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Minireview

# Regulation of ribosome biogenesis within the nucleolus

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**Abstract Ribosome biogenesis is both necessary for cellular adaptation, growth, and proliferation as well as a major energetic and biosynthetic demand upon cells. For these reasons, ribosome biogenesis requires precise regulation to balance supply and demand. The complexity of ribosome biogenesis gives rise to many steps and opportunities where regulation could take place. For *trans*-acting factors involved in ribosome biogenesis in the nucleolus, there may be a dynamic coordination, both spatially and temporally, that regulates their functions from the transcription of rDNA to the assembly and export of preribosomal particles. Here we summarize most of the described regulations on ribosome biogenesis in the nucleolus. However, these may represent only a small fraction of a larger picture. Further studies are required to determine the initial signals, signal transduction pathways utilized, and the specific targets of these regulatory modifications and how these are used to control ribosome biogenesis as a whole. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

*Key words:* Regulation of ribosome biogenesis; rDNA transcription; Pre-rRNA processing and preribosomal particle assembly; Intranuclear trafficking and dynamics

## 1. Introduction

The mature 80S eukaryotic ribosome consists of two subunits, the 60S (large) subunit and 40S (small) subunit. The two subunits together contain approximately 80 ribosomal proteins that are organized around highly modified ribosomal RNAs (rRNAs). The synthesis and assembly of a complete ribosome involves a complex series of pathways that occur throughout the cell. All three types of RNA polymerase are involved in the synthesis of ribosomal components. RNA polymerase I (Pol I) is dedicated to the synthesis of the 28S, 18S, and 5.8S rRNAs, RNA polymerase III synthesizes the remaining 5S rRNA as well as small RNAs that are necessary for various steps of ribosomal biogenesis, and RNA polymerase II synthesizes the pre-mRNAs for ribosomal proteins. The majority of these building blocks come together in the nucleolus where they are assembled into preribosomal particles. These complexes traverse the nucleoplasm, exit the nucleus through nuclear pores, and become mature ribosomes in the cytoplasm (see Fig. 1).

The protein synthesis mediated by ribosomes is crucial to cell growth, proliferation, and adaptation to changing environments. It has been estimated that a growing HeLa cell produces about 7500 ribosomal subunits per minute, which requires approximately 300 000 ribosomal proteins and numerous associations and dissociations with *trans*-acting factors (non-ribosomal proteins) [1], putting an immense demand on cellular machinery. Therefore, ribosomal biogenesis is closely regulated at multiple levels (see Table 1 and Fig. 1). Although the factors and steps involved in ribosomal biogenesis have been extensively studied, the mechanisms that regulate this process in response to growth conditions are not well understood. As other reviews have adequately covered the regulation of ribosomal protein production [2–5] and transcription by RNA polymerase III [6,7], this review will focus on the regulation of ribosome biogenesis within the nucleolus.

## 2. Transcription of ribosomal DNA

Although not all ribosomal DNA (rDNA) repeats are utilized at any given moment, synthesis of pre-rRNA is a highly active process and rRNA can constitute up to 80% of total RNA in a cell [8]. rDNA transcription is believed to occur on the border of fibrillar centers and the dense fibrillar components of nucleoli [9]. Studies using in vitro reconstituted systems demonstrated that initiation of the transcription of rDNA requires selectivity factor 1 (SL-1), upstream binding factor (UBF), RRN3, Pol I subunits, and other cofactors (for complete reviews see [8,10,11]). More recent data using cell-free systems suggest that initiation complex formation is a stepwise process involving chromatin remodeling before or after the initial recruitment of SL-1 to the promoter, followed by association of RRN3 and Pol I subunits to form the pre-initiation complex [12,13]. However, an opposing model envisions that Pol I binds to rDNA and initiates transcription as a preassembled holoenzyme [14–16]. In either model, SL-1 and UBF are believed to remain promoter-bound following promoter clearance by Pol I. Promoter clearance is suggested to be the rate-limiting step in the transcription of rDNA [13,17]. Since these studies have been done predominantly in vitro, the

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*Abbreviations:* rRNA, ribosomal RNA; Pol I, RNA polymerase I; rDNA, ribosomal DNA; SL-1, selectivity factor 1; UBF, upstream binding factor; CKII, casein kinase II; TTF-1, transcription terminator factor 1; pRb, retinoblastoma protein; HAT, histone acetyltransferase; CBP, CREB-binding protein; CPBF, core promoter-binding factor; RENT, regulator of nucleolar silencing and telophase exit; PKC, protein kinase C; FRAP, fluorescence recovery after photobleaching


