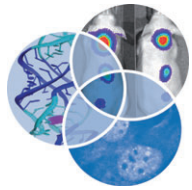
Cotranscriptional events in eukaryotic ribosome synthesis

Advanced Review



Cotranscriptional events in eukaryotic ribosome synthesis

Tomasz W. Turowski and David Tollervey*

Eukaryotic ribosomes are synthesized in a complex, multistep pathway. This begins with transcription of the rDNA genes by a specialized RNA polymerase, accompanied by the cotranscriptional binding of large numbers of ribosome synthesis factors, small nucleolar RNAs and ribosomal proteins. Cleavage of the nascent transcript releases the early pre-40S and pre-60S particles, which acquire export competence in the nucleoplasm prior to translocation through the nuclear pore complexes and final maturation to functional ribosomal subunits in the cytoplasm. This review will focus on the many and complex interactions occurring during pre-rRNA synthesis, particularly in budding yeast in which the pathway is best understood.

How to cite this article:
WIREs RNA 2014. doi: 10.1002/wrna.1263

INTRODUCTION

In rapidly growing yeast cells, protein production and growth are typically limited by the availability of new ribosomes[1] and this is likely to be the case in many other systems. Ribosomal RNA (rRNA) is a basic component of ribosomal subunits and represents about 80% of total cellular RNA.[2] Ribosome biogenesis is highly complex, but must also be very efficient to restock the cell for each division cycle.

Ribosome synthesis is particularly active in budding yeast. A yeast cell is around 37 μm^3 in volume, whereas a human HeLa cells is some 2500 μm^3. Despite this large size discrepancy, total ribosome synthesis is surprisingly similar in these very different cells. Simply for replacement at cell division, yeast cells must generate around 2×10^5 ribosomes per generation (~100 min) from 150 to 200 rDNA repeats, about 50% of which are transcriptionally active. This corresponds to approximately 20 matured, functional ribosomes per transcription unit per minute—or one every three seconds. HeLa cells must generate around 4×10^6 ribosomes per generation (~1200 min) from 300 to 400 rDNA repeats. Again around 50% of the rDNA repeats are active, so this also corresponds to approximately one pre-rRNA transcript per transcription unit every three seconds. Such high levels of production will clearly require specialized features to promote transcriptional efficiency. Moreover, the strikingly overall high rate of ribosome synthesis (around 2000 ribosomes per minute) demands substantial input of resources; around 160,000 ribosomal proteins and 14 Mb of pre-rRNA transcription per minute. The large metabolic costs entailed make it likely that the pathway has been optimized for both efficiency and robustness. Many reports have analyzed specific steps in the ribosome synthesis pathway, but the cotranscriptional events remain the least understood. This is in part due to the short time scale of transcription, the heterogeneous nature of the nascent transcripts and the difficulties in applying conventional biochemical methods to the chromatin-associated nascent pre-ribosomes.

Despite these challenges, significant progress has been made in understanding the cotranscriptional steps in the yeast ribosome biogenesis pathway, and these will be highlighted in this review. The early steps in human pre-rRNA processing have recently been

*Correspondence to: d.tollervey@ed.ac.uk
Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, UK

Conflict of interest: The authors have declared no conflicts of interest for this article.

reviewed in Ref 3 (see also review by Henras et al., WIREs, under revision).

PRE-rRNA TRANSCRIPTION

In almost all Eukaryotes, RNA polymerase I (Pol I) transcribes a single, polycistronic pre-rRNA transcript from tandem rDNA repeats (Figure 1). In yeast, the pre-rRNA represents around 60% of total cellular RNA transcription. This strikingly high overall level of pre-rRNA synthesis is the output of a number of features, including accessibility of rDNA repeats, transcription initiation rate, transcription elongation rate (nucleotides added per min), and processivity of polymerase (the fraction of initiating polymerases that successfully reach the end of the transcription unit). There are distinctive aspects to each of these features for Pol I.

The accessibility of the rDNA plays an important role in regulation of ribosome biosynthesis and is correlated with growth conditions.[4] Around 50% of the rDNA copies are in a chromatin state that is compatible with active transcription, but this is dynamic and in cells with reduced rDNA copy numbers all repeats can be activated.

The Pol I pre-initiation complex contains several specific transcription factors, including Rrn3, UAF (UAS-binding upstream activity factor), and a core factor complex composed of Rrn6, Rrn7, and Rrn11. In addition, the DNA-binding protein TBP is common to all polymerases.[5]

Kinetic labeling indicated that the overall *in vivo* elongation rate for yeast Pol I is around 40 nt second^{-1}.[6] However, analyses of chromatin spreads reveal differences in Pol I density along the rDNA, indicating considerable variation in elongation rate.[7] High Pol I density was observed over the 5′ regions of the 18S and 25S rRNAs, in the vicinity of the sites where compaction of the nascent SSU (small subunit) and LSU (large subunit) particles can be observed (Figure 1). This has led to the suggestion that the transcribing polymerase can be slowed or paused by interactions with the assembling pre-ribosomal complexes, perhaps to facilitate cotranscriptional assembly. Consistent with this idea, alterations in the Pol I transcription elongation rate, directly or indirectly, interfere with normal pre-rRNA processing (reviewed in Ref 8). This suggests that the correct Pol I elongation rate is necessary for normal, cotranscriptional events in pre-rRNA folding and/or assembly.

A number of features in the structure of RNA Pol I facilitate highly processive elongation through the rDNA chromatin.[9] Yeast Pol I is comprised of fourteen subunits; five are common among Pol I, II, and III, while two are shared between Pol I and Pol III and seven are specific to Pol I.[5] Within Pol I a variable DNA-binding cleft is formed between 'core' and 'shelf' modules.[10–12] High processivity is partly maintained by a closed-clamp structure that includes a fixed stalk, which is a detachable element in Pol II. However, despite these features not all Pol I molecules traverse the 35S. Truncated pre-rRNA fragments can be detected and are greatly stabilized by loss of the activity of the exosome and TRAMP nuclear RNA surveillance complexes.[7] Notably, the major 3′ ends are located close to the major Pol I pause sites in the 18S and 25S rRNA 5′ regions, indicating that a fraction of the paused polymerases are terminated at these positions.

COTRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL EVENTS IN RIBOSOME SYNTHESIS

Pre-rRNA processing in yeast is comprised of multiple endonuclease cleavages, in some cases followed by exonuclease trimming. This maturation pathway removes the 5′ and 3′ external transcribed spacers (5′ ETS and 3′ ETS), and internal transcribed spacers 1 and 2 (ITS1 and ITS2) (see Figure 2). Three early cleavages, at sites A0 in the 5′ ETS, A1 at the 5′ end of the mature 18S rRNA and at A2 within ITS1, generate the 20S pre-rRNA and the 5′ end of the 27SA2 pre-rRNA. These cleavages can occur either cotranscriptionally on the nascent pre-rRNA transcript (Figure 2), or posttranscriptionally on the released, 35S pre-RNA.[6,13] In contrast, cleavage at site B0, which generates the 3′ end of the 35S and 27SA2 pre-rRNAs, is likely to always occur cotranscriptionally[14,15] and is followed by cotranscriptional 5′ nuclease digestion that contributes to transcription termination.[16,17] Subsequent cleavages, at sites A3 in ITS1 and at C2 in ITS2, are believed to occur only posttranscriptionally.

