,51]. Like Paf1C, Spt4/Spt5 is a key transcription factor for Pol II. Thus, the direct or indirect nature of these effects on rRNA processing remains an area of investigation.

All of these potential mechanisms for influencing Pol I transcription elongation are reminiscent of a paradigm for molecular orchestration of cotranscriptional events: covalent modification of the Pol II CTD. The heptad-repeated CTD of Pol II is thought to work as a 'landing pad', recruiting Pol II elongation factors through a dynamic series of post-translational modifications, predominantly at serine residues. These modifications on Pol II are thought to coordinate steps of the transcription elongation cycle and grant Pol II the ability to distinguish between regions of genes, modify gene expression, and allow for the effective coordination of transcription termination factors [52–54]. Is it possible that Pol I has lost the necessity for the CTD domain because it does not have to distinguish genes of different lengths? Although other RNA polymerases lack an analogous structure to the Pol II CTD, the research surrounding this structure illustrates how cells have evolved mechanisms to orchestrate sophisticated molecular processes during active transcription elongation.

Finally, the chromatin template can directly affect transcription elongation properties. The effect of chromatin on Pol II transcription elongation has been well characterized and is the topic of recent reviews [55–57]. However, it is unclear how histone modifications are regulated in the nucleolus. In fact, it remains unclear whether histones are present on actively transcribed rDNA repeats at all. Some studies have used chromatin immunoprecipitation to reveal activating histone marks at active rDNA repeats [58–60]. However, other methods suggest that histones are largely absent from the rDNA when Pol I is transcribing it [31,61,62]. This mystery is heightened by the presence of 'nucleosome like' transcription factors, which may interact with rDNA and play a role similar to nucleosomes. For example, histone-like factor, Histone Modifier 1, or Hmo1, binds to the rDNA coding region and seems to at least indirectly have a positive effect on Pol I transcription elongation [63]. Substantial further investigation, *in vitro* and *in vivo*, is needed to reveal a deeper understanding of the chromatin state and coordination of transcription elongation at the rDNA template.

Dynamic Regulation of Ribosome Biogenesis Impacts Cellular Growth Signaling

Though previous studies clearly demonstrated that the rRNA is cotranscriptionally processed, the mechanistic consequences of this functional coupling were not clear. A recent study describes that during times of cellular stress, pre-rRNA cleavage steps occur in an alternative orders to produce 'productive' 20S pre-rRNA through cleavage site A2 and 'nonproductive' 23S rRNA precursor through early cleavage at site A3 [64]. Although these precursors were not described as direct results of modulation of the elongation rate of Pol I, previous work that identified functional coupling between Pol I transcription elongation and rRNA processing revealed the same accumulation of 23S nonproductive rRNA precursor [27]. The choice between productive and nonproductive pre-rRNA processing pathways is governed by both TOR and CK2 signaling pathways. This novel regulatory pathway reveals how downstream pre-rRNA processing events are

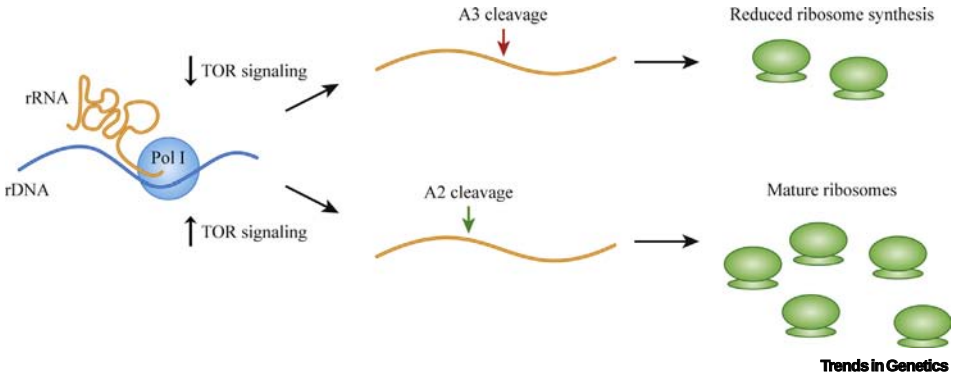

Figure 3. Nutrient Limitations and Stress Result in Alternative Ribosome Biogenesis Pathways. Studies suggest two distinct pathways for rRNA processing (orange line) influenced by TOR signaling in response to nutrient availability and stress.

regulated in concert with transcription of the rDNA to ultimately fine tune ribosome biogenesis to suit cellular metabolic needs (Figure 3) and [64]. Interestingly, TOR kinase has also been described as a Pol I transcription initiation factor. This suggests the possibility of a 'feed-forward' loop, where nutrient-sensing TOR upregulates Pol I initiation, increasing rRNA synthesis while directing that rRNA to productive ribosome biogenesis at the same time.

An additional, well-described control mechanism of ribosome biogenesis is the p53-mediated, apoptotic signaling pathway. In mammalian cells, imbalances in the rate of ribosome biogenesis work as an alarm system that can ultimately result in apoptosis. Free ribosomal proteins (RPs) L11 and L23 (synthesized by Pol II) can bind MDM2, freeing p53 to signal for cell cycle arrest and programmed cell death [65–68]. Thus, modulation of pre-rRNA processing and transcription elongation by Pol I may influence cellular nutrient signaling and stress responses.

