A surfeit of factors

A Surfeit of Factors: Why is Ribosome Assembly So Much More Complicated in Eukaryotes than Bacteria?

Article *in* RNA biology · June 2004

DOI: 10.4161/rna.1.1.932 · Source: PubMed

2 authors:

Aziz El Hage
The University of Edinburgh
13 PUBLICATIONS **662** CITATIONS
SEE PROFILE

David Tollervey
The University of Edinburgh
382 PUBLICATIONS **24,857** CITATIONS
SEE PROFILE

[RNA Biology 1:1, 10-15; May/June 2004]; ©2004 Landes Bioscience

Review

A Surfeit of Factors

Why is Ribosome Assembly so Much More Complicated in Eukaryotes than Bacteria?

Aziz El Hage

David Tollervey*

Wellcome Trust Centre for Cell Biology, Edinburgh, Scotland

*Correspondence to: David Tollervey; Wellcome Trust Centre for Cell Biology; University of Edinburgh, EH9 3JR, UK; Tel.: 44.131.650.7092; Fax: 44.131.650.7040; Email: d.tollervey@ed.ac.uk

Received 03/15/04; Accepted 04/19/04

Previously published online as a *RNA Biology* E-publication: http://www.landesbioscience.com/journals/rnabiology/abstract.php?id=932

KEY WORDS

nucleolus, ribosome, yeast, *E. coli*, RNA processing, RNA-protein interactions, evolution

ACKNOWLEDGEMENTS

We would like to thank Philip Mitchell and Christophe Dez for critical reading of the manuscript.

This work was supported by the Wellcome Trust.

ABSTRACT

Recent years have seen a dramatic increase in the number of ribosome synthesis factors identified in the yeast *Saccharomyces cerevisiae*. Most of these are not predicted to directly catalyze either RNA processing or modification, and they are therefore predicted to function in some sense as assembly factors, promoting the assembly and/or disassembly of the processing and modification machinery, binding of the ribosomal proteins and correct folding of the pre-rRNAs and rRNAs. In contrast, ribosome synthesis in *E. coli*, which has also been extensively analyzed, appears to involve a very small number of potential assembly factors. Here we will consider the differences between eukaryotic and bacterial ribosome synthesis that may underlie this distinction.

The structure and function of ribosomes are believed to be conserved throughout all domains of life, with mRNA decoding functions performed by the bacterial 30S or eukaryotic 40S subunits and peptide bond formation catalyzed by the bacterial 50S or eukaryotic 60S subunit. In all organisms the rRNAs presumably fold into similar, tightly packed, core structures and some, although by no means all, ribosomal proteins (r-proteins) are also conserved throughout evolution. The basic pathway of rRNA synthesis also appears to be remarkably well conserved. The structure and polarity of the pre-ribosomal RNA, in which the small subunit rRNA is cotranscribed with and positioned 5' to the major large subunit rRNAs, is conserved in almost all bacteria, archaea and eukaryotes (see Fig. 1). Similarly, processing of the pre-rRNAs to the mature rRNAs seems rather similar, at least in the few organisms in which this has been examined in any detail. Initial cleavages separate the individual rRNA precursors, which then undergo subsequent 5' and 3' processing to generate the mature rRNAs. Moreover, several of the pre-rRNA processing enzymes are clearly homologous in *Escherichia coli* and the budding yeast *Saccharomyces cerevisiae*, the two organisms in which these have been best characterized (reviewed in refs. 1 and 2). In all organisms studied ribosome assembly proceeds via a complex multi-step pathway but there appear to be substantial differences in subunit assembly in yeast and bacteria.

In *E. coli*, functional subunits can be assembled in vitro from purified rRNA and r-proteins, but this requires conditions that are far from physiological (reviewed in refs. 3 and 4). In vivo, genetic and biochemical analyses have identified many rRNA processing and modifying enzymes,[4,5] but only a small number of nonribosomal factors have been reported to participate in ribosome assembly (reviewed in ref. 3) (Table 1). Deletion or mutation of two protein chaperones DnaK or GroEL or two putative RNA helicases CsdA or SrmB causes defects in ribosome assembly at restrictive temperature, that are characterized by the accumulation of discrete pre-ribosomal particles (Iost I, personal communication and refs. 6–8). An additional helicase DbpA binds to a specific region of the 23S rRNA and is therefore also likely to function in 50S assembly.[9,10] Two putative RNA chaperones RbfA and RimM, and the GTPase Era, may play roles in pre-16S rRNA processing and late 30S assembly.[11,12] The overexpression of two further GTPases ObgE/YhbZ and EngA can suppress a ribosome synthesis defect caused by defective 23S rRNA modification, but their roles in ribosome synthesis have not otherwise been assessed.[13] Strikingly, all of these factors are dispensable for ribosome synthesis in vivo. They may therefore act to facilitate ribosome assembly, and are probably of particular importance when physiological conditions are not optimal. The RNA helicases and the RNA chaperone RbfA become essential at low temperatures,[7,12] whereas the protein chaperones are essential for ribosome assembly at elevated temperatures.[6,8] In vivo ribosome synthesis in *E. coli* is believed to proceed via an "assembly gradient", in which r-protein binding to the nascent rRNA transcript is coupled to its folding and maturation (Fig. 1A).[14] This probably restricts the possibilities

for misassembly and forces maturation down the productive pathway, thus limiting the need for nonribosomal assembly factors in vivo. Loss of this organizing principle for the assembly pathway during in vitro reconstitution might explain, in part, the requirement for non-physiological conditions.[4]