In addition to RNA cleavage, many other maturation steps occur during transcription of the 7 kb long pre-rRNA transcript. Ribosome assembly in yeast involves 80 ribosomal proteins (r-proteins), many of which assemble cotranscriptionally (reviewed in Ref 18). However, distinct subsets of r-proteins, from both the 40S and 60S ribosomal subunits, assemble only with late cytoplasmic pre-ribosomes.

In addition, each pre-rRNA is transiently bound by some 75 different small nucleolar RNA (snoRNA) species. Three of these (U3, U14, and snR30/U17) are required for cleavage at sites A0-A2, while a further species (snR10) is important for the efficiency of these cleavages. Since processing at these

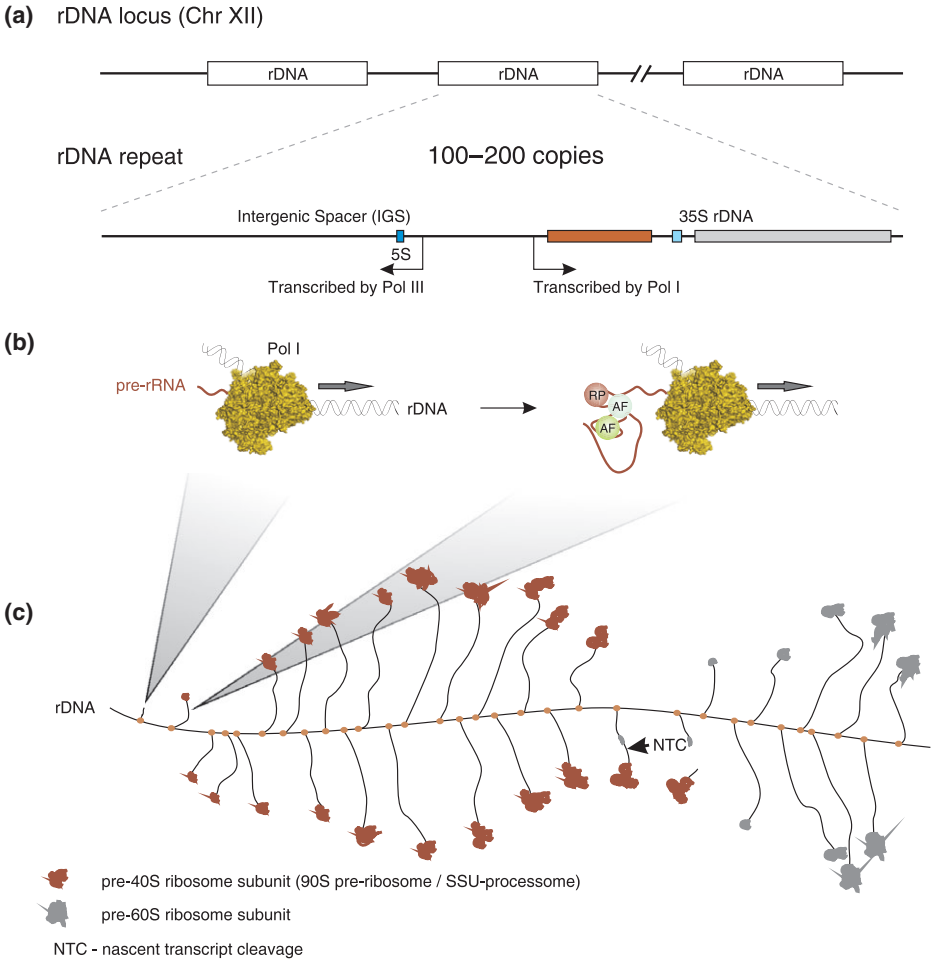


FIGURE 1 | Structure of the yeast rDNA and pre-rRNA. (a) Structure of the rDNA locus, which consists of a single tandem array of 150–200 repeats of 9.8 kb rDNA units located on chromosome XII. Within each repeat the 35S pre-rRNA is transcribed by RNA Pol I, whereas the 5S rRNA is transcribed in the opposite direction by RNA Pol III. The Pol I transcription units are separated by intergenic spacer (IGS) regions that are transcribed by RNA Pol II into noncoding RNAs (ncRNAs). (b and c) Schematic of transcription by Pol I. The nascent transcripts are cotranscriptionally packaged by the small subunit (SSU) processome, and a less well characterized, large subunit (LSU) packaging complex.

sites is cotranscriptional, the snoRNAs must presumably also function cotranscriptionally and prior to cleavage. The remaining snoRNAs generally function to direct site-specific nucleotide modification of the pre-rRNA. The snoRNA-directed methylation of the 18S rRNA component of the 40S ribosome subunits is predominately cotranscriptional.[6,13] Methylation of the 25S rRNA component of 60S ribosomes is also substantially cotranscriptional, but to a lesser extent that for 18S. This may simply reflect the lack of time available between transcription of the 3′ region of the 25S rRNA and the cotranscriptional cleavage at site B0 that releases the nascent pre-rRNA. In contrast, rRNA base methylation, which is performed by proteins acting independently of the snoRNAs, generally takes place later in the processing pathway, following export of the pre-ribosomal particles to the cytoplasm. However, an exception is the methlytransferase Bud23 which interacts with components of the SSU processome.[19] The conversion of uridine to pseudouridine cannot be followed by metabolic labeling, but it seems likely that this will also predominately occur cotranscriptionally.

In addition to the r-proteins, more than 200 additional protein factors participate in ribosome synthesis.[20–23] Many of the early cotranscriptional steps—processing, assembly, and modification on the pathway of 40S subunit synthesis—are likely to be coordinated by a 2.2 MDa ribonucleoprotein (RNP) complex termed the small subunit (SSU) processome[24]; and reviewed in.[22,18] This complex can be visualized by electron microscopy (EM), as 'terminal balls' that are seen to compact the nascent pre-rRNA transcripts in chromatin spreads of the rDNA[13] (Figure 1). The composition of the SSU processome is summarized in Table 1.