Mammalian rRNA Processing versus Lower Eukaryotes

Transcription elongation in higher eukaryotes has not been as well characterized as transcription elongation in lower eukaryotes. *In vivo* evidence suggests notable similarities and differences between control mechanisms that influence Pol I and pre-rRNA processing in higher eukaryotes compared with yeast [69]. At present, detailed biochemical characterization of these events in mammalian cells are not yet well defined.

One notable similarity between higher and lower eukaryotes involves the rRNA processing proteins, U three proteins (Utps). Conservation of the Utps among higher and lower eukaryotes suggests that rRNA processing pathways are at least partially conserved [25]. It was shown in yeast that when Utps are depleted, transcription of the rDNA is reduced [25]. This processing pathway is also found in plants [70,71]. Further, a systematic screening strategy determined that Utps are likewise necessary for correct rRNA processing in HeLa cells [72]. While these results are not identical to observations made in yeast, they do hint at functional conservation. Conservation of this pathway provides evidence for potential conservation of coupling between transcription and rRNA processing among diverse eukaryotic species.

The most convincing evidence that transcription elongation rate is dynamically controlled in higher eukaryotes comes from studies of the human and murine transcription initiation and elongation factor UBF. UBF has many roles in transcription of the mammalian rDNA. It binds at the rDNA promoter to enhance transcription initiation, it influences promoter escape, and is also thought to bind throughout the rDNA to enhance transcription elongation [31]. UBF is a target of the ERK

growth factor pathway, and phosphorylation of UBF results in an increase in its occupancy on the rDNA and increased Pol I transcription [73,74]. UBF binds throughout rDNA genes and has been suggested to serve as a replacement for histones on the rDNA [31]. Further, UBF is suggested to directly affect transcription elongation, as phosphorylation of UBF by ERK results in rapid changes (in under 10 minutes) in Pol I activity in mammalian cell culture [75]. The mechanism by which UBF directly impacts transcription elongation kinetics and how this activity might affect processing of the nascent rRNA remains a topic of active investigation.

While there exist a number of similarities and differences between rDNA transcription and rRNA processing in lower and higher eukaryotes, the degree to which functional coupling of these processes is conserved remains somewhat unclear. Importantly, the conservation of direct coupling of Pol I elongation rate to rRNA processing, and how this is coordinated in complex multicellular organisms, has yet to be probed. More analysis of the biophysical and biochemical properties of transcription elongation by Pol I in both higher and lower eukaryotes is needed to glean a better understanding of the extent to which the dynamic nature of rRNA processing is conserved.

Imbalances in rRNA Synthesis and RP Synthesis Can Result in Disease

Alterations in rRNA synthesis have long been connected with a variety of diseases. Changes in nucleolar organization, size, and the number of nucleoli was one of the earliest histological indicators of cancer [76]. As such, transcription by RNA Pol I is now viewed as a target for cancer therapeutics. While it is debatable whether or not ribosome biogenesis is a driver of cancer or a response, inhibition of rRNA synthesis clearly inhibits cancer cell growth [77]. Two compounds that inhibit ribosome biogenesis have been well described as potential cancer therapies (Figure 4). The first, CX-5461, was initially characterized as an inhibitor of Pol I initiation on the rDNA. It inhibits Pol I initiation by blocking transcription initiation factor SL1 binding to Pol I, which stops the polymerase from binding to the rDNA promoter [78]. In recent literature, CX-5461 was also shown to affect transcription through interactions with DNA damage response pathways and G-quadruplexes [77–84]. The second compound, BMH-21 is a DNA intercalator that binds preferentially in GC-rich regions of DNA and impairs transcription elongation by Pol I, ultimately inducing degradation of the largest subunit of Pol I [85–87]. While these two inhibitors apparently