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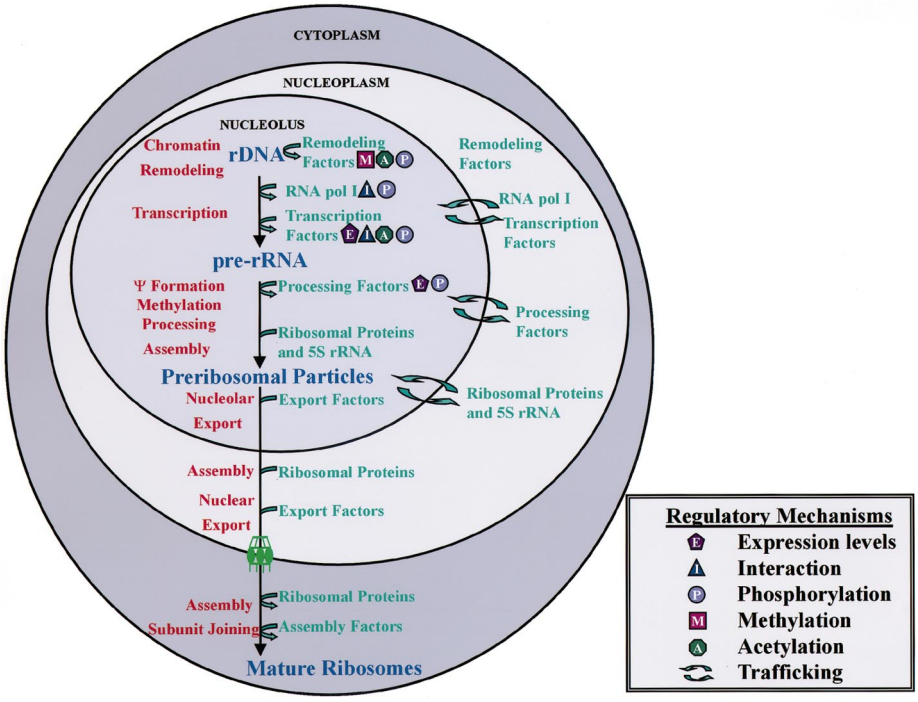


Fig. 1. Diagrammatic representation of the steps and corresponding points of regulation in ribosome biogenesis. Functional steps are listed in red with the *trans*-acting factors involved shown to their right in green. The substrates/products of the biogenic steps are listed in blue.

precise mechanism of the transcription of rDNA in vivo remains to be clarified.

The regulation of pre-rRNA synthesis is a major control point in ribosome biogenesis. Pre-rRNA synthesis is downregulated in response to nutrient starvation, differentiation, or inhibition of protein synthesis, and is upregulated upon re-addition of nutrients, or growth or proliferation stimuli (for reviews see [8,10,11]). Regulation could take place at multiple stages in the process, including chromatin remodeling, transcriptional factor activation, initiation, elongation, and termination. Studies in the past few years have begun to address the mechanisms that act at these regulatory points and their relationship to the level of ribosome biogenesis.

There are excess amounts of the Pol I transcriptional factors, but it is apparent that not all of these proteins are functionally active at all times. The functional status of a protein could be regulated through a number of mechanisms. Those so far described include phosphorylation, acetylation, and protein–protein interactions. Many of the factors involved in the transcription of rDNA are phosphoproteins. For example, the multifunctional protein nucleolin, which is also involved in pre-rRNA processing, binds to the spacer regions between rDNA repeats and increases the specificity of the transcription of rDNA, apparently by remodeling chromatin [18]. This function requires phosphorylation of nucleolin by casein kinase II (CKII), an event coordinated with cell growth and regulated by signal transduction pathways [18]. CKII has also been shown to phosphorylate UBF [19–21], resulting in increased transcription of rDNA in interphase cells, apparently by enhancing the interaction of UBF and SL-1 [21,22]. Phosphorylation of UBF [23,24] and SL-1 [25,26] by cdc2 kinase during mitosis and early $G_1$ has the opposite effect and inhibits the interaction of these proteins resulting in inhibition of the transcription of rDNA. Cdc2 kinase also phosphorylates the initiation and transcription termination factor TTF-1, reducing its chromatin-binding affinity and contributing to mitotic rDNA silencing [27]. Pol I itself can be purified as a holoenzyme containing CKII [28] and CKII may directly phosphorylate Pol I, though the effect of this phosphorylation on Pol I activity is not yet clear [8,28]. In addition, DNA-activated protein kinase, a trimeric protein that contains the rDNA promoter-binding protein Ku, can inhibit initiation of the transcription of rDNA through modification of an as yet unidentified substrate [29,30]. These studies demonstrate that phosphorylation of proteins involved in the transcription of rDNA provides both positive and negative regulation of pre-rRNA synthesis.