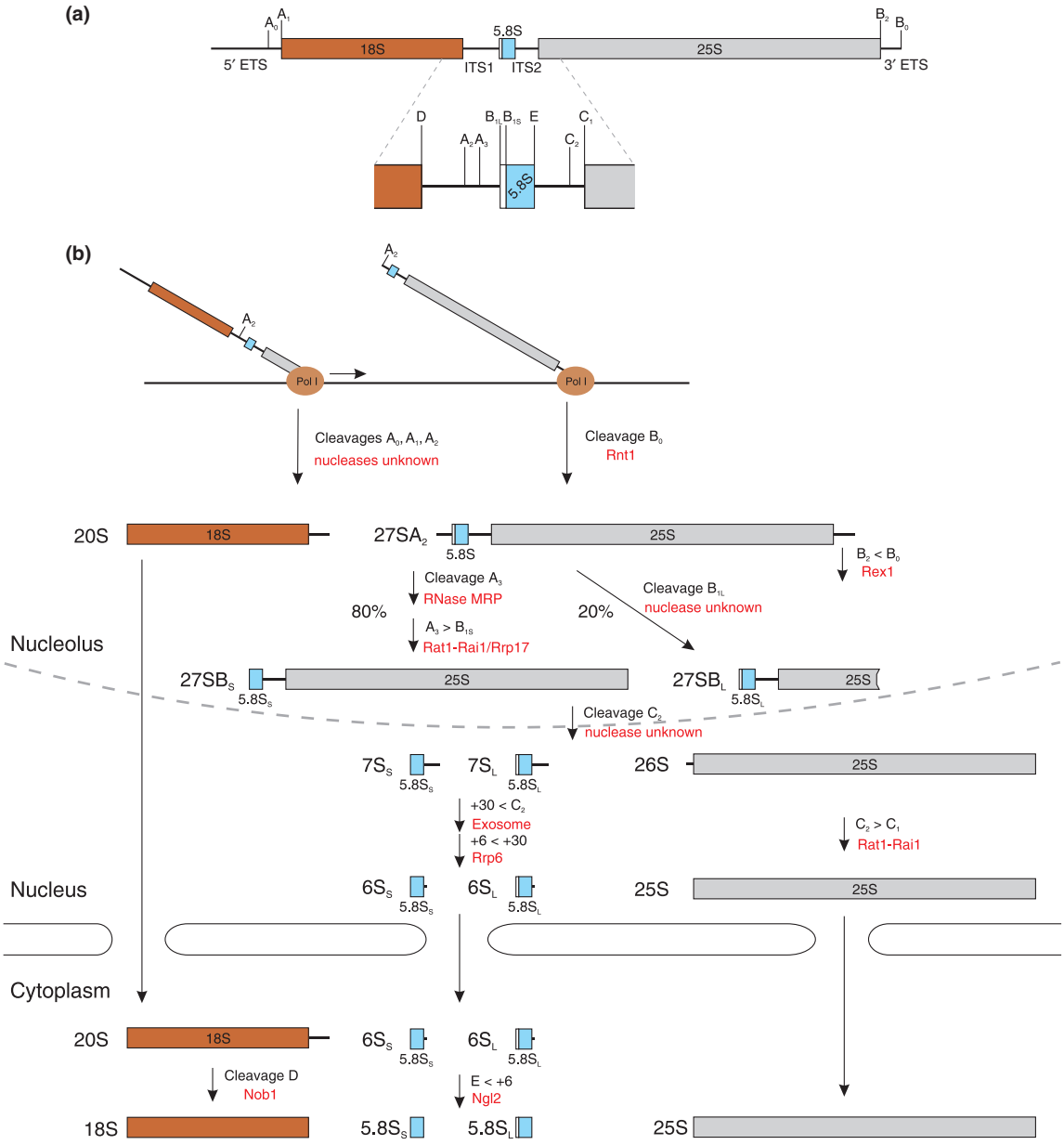


FIGURE 2 | Pre-rRNA processing pathway. (a) Schematic of the 35S pre-rRNA showing the processing sites (a–e). (b) Simplified overview of yeast pre-rRNA processing. RNA processing enzymes acting at specific steps are indicated in red.

Compaction of the 5′ region of the 27S pre-rRNA is also observed in EM, as knobs that remain after cleavage at sites A0-A2 and release of the pre-40S particles.[13] However, the composition of these large subunit (LSU) assembly complexes is less well characterized than the SSU processome.

PRE-rRNA PROCESSING

Cleavage at Sites A0, A1, and A2

The overall yeast pre-rRNA processing pathway will not be discussed in detail here, but a summary is presented in Figure 2. Pre-rRNAs generated by cotranscriptional and posttranscriptional pathways cannot be distinguished by steady state RNA analyses, since the products are the same in each case. However, two methods are currently available that distinguish these pathways. Cleavage of the nascent transcripts can be directly visualized in 'Miller' chromatin spreads.[13] Visualization of rDNA chromatin by EM showed a dense knob at the 5′-end of nascent transcripts, which was lost on truncation of the nascent transcript by cleavage at site A2. There was no evidence from EM analyses for cleavage at sites

TABLE 1 | Composition of the SSU Processome

Complex	Subcomplex	Protein Names
U3 snoRNP	Box C/D	Nop1, Nop56, Nop58, Snu13
	Mpp10	Imp3, Imp4, Mpp10
		Rrp9
UtpA/t-Utp		t-Utp4, t-Utp5, t-Utp8, t-Utp9, t-Utp10, t-Utp15, t-Utp17
UtpB		Utp1, Utp6, Utp12, Utp13, Utp18, Utp21
UtpC		Rrp7, Utp22
		Rrp36
	CK II	Cka1, Cka2, Ckb1, Ckb2
Other		Utp2, Utp3, Utp7, Utp11, Utp14, Utp16, Noc4, Utp20, Utp23, Utp24, Utp25, Utp30, Bms1, Dbp8, Dhr1, Dhr2, Emg1, Krr1, Rcl1, Rok1, Rrp3, Rrp5, Sof1, Dbp4, Enp1, Esf1, Esf2, Fal1, Fyv7, Gno1, Has1, Kre33, Lcp5, Ltv1, Mrd1, Nop9, Nsr1, Pfa1, Prp43, Sgd1, Slx9, Ygr251

A0 and A1 prior to A2 cleavage and, conversely, RNA analyses have not detected the accumulation of RNAs that are cleaved at A2 but not at A0 or A1. Moreover, mutational analyses strongly indicate that processing of the three sites is tightly coupled since neither mutations in the pre-rRNA[25] nor ribosome synthesis factors (see Ref 23) lead to cleavage at site A2 without prior cleavage at A1. Together this strongly indicates that sites A0, A1, and A2 are cotranscriptionaly cleaved over a very short period. In an alternative approach, the occurrence of cotranscriptional cleavage was indirectly inferred from the timing of the appearance of the cleaved RNA product relative to transcription. This was based on kinetic analyses of *in vivo* labeled pre-rRNAs supported by mathematical modeling.[6,26] Both approaches indicate that in wild-type cells around approximately 70% of the nascent pre-rRNA transcripts are cleaved cotranscriptionally at sites A0, A1 and A2.[6,13]

Notably, both approaches also revealed that, while the cleavages occur on the nascent transcripts, they are not coincident with transcription through the cleavage site. Rather cleavage occurs when Pol I transcribes through the 5′ region of the 25S rDNA approximately 1.2 to 1.5 kb downstream of site A2. The features that establish this timing remain to be determined, but may include interactions with the assembling LSU complex and/or binding of the large, highly conserved ribosome synthesis factor Rrp5. It has long been observed that depletion of early acting factors on the 60S maturation pathway delay processing on the 40S pathway, as shown by 35S pre-rRNA accumulation, while not clearly reducing synthesis of mature 18S rRNA.[20–23] A possible explanation for this would be the loss of cotranscriptional, but not posttranscriptional, processing. This model was supported by kinetic labeling data for the 5′ exonuclease Rat1,[26] which is required catalytically only following the posttranscriptional cleavages at sites A3 and C2, but binds the pre-rRNA prior to cleavage at site A2.[27] Rrp5 is unusual in being required for both cotranscriptional cleavage at sites A0-A2 and for posttranscriptional cleavage at site A3. Rrp5 binds to the 25S rRNA region[28] (Figure 3) and potentially also participates in setting the timing for cotranscriptional cleavage.