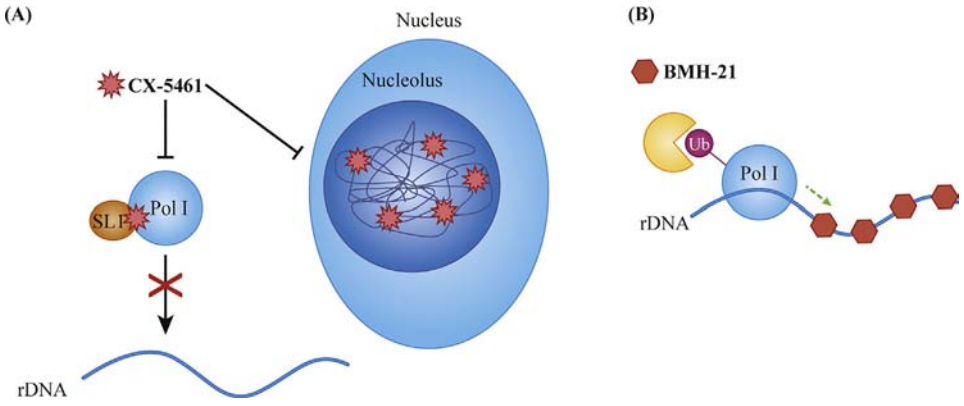

Figure 4. Ribosome Biogenesis Inhibitors CX-5461 and BMH-21 Act through Distinct Mechanisms. (A) CX-5461 (red star) works by inhibiting two activities: (i) CX-5461 prevents SL1 (orange oval) binding to Pol I (blue circle), which results in lack of Pol I binding to the rDNA; (ii) CX-5461 works in the nucleolus to stabilize G-quadruplexes and also results in DNA damage response pathways, likely by interacting with the rDNA. (B) BMH-21 (red hexagon) is a DNA intercalator and is proposed to bind to the rDNA and inhibit Pol I elongation. Subsequent to impaired transcription elongation by BMH-21, the largest subunit of Pol I is subject to ubiquitination and proteasome-mediated degradation (yellow pacman).

influence different steps in rRNA synthesis, they both have been shown to directly and selectively inhibit Pol I activity on the rDNA in cancer cells. Importantly, many changes occur at the rDNA during cell transformation, including dramatic reductions in the cancer cell's rDNA copy number [88,89]. However, it is unknown whether pre-rRNA processing is coupled to transcription elongation by Pol I under these conditions. Further, it is not known whether emerging inhibitors, such as BMH-21, which affects transcription elongation, perturb pre-rRNA processing as well as rDNA transcription.

Emerging Technologies for the Continued Understanding of rRNA Processing

There have been technological advances both *in vivo* and *in vitro* that have resulted in a better understanding of the nature of rRNA cotranscriptional processing. For example, cryo-EM studies were used to create a 3D reconstruction of transcription of an entire rDNA repeat [26]. From this high-resolution structure of a Miller chromatin spread, pre-rRNA processing was observed on the nascent transcripts and the images suggested that individual polymerases on the rDNA repeat do not physically interact with one another. This conclusion is contrary to the previous models that sequential polymerases physically interact and influence transcription efficiency [90]. This methodology may help define the structural consequences of defects in a wide array of transcription factors or rRNA processing factors during endogenous transcription of the rDNA.

In order to interrogate the influence of rDNA sequence elements on Pol I transcription elongation and potentially pre-rRNA processing, native elongating transcript sequencing (NETSeq) was recently adapted for the study of Pol I transcription in yeast [91]. This technique revealed heterogeneous occupancy of the rDNA by Pol I, consistent with previous EM-based studies. With NETSeq, however, the influence of DNA sequence elements can be analyzed. In the recent work, there were no identifiable DNA sequence elements that correlated with Pol I occupancy/pausing; however, there are observable nucleotide preferences within the nascent transcript. Future studies using this methodology will reveal how transcription factors, DNA sequence elements, or cell nutrition/stress conditions impact transcription elongation by Pol I in live cells.

Lastly, single-molecule *in vitro* analysis of rRNA folding is a rapidly developing area of research [29,92–95]. While these studies are new and currently utilized only for prokaryotic RNAP systems, the capability of quantitatively coupling transcription elongation to rRNA processing to probe how proteins bind to the rRNA cotranscriptionally is potentially ground-breaking. Recent work has elucidated that cotranscriptional folding of rRNA in prokaryotes favors the dwell time of rRNAs in their correctly folded conformations. Further, cotranscriptional binding of RPs helps mature rRNAs reach their properly folded conformation more rapidly. In another recent publication, zero-mode waveguide (ZMW) single-molecule förster resonance energy transfer (FRET) was utilized to monitor the transcription rate of prokaryotic RNAP and binding of ribosomal subunits was quantified cotranscriptionally [29]. The study suggested that nascent RNAs were better substrates for binding than prefolded RNAs. This observation is biologically important, as it points to the energetic advantage that cells gain by cotranscriptionally coupling ribosome biogenesis to rRNA processing.

Collectively, these studies demonstrate the level of precision that can be achieved using modern and emerging technologies. These analyses allow for direct interrogation of the coupling between transcription elongation and RNA processing, but also reveal the dynamics between individual events that occur on each RNA. The future is bright for this area of investigation.

Concluding Remarks

It is clear that although recent advances in the study of Pol I activity and ribosome biogenesis have revealed some of the mechanisms by which these processes are orchestrated, there is much that

Outstanding Questions

How well conserved are Pol I covalent modifications in higher and lower eukaryotes?

What is the chromatin state of active versus inactive rDNA repeats?

How does the evolutionary divergence of the Pols relate to their specialized roles in transcription?

How and why do alterations in cellular signaling for ribosome biogenesis throughout stages in development and disease occur?

remains to be discovered (see Outstanding Questions). Excitement in recent years has been partially fueled by the emergence of Pol I as a potential chemotherapeutic target, but the advances made in studying fundamental properties of Pol I activity and its regulation also provide key answers to a number of basic evolutionary and biochemical questions. As new technologies emerge, the sophisticated mechanisms that govern transcription elongation and its coupling to pre-rRNA processing will be continually revealed and refined.

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