Covalent modification through acetylation and methylation also plays roles in regulating the transcription of rDNA at multiple levels. At the chromatin level, hypermethylated DNA and histone deacetylation contribute to rDNA silencing [8,31] while, conversely, histone hypomethylation and histone acetylation result in the activation of rDNA transcription [32–34]. Though the factors that mediate methylation-induced rDNA silencing are unknown, there are several linkages between Pol I transcription factors and histone-modifying enzymes. Both the tumor suppressor retinoblastoma protein (pRb) [35] and the regulator of nucleolar silencing and telophase exit (RENT) complex component Net1 [36], which directly associate with components of the Pol I machinery, bind to and may recruit histone deacetylases to rDNA repeats, resulting in rDNA silencing. In opposition to the silencing effects of deacetylases, a histone acetyltransferase (HAT) has been copurified with Pol I from *Xenopus* [37], though modification of rDNA by this HAT has not yet been demonstrated. More recently, acetylation of Pol I transcription factors has also been documented and overexpression of several transacetylases has been shown to stimulate rDNA transcription [34].

UBF is acetylated in vivo [34] and can be acetylated in vitro by the acetyltransferase CREB-binding protein (CBP) [38]. The acetylated UBF is more active leading to enhanced initiation of the transcription of rDNA [38]. In addition, SL-1 can be acetylated by p300/CBP-associated factor, an acetyltransferase recruited by TTF-1. Acetylated SL-1 initiates the transcription of rDNA more efficiently than the non-acetylated form [39]. Based on these studies, it appears that acetylation of both rDNA-associated histones and Pol I transcription factors enhances the transcription of rDNA.

There are also two prominent but less well-characterized examples of apparent covalent modifications that affect the transcription of rDNA. The Pol I cofactor RRN3, which is required for initiation, is active in vitro when isolated from exponentially growing cells but is inactive when isolated from starved or cycloheximide-treated cells [40]. This indicates that its activity may be post-translationally regulated, though the mechanism is unclear. The core promoter-binding factor (CPBF)/USF1, which interacts with Ku, forms heterodimers with USF2 [41]. While the DNA-binding affinity of USF proteins can be regulated by cdc2 kinase [42], their role in regulation of the transcription of rDNA appears to be mediated by their dimerization. Heterodimers of USF1 and 2 enhance the transcription of rDNA both in vitro and in vivo while homodimers of either protein inhibit transcription and cellular proliferation [43,44]. The importance of these factors in regulation of Pol I and the interplay between them requires further study.

Table 1
List of nucleolar substrates and factors involved in ribosomal biogenesis, their function, the mechanisms used to regulate them and the effect of these regulations on the listed function