The nucleases responsible for the cotranscriptional cleavages at sites A0 to A2 have not been clearly identified. Candidates include the RNA cyclase-related protein Rcl1, which was proposed to cleave at A2,[29] and the PIN domain proteins Utp23 and 24, which are structurally related to endonucleases and were proposed to cleave sites A1 and A2.[30]

Cleavage at Site B0

In striking contrast to the elaborate SSU processome assembly required for the early cleavages at A0 to A2, cotranscriptional cleavage at site B0 by Rnt1 (RNase three) requires no known cofactors.[14,15] Like other RNase III family members, yeast Rnt1 cleaves both sides of a stem-structure, at target sites that are partly defined by the sequence of the terminal loop in the stem.[31] Rnt1 also targets many other sites in the precursors to snRNAs and snoRNAs, as well as sites within some pre-mRNA introns.

Cleavage at B0 releases both 35S/27SA2 and a 3′ nascent transcript with a free 5′ end. The 3′ residual nascent transcript is rapidly degraded by the 5′ exonuclease Rat1, which chases and catches the transcribing polymerase, contributing to transcription termination.[16,17] This resembles the 'torpedo' mechanism for transcription termination of Pol II, which similarly involves degradation of the cleaved, nascent transcript by Rat1. It is notable that cotranscriptional cleavage at site A2 is not followed by similar Rat1 degradation, presumably due to protection of the free 5′ end of the 27SA2 pre-rRNA by factors that are likely to include Rrp5.

The 5′ products of site B0 cleavage extend only to sites 14 and 49 nts beyond the 3′ end of the 25S rRNA sequence.[15] This region is trimmed by the 3′ exonuclease Rex1, a member of the RNase D family that, like Rnt1, has numerous other substrate RNAs.[32,33]

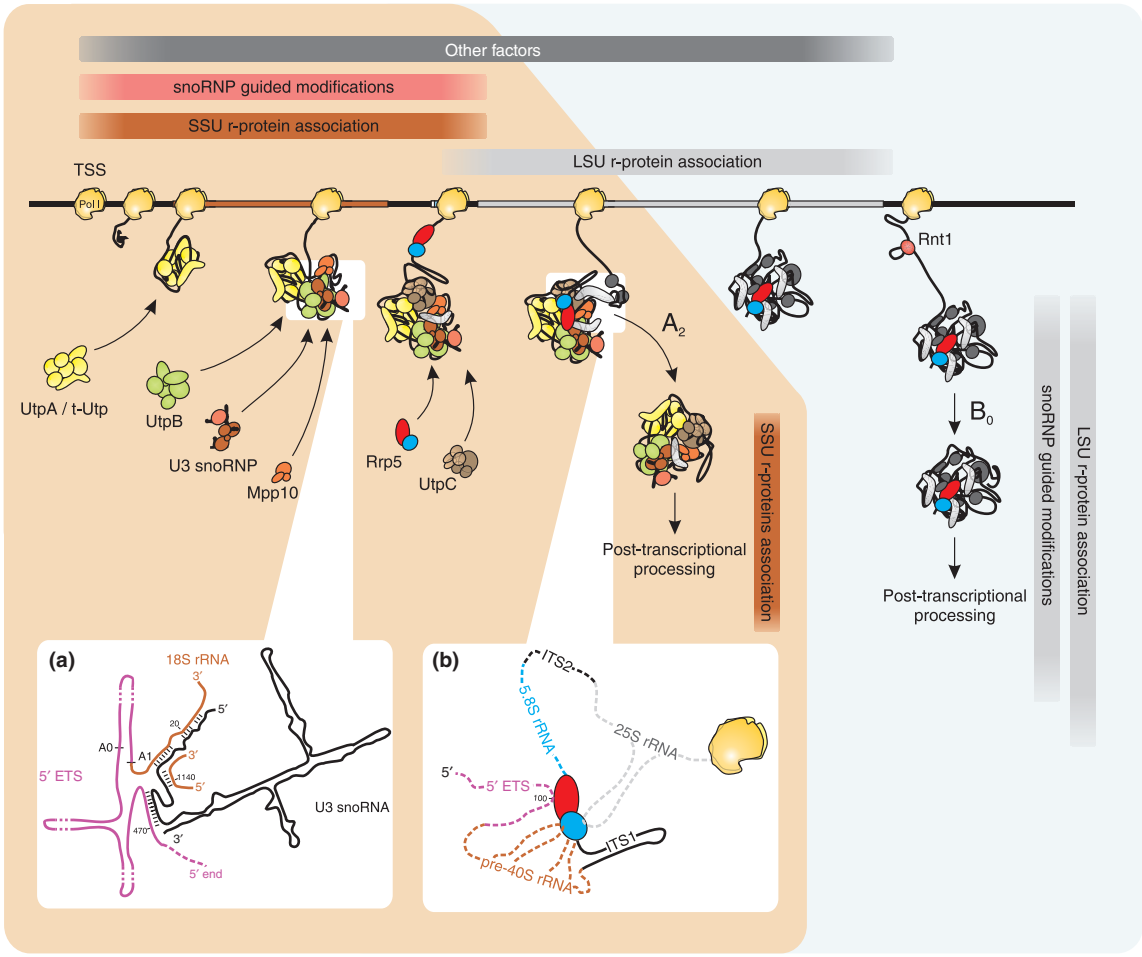


FIGURE 3 | Cotranscriptional events in ribosome synthesis. Schematic showing the sequence of loading of the Utp complexes and other components of the large SSU processome, and the cotranscriptional cleavages at A2 and B0 that release the pre-40S and pre-60S particles, respectively. Insets indicate the multiple binding sites identified for (a) U3 snoRNA and (b) Rrp5, which are likely to contribute to pre-rRNA compaction. See Table 1 for composition of SSU processome subcomplexes.

COVALENT NUCLEOTIDE MODIFICATION

About 2% of nucleotides in the yeast 18S and 25S rRNAs undergo covalent modification.[34–36] The predominant modifications are methylation of the 2′-hydroxyl of the ribose (2′-*O*-methylation) and conversion of uridine to pseudouridine by base rotation. Both types of modifications are performed by proteins that are associated with guide RNAs; termed box C/D and box H/ACA snoRNPs, respectively, reflecting conserved sequence motifs present in the respective classes.[37,38]

C/D box snoRNAs associate with four conserved proteins; the methyltransferse Nop1 (fibrillarin in humans), together with Nop56, Nop58 and Snu13. Ribose methylation increases the stability of RNA–RNA base-pairing and decrease mobility restricting conformational flexibility on the angstrom scale.[39]

H/ACA box snoRNAs also associate with four proteins; the pseudouridine synthase Cbf5 (dyskerin in humans), together with Gar1 and Nhp2, Nop10. Pseudouridine has an additional free NH residue relative to uridine, and therefore has the potential to form an additional hydrogen bond, which might alter or strengthen tertiary structure interactions.

The sites of methylation and pseudouridine formation are clustered in key functional regions of rRNA and well conserved in evolution, strongly suggesting that they play important roles.[40,41] However, snoRNA-directed modifications are all individually dispensable, although growth defects are seen following the loss of multiple snoRNAs.[38] In human cells, mutations in dyskerin reduce levels of pseudouridine and cause the inherited disease

Dyskeratosis congenital. This has been linked to defects in translation of mRNAs that have internal translation initiation sites.[42] In addition to directly affecting rRNA folding, cotranscriptional modifications may promote the association of assembly factors and/or ribosomal proteins. It could also be envisaged that base-pairing by the snoRNPs might directly modulate pre-ribosome structure or folding. Modification might then be a secondary consequence of binding and/or act as sign that binding has occurred and the snoRNA is ready for removal. Surprisingly, recent results indicate that, at many sites, modification levels are less than 100%,[43] implying that distinct sub-populations of ribosomes will be present that differ in their modification patterns, but the functional consequences of this are not yet clear.

Based on EM studies, rough estimates of pre-rRNA/snoRNA association times during modification were very low (~50 milliseconds).[13] This may reflect rapid removal of snoRNPs by helicases.[44] In general, it is assumed that helicases regulate snoRNPs release or association with pre-ribosomes. Particularly in the case of the box C/D snoRNAs, their interactions with the pre-rRNA appear to be far too stable to spontaneously dissociate in a time frame that is compatible with the timing of the ribosome synthesis pathway. Some 18 different ATP-dependent RNA helicases participate in ribosome synthesis. Most of these are essential for ribosome maturation, showing that they have nonredundant functions. However, for most snoRNAs it has proved impossible to identify a simple dependency on a specific helicase for snoRNA dissociation. This suggests that, while each helicase has a specific, essential role in ribosome maturation, they also show redundancy in their activities in cotranscriptional dissociation of snoRNP/pre-rRNA complexes.

Pre-Ribosome Assembly

Analyses of the steps leading to the assembly of proteins and RNP subcomplexes onto the nascent pre-rRNA to form the SSU processome (Figure 3) have been reported in several publications (reviewed in Ref 18).

Many proteins and at least three snoRNAs (U3, U14, and snR30/U17) contribute to SSU processome assembly. However, the U3 snoRNP and the large, highly conserved protein Rrp5 are likely to play key roles. In cells depleted for either U3 snoRNA or Rrp5, the 5′ terminal knobs are largely lost from the nascent pre-rRNA in chromatin spreads.[13,28,45]

U3 base-pairs to around 6 sites in the 5′ region of the pre-rRNA, dispersed over approximately 1.5 kb of primary sequence (Figure 3). Binding of the 311 nt U3 molecule to all of these sites presumably helps bring them together to promote complex assembly. Binding of U3 to the pre-rRNA is promoted by Dhr1/Ecm16, an ATP-dependent RNA helicase of the DEAH family (named for a conserved amino acid motif) and Imp3, which forms a U3-specific snoRNP together with Mpp10 and Imp4[46] (R. Sardana, X. Liu, S. Granneman, J. Zhu, M. Gill, D. Tollervey, C.C. Correll and A. Johnson, submitted). Similarly, Rrp5 binds dispersed sites across 3 kb, using its 12 S1 RNA-binding domains[28,47–49] (Figure 3). Rrp5 additionally contains seven TPR protein–protein interaction domains and interacts with numerous ribosome synthesis factors.[28,50,51] These include both very large, structural proteins and nucleotide triphosphate binding factors, and it has been proposed that this megadalton complex forms a structural framework for large-scale remodeling of the early pre-ribosomes assembling on the nascent transcript. The snoRNA-based modification system predominately targets the highly folded core regions of the ribosomal subunits. This requires that the pre-rRNA initially be maintained in an open, accessible conformation to allow base-pairing of the 75 (in yeast) snoRNAs, before refolding into the highly structured mature conformation. We predict that this is one key function of the SSU processome complex.

The SSU processome is formed in an ordered assembly pathway, in which the binding of some, but not all, factors shows specific dependencies (reviewed in Refs 18, 20). An outline of the SSU processome assembly pathway is shown in Figure 3 and the components are summarized in Table 1. The incorporation of the 80 r-proteins also follows an ordered pathway (see Refs 52, 53 and references therein). Most are incorporated into pre-ribosomes in the nucleolus, probably associating with the nascent transcript, but a substantial minority is incorporated into late pre-ribosomes in the cytoplasm.

LINKS BETWEEN POL I TRANSCRIPTION ELONGATION AND COTRANSCRIPTIONAL RIBOSOME MATURATION

The efficiency of processing of mRNA precursors (pre-mRNAs) is enhanced by coupling of transcription by RNA polymerase II (Pol II) to cotranscriptional RNA processing events. Among the best characterized examples is the coupling of cotranscriptional recruitment of splicing factors onto nascent pre-mRNAs with Pol II elongation pausing.[54–56] Growing evidence indicates that links also exist between cotranscriptional processing of ribosomal RNA precursors

(pre-rRNA) and transcription elongation by RNA Polymerase I (Pol I), but these remain much less well characterized.

Functional ribosomal subunits can be synthesized by transcription of the pre-rRNA by RNA Pol II,[57] indicating that there are no essential links between the assembling ribosome synthesis factors and RNA Pol I. There is, however, evidence for functional links; mutations that impair the elongation rate of Pol I (reduced by 90% as measured *in vitro*) cause the accumulation of the 23S pre-rRNA.[58] This species is generated by cleavage a site A3 in the absence of prior cleavage at sites A0, A1, and A2, possibly reflecting the loss of cotranscriptional processing of these sites. This is perhaps a surprising result, as slowed elongation should allow more time for processing of the nascent transcript and might have been expected to favor efficient cotranscriptional processing at these sites. Moreover, Pol I pausing was observed in the 5′ region of 18S, and to a lesser extent in the 5′ region of the 25S both in chromatin spreads visualized by EM and in chromatin immunoprecipitation (ChIP) analyses of Pol I occupancy.[7] The transcription pause sites show an approximate correlation with the major cotranscriptional assembly events of SSU compaction on the 18S and LSU complex appearance on the 25S rRNA. This suggests that pausing might be causally linked to the pre-ribosome assembly on the nascent transcript.