| Substrate/factor | Function | Regulatory mechanisms | Functional effect |
|---|---|---|---|
| *Pol I transcription* | | | |
| Chromatin remodeling of rDNA | | | |
| rDNA | Template for rRNA synthesis | Methylation [8,31–34] | − |
| Histones | Activation of rDNA transcription | Acetylation [8,31–34] | + |
| Nucleolin (Nsr1p) | Binds rDNA spacer regions, enhances Pol I specificity | Phosphorylation [18] | + |
| TTF-1 | Termination and initiation factor | Phosphorylation [27] | +/− |
| Pol I and cofactors | | | |
| RNA polymerase I | Transcription of rDNA | Phosphorylation [8,28] | + |
| | | Protein–protein interaction [36,45,46] | Unclear |
| | | Trafficking [PC] | Unclear |
| UBF | Transcription factor required for Pol I initiation | Phosphorylation [19–24] | +/− |
| | | Acetylation [34,38] | +/− |
| | | Protein–protein interaction [47–51] | +/− |
| | | Trafficking [74] | Unclear |
| SL-1 | Transcription factor required for Pol I initiation | Phosphorylation [19,20] | − |
| | | Acetylation [30] | + |
| | | Protein–protein interaction [52] | − |
| RRN3 | Required for Pol I initiation | Unclear [40] | Unclear |
| CPBF (USF1) and USF2 | rDNA promoter-binding proteins | Phosphorylation [42–44] | Unclear |
| *rRNA processing, assembly and trafficking* | | | |
| Nucleases | | | |
| Csl4p | 3′→5′ Exoribonuclease | Expression level [55] | − (Unclear) |
| Pop3p | Component of RNase P and RNase MRP | Expression level [55] | − (Unclear) |
| Rpp29 | Component of RNase MRP | Trafficking [74] | Unclear |
| snoRNP proteins | | | |
| Imp4p | U3 snoRNP component | Expression level [55] | +/− (Unclear) |
| Nop1p (fibrillarin) | C/D box snoRNP component, putative methyltransferase | Expression level [58] | +/− (Unclear) |
| | | Trafficking [74–76] | Unclear |
| Nop56p/Sik1p | C/D box snoRNP component | Expression level [58] | − (Unclear) |
| Nop58p/Nop5p | C/D box snoRNP component | Expression level [58] | − (Unclear) |
| Nhp2p | H/ACA box snoRNP component | Expression level [58] | − (Unclear) |
| Srp40p (Nopp140) | Putative snoRNP chaperone | Phosphorylation [46,61] | Unclear |
| Helicases | | | |
| Dbp3p | Acts in 60S subunit synthesis | Expression level [58] | − (Unclear) |
| Other factors | | | |
| Ebp2 | Involved in processing and 60S assembly, required for PKC-mediated response to secretory defects | Unclear [67] | Unclear |
| Erb1p (Bop1p) | Involved in 60S subunit processing and assembly | Expression level [56] | − (Unclear) |
| Nip7p | Putatively involved in late preribosomal particle assembly | Expression level [58] | − (Unclear) |
| Nsr1p (nucleolin) | Involved in processing, assembly and possibly trafficking | Expression level [55,58–60] | +/− (Unclear) |
| | | Phosphorylation [62,64] | Unclear |
| | | Trafficking [65] | Unclear |
| Nucleophosmin/B23/NO38 | Binds preribosomal particles, may act in trafficking | Expression level [55] | + (Unclear) |
| | | Phosphorylation [54,65] | + (Unclear) |
| | | Trafficking [74] | Unclear |
| Rrs1 | Involved in processing and 60S assembly, required for PKC-mediated response to secretory defects | Unclear [68] | Unclear |

Numbers in brackets in the Regulatory mechanisms column are citations, PC stands for personal communication. In the Functional effect column, '+' indicates an enhancement of the given function, '−' indicates impairment of function, and '+/−' indicates that the mechanism can either enhance or impair protein function. The presence of 'Unclear' indicates that the mechanism/effect is either unclear (if not in parentheses) or has not been experimentally tested (if in parentheses).

In addition, the functional status of a transcription factor can also be regulated through protein–protein interactions that either enhance or inhibit its activity. For example, direct interaction with Net1 appears necessary to position Pol I at sites of transcription. Net1, along with other components of the RENT complex, Sir2 and Cdc14, is required to maintain nucleolar structure and enhances the transcription of rDNA in vivo [36,45]. Nopp140 (Srp40p in yeast) also binds to Pol I and possibly enhances Pol I activity, as the transcription of rDNA is inhibited when full-length or dominant-negative Nopp140 is overexpressed [46]. In contrast, the binding of the interferon-inducible protein p204 [47] to UBF inhibits the transcription of rDNA and may prevent the transcription factor from binding DNA. Other studies have shown that the binding of pRb to UBF, either by reducing the DNA-binding affinity of UBF [48] or by preventing the interaction between SL-1 and UBF [49], can inhibit rDNA transcription in vitro [48] and in vivo [50,51]. Another tumor suppressor, p53, has been shown to bind SL-1 directly and also prevents the interaction between SL-1 and UBF, thereby suppressing initiation of the transcription of rDNA [52]. The interactions between Pol I transcriptional factors and pRb or p53 provide contact points with proteins which have widespread effects on cellular processes and are themselves regulated by multiple signaling pathways. Thus, although these interactions have not been fully characterized in vivo, they may provide important linkages between numerous signaling pathways and the transcription of rDNA.