The earliest-associating components of the SSU processome are termed the UtpA or t-Utp (transcription associated, U3 binding) complex, and were proposed to interact with Pol I to promote transcription.[59] This might be analogous to the interactions of pre-mRNA binding factors with RNA Pol II. However, Pol I lacks a region that is evidently related to the C-terminal domain (CTD) of the large subunit of RNA Pol II, which plays key roles in linking RNA-binding proteins with the polymerase, and site(s) of Pol I interaction with t-Utps remain to be identified. The human homologue of the yeast ribosome synthesis factor Kre33 is also known as hALP (human acetyltransferase-like protein). hALP was reported to associate with U3-containing complexes and acetylate UBF (upstream binding factor), a key Pol I transcription activator.[60] Acetylation of UBF stimulates transcription activation, and the histone acetyltransferase CBP (CREB-binding protein) acetylates and activates UBF, whereas Rb (Retinoblastoma) binding to UBF leads to its deacetylation by HDAC1 and the inhibition of pre-rRNA synthesis.[61] Notably, t-Utps are recruited to human 'pseudo-NORs'; chromosomally integrated, artificial arrays that mimic the specialized chromatin structure of the rDNA and recruit UBF, but are transcriptionally inactive.[62] This suggests the possibility that human UBF might help recruit t-Utps to the rDNA, and then be stimulated by their association with the pre-rRNA. In addition, yeast Rrp5 directly interacts with two largest subunit of Pol I, Rpa190 and Rpa135, as well as early synthesis factors, and is therefore a candidate to also play a role in transcriptional coupling.[28]

EVOLUTIONARY CONSERVATION OF COTRANSCRIPTIONAL PRE-rRNA PROCESSING

While the ribosome synthesis pathway in general, and the cotranscriptional events in particular, remains less well characterized in other systems, it seems likely that fundamental features uncovered in yeast will be conserved in many or all Eukaryotes. Terminal knobs are visible on 'Miller' chromatin spreads from yeast to mammals[63,64] suggesting cotranscriptional assembling and compaction in other eukaryotic systems. Moreover, Rrp5, other SSU processome components, and the U3 snoRNP are highly conserved throughout Eukaryotes.[3,65] U3 forms equivalent interactions with the 5′ETS, and is important for early pre-rRNA processing, in organisms as diverse as humans and Trypanosomes.[66] It is, however, likely that there are also significant differences. Inspection of chromatin spreads from human and other metazoan cells has not been reported to reveal the occurrence of cotranscriptional cleavage. Thus, although related SSU processome complexes may assemble cotranscriptionally in many Eukaryotes,[67] the activation of the endonuclease(s) is apparently different. This may be related to differences in pathways of ITS1 processing,[3,68] but it is not evident that the major human pathway is more closely related to the posttranscriptional pathway in yeast.

CONCLUSION

Cells have developed many mechanisms to avoid the accumulation of unprocessed transcripts, which will potentially bind and sequester both cognate and noncognate processing factors, and surveillance systems help ensure that nascent precursor RNAs are either rapidly processed or degraded. This is likely to be particularly important for the pre-rRNAs, which are both very long and extremely abundant transcripts. We have previously proposed that pre-rRNA surveillance involves some (or all) of the many NTPases in the ribosome synthesis pathway, functioning in an

active, kinetic proofreading mechanism.[69] An effective means of avoiding the accumulation of precursor RNA is the packaging and processing of the nascent transcript, and this appears to be a universal feature of ribosome synthesis pathways from Bacteria to mammals. The complex 3D structure of the mature ribosomal subunits in eukaryotes must be established from an initial, open structure that is accessible to the large numbers of snoRNPs. The large-scale reorganization entailed is likely to be a key function of the cotranscriptionally assembling ribosome synthesis machinery, particularly the SSU processome.

To facilitate robust cotranscriptional preribosome assembly, it seems likely that some signaling system links ribosome synthesis factors to the elongating polymerase, perhaps involving the t-Utps, in order to induce pausing until some (maybe structural) checkpoint is satisfied. When the correct structure is achieved, transcription is resumed; otherwise the nascent pre-rRNA is released and degraded in an exosome dependent pathway.

Despite substantial progress in understanding the cotranscriptional events in ribosome synthesis, many outstanding questions remain: (1) How do the SSU and/or LSU processome complexes communicate with Pol I? (2) What intermediates during ribosome synthesis are subject to surveillance? (3) How is the timing of pre-40S cotranscriptional release achieved, and what roles do pre-60S factors play in this process?

ACKNOWLEDGMENTS

This work was supported by a Mobility Plus program to TWT (1069/MOB/2013/0) and a Wellcome Trust Fellowship to DT [077248]. Work in the Wellcome Trust Centre for Cell Biology is supported by Wellcome Trust core funding [092076].

REFERENCES

1. Shah P, Ding Y, Niemczyk M, Kudla G, Plotkin JB. Rate-limiting steps in yeast protein translation. *Cell* 2013, 153:1589–1601.
2. Warner JR. The economics of ribosome biosynthesis in yeast. *Trends Biochem Sci* 1999, 24:437–440.
3. Sloan KE, Bohnsack MT, Schneider C, Watkins NJ. The roles of SSU processome components and surveillance factors in the initial processing of human ribosomal RNA. *RNA (New York, NY)* 2014, 20:540–550.
4. Hamperl S, Wittner M, Babl V, Perez-Fernandez J, Tschochner H, Griesenbeck J. Chromatin states at ribosomal DNA loci. *Biochim Biophys Acta* 2013, 1829:405–417.
5. Vannini A, Cramer P. Conservation between the RNA polymerase I, II, and III transcription initiation machineries. *Mol Cell* 2012, 45:439–446.
6. Kos M, Tollervey D. Yeast pre-rRNA processing and modification occur cotranscriptionally. *Mol Cell* 2010, 37:809–820.
7. El Hage A, French SL, Beyer AL, Tollervey D. Loss of Topoisomerase I leads to R-loop-mediated transcriptional blocks during ribosomal RNA synthesis. *Genes Dev* 2010, 24:1546–1558.
8. Turowski TW. The impact of transcription on posttranscriptional processes in yeast. *Gene* 2013, 526:23–29.
9. Schneider DA. RNA polymerase I activity is regulated at multiple steps in the transcription cycle: recent insights into factors that influence transcription elongation. *Gene* 2012, 493:176–184.
10. Engel C, Sainsbury S, Cheung AC, Kostrewa D, Cramer P. RNA polymerase I structure and transcription regulation. *Nature* 2013, 502:650–655.
11. Fernández-Tornero C, Moreno-Morcillo M, Rashid UJ, Taylor NMI, Ruiz FM, Gruene T, Legrand P, Steuerwald U, Müller CW. Crystal structure of the 14-subunit RNA polymerase I. *Nature* 2013, 502:644–649.
12. Zomerdijk J. Structural biology: Pivotal findings for a transcription machine. *Nature* 2013, 502:629–630.
13. Osheim YN, French SL, Keck KM, Champion EA, Spasov K, Dragon F, Baserga SJ, Beyer AL. Pre-18S ribosomal RNA is structurally compacted into the SSU processome prior to being cleaved from nascent transcripts in Saccharomyces cerevisiae. *Mol Cell* 2004, 16:943–954.
14. Elela SA, Igel H, Ares M Jr. RNase III cleaves eukaryotic preribosomal RNA at a U3 snoRNP-dependent site. *Cell* 1996, 85:115–124.
15. Kufel J, Dichtl B, Tollervey D. Yeast Rnt1p is required for cleavage of the pre-ribosomal RNA in the 3′ ETS but not the 5′ ETS. *RNA (New York, NY)* 1999, 5:909–917.
16. El Hage A, Koper M, Kufel J, Tollervey D. Efficient termination of transcription by RNA polymerase I requires the 5′ exonuclease Rat1 in yeast. *Genes Dev* 2008, 22:1069–1081.

17. Kawauchi J, Mischo H, Braglia P, Rondon A, Proudfoot NJ. Budding yeast RNA polymerases I and II employ parallel mechanisms of transcriptional termination. *Genes Dev* 2008, 22:1082–1092.
18. Woolford JL Jr, Baserga SJ. Ribosome biogenesis in the yeast Saccharomyces cerevisiae. *Genetics* 2013, 195:643–681.
19. Sardana R, White JP, Johnson AW. The rRNA methyltransferase Bud23 shows functional interaction with components of the SSU processome and RNase MRP. *RNA (New York, NY)* 2013, 19:828–840.
20. Kressler D, Hurt E, Bassler J. Driving ribosome assembly. *Biochim Biophys Acta* 2010, 1803:673–683.
21. Lempiäinen H, Shore D. Growth control and ribosome biogenesis. *Curr Opin Cell Biol* 2009, 21:855–863.
22. Phipps KR, Charette JM, Baserga SJ. The small subunit processome in ribosome biogenesis—progress and prospects. *WIREs RNA* 2011, 2:1–21.
23. Venema J, Tollervey D. Ribosome synthesis in Saccharomyces cerevisiae. *Annu Rev Genet* 1999, 33:261–311.
24. Dragon F, Gallagher JEG, Compagnone-Post PA, Mitchell BM, Porwancher KA, Wehner KA, Wormsley S, Settlage RE, Shabanowitz J, Osheim Y, et al. A large nucleolar U3 ribonucleoprotein required for 18S ribosomal RNA biogenesis. *Nature* 2002, 417:967–970.
25. Sharma K, Venema J, Tollervey D. The 5′ end of the 18S rRNA can be positioned from within the mature rRNA. *RNA (New York, NY)* 1999, 5:678–686.
26. Axt K, French SL, Beyer AL, Tollervey D. Kinetic analysis demonstrates a requirement for the Rat1 exonuclease in cotranscriptional pre-rRNA cleavage. *PLoS One* 2014, 9:e85703.
27. Sahasranaman A, Dembowski J, Strahler J, Andrews P, Maddock J, Woolford JL Jr. Assembly of Saccharomyces cerevisiae 60S ribosomal subunits: role of factors required for 27S pre-rRNA processing. *EMBO J* 2011, 30:4020–4032.
28. Lebaron S, Segerstolpe A, French SL, Dudnakova T, de Lima Alves F, Granneman S, Rappsilber J, Beyer AL, Wieslander L, Tollervey D. Rrp5 binding at multiple sites coordinates pre-rRNA processing and assembly. *Mol Cell* 2013, 52:707–719.
29. Horn DM, Mason SL, Karbstein K. Rcl1 protein, a novel nuclease for 18S ribosomal RNA production. *J Biol Chem* 2011, 286:34082–34087.
30. Bleichert F, Granneman S, Osheim YN, Beyer AL, Baserga SJ. The PINc domain protein Utp24, a putative nuclease, is required for the early cleavage steps in 18S rRNA maturation. *Proc Natl Acad Sci USA* 2006, 103:9464–9469.
31. Hartman E, Wang Z, Zhang Q, Roy K, Chanfreau G, Feigon J. Intrinsic dynamics of an extended hydrophobic core in the S. cerevisiae RNase III dsRBD contributes to recognition of specific RNA binding sites. *J Mol Biol* 2013, 425:546–562.
32. Piper PW, Stråby KB. Processing of transcripts of a dimeric tRNA gene in yeast uses the nuclease responsible for maturation of the 3′ termini upon 5 S and 37 S precursor rRNAs. *FEBS Lett* 1989, 250:311–316.
33. Van Hoof A, Lennertz P, Parker R. Three conserved members of the RNase D family have unique and overlapping functions in the processing of 5S, 5.8S, U4, U5, RNase MRP and RNase P RNAs in yeast. *EMBO J* 2000, 19:1357–1365.
34. Czerwoniec A, Dunin-Horkawicz S, Purta E, Kaminska KH, Kasprzak JM, Bujnicki JM, Grosjean H, Rother K. MODOMICS: a database of RNA modification pathways. 2008 update. *Nucleic Acids Res* 2009, 37:D118–121.
35. Dunin-Horkawicz S, Czerwoniec A, Gajda MJ, Feder M, Grosjean H, Bujnicki JM. MODOMICS: a database of RNA modification pathways. *Nucleic Acids Res* 2006, 34:D145–149.
36. Machnicka MA, Milanowska K, Osman Oglou O, Purta E, Kurkowska M, Olchowik A, Januszewski W, Kalinowski S, Dunin-Horkawicz S, Rother KM, et al. MODOMICS: a database of RNA modification pathways—2013 update. *Nucleic Acids Res* 2013, 41:D262–267.
37. Ge J, Yu Y-T. RNA pseudouridylation: new insights into an old modification. *Trends Biochem Sci* 2013, 38:210–218.
38. Watkins NJ, Bohnsack MT. The box C/D and H/ACA snoRNPs: key players in the modification, processing and the dynamic folding of ribosomal RNA. *WIREs RNA* 2012, 3:397–414.
39. Motorin Y, Helm M. tRNA stabilization by modified nucleotides. *Biochemistry* 2010, 49:4934–4944.
40. Jack K, Bellodi C, Landry DM, Niederer RO, Meskauskas A, Musalgaonkar S, Kopmar N, Krasnykh O, Dean AM, Thompson SR, et al. rRNA pseudouridylation defects affect ribosomal ligand binding and translational fidelity from yeast to human cells. *Mol Cell* 2011, 44:660–666.
41. Motorin Y, Helm M. RNA nucleotide methylation. *WIREs RNA* 2011, 2:611–631.
42. Rocchi L, Pacilli A, Sethi R, Penzo M, Schneider RJ, Treré D, Brigotti M, Montanaro L. Dyskerin depletion increases VEGF mRNA internal ribosome entry site-mediated translation. *Nucleic Acids Res* 2013, 41:8308–8318.
43. Buchhaupt M, Sharma S, Kellner S, Oswald S, Paetzold M, Peifer C, Watzinger P, Schrader J, Helm M, Entian K-D. Partial methylation at Am100 in 18S rRNA of baker's yeast reveals ribosome heterogeneity on the level of eukaryotic rRNA modification. *PLoS One* 2014, 9:e89640.
44. Granneman S, Baserga SJ. Ribosome biogenesis: of knobs and RNA processing. *Exp Cell Res* 2004, 296:43–50.