## 3. Regulation of pre-rRNA processing and preribosomal particle assembly

Once pre-rRNA has been synthesized as a large precursor molecule, multiple cleavages, modifications, and assembly steps are required to produce mature rRNAs that are associated with the proper ribosomal proteins (for comprehensive reviews see [53,54]). Conserved sites on pre-rRNA are cleaved by endo- and exonucleases and methylation is carried out by either conserved methyltransferases or C/D box small nucleolar ribonucleoproteins (snoRNPs) consisting of stable snoRNAs and protein components. Particular residues are pseudouridylated by the H/ACA box snoRNPs. RNA helicases of the DEAD box and related families are necessary for processing and assembly, possibly by unwinding RNA duplexes, displacing protein factors, and/or creating RNA structure necessary for subsequent steps [53]. While the processing of pre-rRNA proceeds, both the *trans*-acting factors and the majority of the ribosomal proteins are assembled onto the pre-rRNA in a cooperative manner. These preribosomal particles are thought to move from the dense fibrillar component to the granular component parallel with their processing and the particles enter the nucleoplasm prior to subunit separation and export.

Although the steps and mechanisms involved in processing pre-rRNA have been extensively investigated, the overall regulation of and coordination between the processing and assembly of preribosomal particles is not well understood. The involvement of a large number of cellular components and of multiple steps provides numerous opportunities for coordination and regulation. Since processing, assembly, and trafficking cannot be reconstituted in vitro, nor can they be easily separated or manipulated, the interplay among the regulations of many processing and assembly factors is still unclear. Most of the evidence for the regulation of processing of pre-rRNA and preribosomal particle assembly comes from studies using the yeast genetic system, thus yeast nomenclature will be used in this section.

Regulation at the expression levels of components involved in processing of pre-rRNA preribosomal particle assembly has been shown to occur in response to many cellular signals. For example, when yeast enter stationary phase and ribosome biosynthesis is inhibited, there is a significant decrease of the level of mRNA encoding the essential $3' \rightarrow 5'$ exoribonuclease Csl4p, the RNase P and RNase MRP component Pop3p, and the late preribosomal RNA processing and particle assembly factor Nsr1p (nucleolin) [55]. When ribosome biosynthesis is inhibited by serum starvation of mammalian cells, the mRNA levels of the processing and assembly protein Erb1p (Bop1p) are reduced [56]. A recent study has demonstrated that expression of dominant-negative Bop1p can induce a p53-mediated cell cycle arrest [57], providing a possible linkage between multiple steps of ribosome biogenesis and the cell cycle. Other treatments that can inhibit ribosome biogenesis, including glucose depletion, heat shock, and nitrogen starvation, decrease the mRNA level of the U3 snoRNP component Imp4p [55]. In addition, treatment of cells with the alkylating agent methyl methanesulfonate, which creates DNA damage and stabilizes p53, reduces the mRNA level of C/D box proteins Nop1p (fibrillarin), Nop56p/Sik1p, Nop58p/Nop5p, the H/ACA box protein Nhp2p, the DEAD box helicase Dbp3p, and the late preribosomal RNA processing and particle assembly factors Nip7p and Nsr1p (nucleolin) [58]. Such regulation may represent a multifaceted inhibition of ribosome biogenesis in response to DNA damage. On the other hand, transcription factor Myc can increase the mRNA level of both Nsr1p/nucleolin and nucleophosmin (B23/NO38), another multifunctional protein involved in both assembly and intranuclear trafficking of preribosomal particles [59,60]. In general, changes in the level of expression of processing factors coincide with the level of ribosome biogenesis, but the specific effects of such alterations on ribosome biogenesis are not clear. In addition, the signaling pathways and components that link cellular changes to regulation of the expression of these factors also need to be addressed.

Control through covalent modification may also play a role in regulating processing of pre-rRNA and assembly of preribosomal particles, since several of the *trans*-acting factors are phosphoproteins. For example, the H/ACA box and C/D box snoRNP-associated protein Srp40p (Nopp140), which may act as a snoRNP chaperone, interacts with and is phosphorylated by CKII [61]. Srp40p can be extensively phosphorylated in both yeast and mammalian cells, but how phosphorylation affects its interaction with snoRNPs or its functional status is unclear [46]. Like Srp40p/Nopp140, Nsr1p/nucleolin [62] and nucleophosmin [63] are phosphorylated by CKII in interphase cells. However, unlike Srp40p/Nopp140, Nsr1p/nucleolin [64] and nucleophosmin [65] are also phosphorylated by cdc2 kinase during mitosis when ribosome biogenesis is inhibited. Phosphorylation by different kinases at different stages of the cell cycle, therefore, could have opposite effects on ribosome biogenesis. Since both CKII and cdc2 kinase also phosphorylate factors involved in the transcription of rDNA, it

will be of interest to explore both the individual and cumulative effects of these kinases and the upstream signaling pathways that lead to these modifications.

One signaling pathway that has been shown to regulate processing of pre-rRNA and preribosomal particle assembly is the protein kinase C (PKC) pathway [66]. Defects in the secretory pathway that is required for membrane synthesis during growth have been found to inhibit ribosome biogenesis in yeast through the PKC pathway. Genetic analyses demonstrate that this inhibitory activity requires the processing and assembly factors Ebp2 and Rrs1 [67,68]. However, the connections between PKC and these proteins as well as their regulatory modifications that result in the inhibition of processing and assembly are not yet understood.

Once preribosomal particles are sufficiently processed and assembled in the nucleolus, they move into the nucleoplasm prior to nuclear export. Using a yeast genetic system, a recent study by Milkereit et al. [69] demonstrated that the translocation of the 66S preribosomal particle into the nucleoplasm might be mediated by the transition from Noc1p- and Noc2p-associated particles to Noc2p- and Noc3p-associated particles. The factors involved in 43S preribosomal particle trafficking and export have not yet been identified. Since ribosome biogenesis is highly spatially defined, the intranuclear trafficking of assembled preribosomes may also represent an important regulatory stage in this process.

## 4. Intranuclear trafficking

Although there is an increasing amount of evidence demonstrating the elaborate and complex regulation of the transcription of rDNA, processing of pre-rRNA, and preribosomal particle assembly, the spatial organization of the covalent modifications and protein–protein interactions as described above remains unclear. Since most of the factors involved in ribosome biogenesis localize predominantly to the nucleolus at steady state whereas many of the known modifiers, including CKII [70], CBP [71], pRb [72] and p53 [73], are predominantly nucleoplasmic, it is difficult to envision the spatial organization of these modifications or interactions. Several recent studies have begun to address this aspect in vivo. Fluorescence recovery after photobleaching (FRAP) analyses using green fluorescent protein-tagged proteins in living cells showed that nucleolar proteins involved in the transcription of rDNA, processing of pre-rRNA, and assembly of preribosomal particles cycle rapidly between nucleoli and the nucleoplasm. This intranuclear shuttling appears to be related to the functions of these proteins, as inhibition of the transcription of rDNA affects their shuttling kinetics. The transcriptional factors examined include UBF [74] and Pol I components (Dundr and Misteli, personal communication). The proteins involved in pre-rRNA processing and preribosomal particle assembly that were examined include Nop1p/fibrillarin [74–76], Nsr1p/nucleolin, RNase MRP protein, and nucleophosmin [74]. It is our view (Fig. 1) that the relatively rapid exchange of these factors between the densely packed nucleolus and nucleoplasm may represent a stage of regulation for these factors. The exchange provides spatial and temporal opportunities for contact between the *trans*-acting factors and their modifiers. After each round of function, factors would exit nucleoli to be modified, reactivated and/or form complexes with other cellular factors in the nucleoplasm. The functionally active form of the *trans*-acting factors would be competent to reenter nucleoli and to engage in transcriptional activation, processing of pre-rRNA, or preribosomal particle assembly. This model is supported by the finding that a C-terminal deletion of UBF1, which abolishes its function in transcriptional activation and prevents the protein from entering the nucleolus [77], also significantly impairs its mobility as measured by FRAP analyses (D. Chen and S. Huang, unpublished observations). Whether this model truly represents the regulatory activities that take place in the nucleus requires future testing.

## 5. Summary

Ribosome biogenesis is a complex and tightly regulated process. There are numerous points and steps where regulations could take place. To incorporate all these regulations may require elaborate temporal and spatial coordination of a large number of processes. The experimental data we have summarized here begin to reveal the complexity of the regulation of ribosome biogenesis. However, they may only represent a small fraction of a much larger picture. Further studies are needed to determine the initial signals, signal transduction pathways utilized, and the specific targets of these regulatory modifications on the steps of ribosome biogenesis and the process as a whole.

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