45. Savino R, Gerbi SA. In vivo disruption of Xenopus U3 snRNA affects ribosomal RNA processing. *EMBO J* 1990, 9:2299–2308.

46. Shah BN, Liu X, Correll CC. Imp3 unfolds stem structures in pre-rRNA and U3 snoRNA to form a duplex essential for small subunit processing. *RNA (New York, NY)* 2013, 19:1372–1383.

47. De Boer P, Vos HR, Faber AW, Vos JC, Raué HA. Rrp5p, a trans-acting factor in yeast ribosome biogenesis, is an RNA-binding protein with a pronounced preference for U-rich sequences. *RNA (New York, NY)* 2006, 12:263–271.

48. Torchet C, Jacq C, Hermann-Le Denmat S. Two mutant forms of the S1/TPR-containing protein Rrp5p affect the 18S rRNA synthesis in Saccharomyces cerevisiae. *RNA (New York, NY)* 1998, 4:1636–1652.

49. Venema J, Tollervey D. RRP5 is required for formation of both 18S and 5.8S rRNA in yeast. *EMBO J* 1996, 15:5701–5714.

50. Hierlmeier T, Merl J, Sauert M, Perez-Fernandez J, Schultz P, Bruckmann A, Hamperl S, Ohmayer U, Rachel R, Jacob A, et al. Rrp5p, Noc1p and Noc2p form a protein module which is part of early large ribosomal subunit precursors in S. cerevisiae. *Nucleic Acids Res* 2013, 41:1191–1210.

51. Young CL, Khoshnevis S, Karbstein K. Cofactor-dependent specificity of a DEAD-box protein. *Proc Natl Acad Sci USA* 2013, 110:E2668–2676.

52. Jakob S, Ohmayer U, Neueder A, Hierlmeier T, Perez-Fernandez J, Hochmuth E, Deutzmann R, Griesenbeck J, Tschochner H, Milkereit P. Interrelationships between yeast ribosomal protein assembly events and transient ribosome biogenesis factors interactions in early pre-ribosomes. *PLoS One* 2012, 7:e32552.

53. Ohmayer U, Gamalinda M, Sauert M, Ossowski J, Pöll G, Linnemann J, Hierlmeier T, Perez-Fernandez J, Kumcuoglu B, Leger-Silvestre I, et al. Studies on the assembly characteristics of large subunit ribosomal proteins in S. cerevisae. *PLoS One* 2013, 8: e68412.

54. Alexander RD, Innocente SA, Barrass JD, Beggs JD. Splicing-dependent RNA polymerase pausing in yeast. *Mol Cell* 2010, 40:582–593.

55. Carrillo Oesterreich F, Preibisch S, Neugebauer KM. Global analysis of nascent RNA reveals transcriptional pausing in terminal exons. *Mol Cell* 2010, 40: 571–581.

56. Alexander R, Beggs JD. Cross-talk in transcription, splicing and chromatin: who makes the first call? *Biochem Soc Trans* 2010, 38:1251–1256.

57. Nogi Y, Yano R, Nomura M. Synthesis of large rRNAs by RNA polymerase II in mutants of Saccharomyces cerevisiae defective in RNA polymerase I. *Proc Natl Acad Sci USA* 1991, 88:3962–3966.

58. Schneider DA, Michel A, Sikes ML, Vu L, Dodd JA, Salgia S, Osheim YN, Beyer AL, Nomura M. Transcription elongation by RNA polymerase I is linked to efficient rRNA processing and ribosome assembly. *Mol Cell* 2007, 26:217–229.

59. Gallagher JEG, Dunbar DA, Granneman S, Mitchell BM, Osheim Y, Beyer AL, Baserga SJ. RNA polymerase I transcription and pre-rRNA processing are linked by specific SSU processome components. *Genes Dev* 2004, 18:2506–2517.

60. Kong R, Zhang L, Hu L, Peng Q, Han W, Du X, Ke Y. hALP, a novel transcriptional U three protein (t-UTP), activates RNA polymerase I transcription by binding and acetylating the upstream binding factor (UBF). *J Biol Chem* 2011, 286:7139–7148.

61. Pelletier G, Stefanovsky VY, Faubladier M, Hirschler-Laszkiewicz I, Savard J, Rothblum LI, Côté J, Moss T. Competitive recruitment of CBP and Rb-HDAC regulates UBF acetylation and ribosomal transcription. *Mol Cell* 2000, 6:1059–1066.

62. Prieto J-L, McStay B. Recruitment of factors linking transcription and processing of pre-rRNA to NOR chromatin is UBF-dependent and occurs independent of transcription in human cells. *Genes Dev* 2007, 21:2041–2054.

63. Scheer U. Contributions of electron microscopic spreading preparations ('Miller spreads') to the analysis of chromosome structure. *Results Probl Cell Differ* 1987, 14:147–171.

64. Scheer U, Benavente R. Functional and dynamic aspects of the mammalian nucleolus. *BioEssays* 1990, 12:14–21.

65. Srivastava A, Ahamad J, Ray AK, Kaur D, Bhattacharya A, Bhattacharya S. Analysis of U3 snoRNA and small subunit processome components in the parasitic protist Entamoeba histolytica. *Mol Biochem Parasitol* 2014, 193:82–92.

66. Hartshorne T, Toyofuku W. Two 5′-ETS regions implicated in interactions with U3 snoRNA are required for small subunit rRNA maturation in Trypanosoma brucei. *Nucleic Acids Res* 1999, 27:3300–3309.

67. Mougey EB, O'Reilly M, Osheim Y, Miller OL Jr, Beyer A, Sollner-Webb B. The terminal balls characteristic of eukaryotic rRNA transcription units in chromatin spreads are rRNA processing complexes. *Genes Dev* 1993, 7:1609–1619.

68. Sloan KE, Schneider C, Watkins NJ. Comparison of the yeast and human nuclear exosome complexes. *Biochem Soc Trans* 2012, 40:850–855.

69. Houseley J, Tollervey D. The many pathways of RNA degradation. *Cell* 2009, 136:763–776.