Ribosome assembly appears to be quite different in eukaryotes. Successful reconstitution of a functional eukaryotic subunit from rRNAs and r-proteins in vitro has never been reported, and in vivo ribosome synthesis requires a startlingly large number of trans-acting factors. In yeast, around 170 proteins and ~70 small nucleolar RNAs have been shown to participate in the post-transcriptional steps of ribosomal subunit synthesis. At least 100 proteins that are essential for ribosome production have no known, direct role in the maturation or modification of the rRNAs, and they are therefore assumed to function in the assembly and/or transport of the pre-ribosomes (reviewed in refs.15–19) (Table 1).

WHY ARE THE YEAST AND BACTERIAL SYSTEMS SO DIFFERENT?

One clear difference stems from the use of modification guide small nucleolar RNAs (snoRNAs) in eukaryotes. The description of the guide particles as small nucleolar ribonucleoprotein (snoRNP) complexes gives a slightly misleading impression of their size. Each box C/D snoRNP contains five proteins (two copies of the methyl transferase Nop1p/fibrillarin and one copy each of Nop56p/Sik1p, Nop58p/Nop5p and Snu13p/15.5 K)[20] with a single RNA, generally around 100nt in length, and has a total mass of ~230 KDa (reviewed in ref. 21). Each box H/ACA snoRNP contains eight proteins (two copies each of Nhp2p, Nop10p, Gar1p and the pseudouridine synthase Cbf5p/dyskerin) with one RNA, generally around 200 nt, and a total mass of ~260 KDa (reviewed in ref. 21). Every yeast 35S pre-rRNA molecule will associate with around 70 different snoRNPs, which would have a total mass (~17 MDa) that is much greater than mature 40S subunits (~1 MDa) or 60S subunits (~2 MDa) (Fig. 1B). Most snoRNPs bind to conserved regions of the rRNAs, which will subsequently form the tightly packed cores of the mature subunits. Labeling data from yeast indicates that methylation directed by the box C/D snoRNPs is not cotranscriptional but occurs immediately after the completion of pre-rRNA transcription (Fig. 1B).[22] Despite this observation, the snoRNPs might bind to their complementary sites as soon as they are transcribed, thereby preventing folding of the pre-rRNA. However, rapidly growing yeast cells synthesize around 2000 ribosomes per minute, while the abundance of most modification guide RNAs is estimated at less than 1000 molecules per cell. Transcription of the 7 Kb pre-rRNA requires around 4–5 minutes, corresponding to ~8000 nascent transcripts. This suggests that the snoRNAs cannot generally remain associated with the pre-rRNAs throughout transcription, at least in yeast. We propose that the yeast pre-rRNAs are actively prevented from fully folding during transcription, in order to allow the subsequent binding of the huge number of large snoRNP particles.

Following modification and snoRNP dissociation, the pre-rRNAs must presumably be refolded into radically different structures, and it seems likely that these structural rearrangements will require the input of energy. Moreover, the many trans-acting protein factors must rapidly and efficiently bind to, and dissociate from, the pre-ribosomal particles and this is also likely to require energy input. Consistent with a requirement for energy-dependent remodeling, many yeast ribosome synthesis factors are predicted to hydrolyze nucleotide tri-phosphates—either ATP or GTP. Ribosome synthesis factors in both yeast and *E. coli* include putative ATP-dependent RNA helicases and GTPases, but the numbers of these factors are significantly different. Eighteen putative helicases and 6 GTPases are required for yeast ribosome synthesis, in contrast to the 3 helicases and 3 GTPases that play roles in ribosome synthesis in *E. coli* (see Table 1).

The "RNA helicases" are large family of RNA-stimulated ATPases, very few of which have actually been shown to display a processive RNA unwinding activity (reviewed in ref. 23). For no "helicase" is the actual function in ribosome synthesis known, but it is likely that they function in remodeling RNA structures and RNA-protein interactions. In addition, the box C/D snoRNAs, in particular, form extended base-paired interactions with the pre-rRNA, which are predicted to be very stable at physiological temperatures and presumably require specific helicase-like activities for their dissociation.

GTPases function in the regulation of almost all aspects of cell metabolism and generally undergo large-scale structural alterations in response to GTP binding and hydrolysis. GTPases can be used to drive structural alterations and frequently have "proof-reading" functions, in which the timing of GTP hydrolysis determines the time allowed for productive processing. For example, during protein synthesis the binding and GTPase activity of the elongation factors EF-Tu and EF-G is dependent on correct binding of the A and P-site tRNAs and drives the physical movement of the ribosomal subunits and mRNA during the translation cycle (reviewed in refs. 24 and 25). Similarly, it can be envisaged that correct binding of ribosome synthesis factors and r-proteins or pre-rRNA processing is monitored by GTPases, with GTP hydrolysis allowing structural alterations that are required for subsequent steps in assembly and processing. Many GTPases function together with GTPase activating proteins (GAPs) and GDP/GTP exchange factors (GEFs). Whether other ribosome synthesis factors act as GAPs or GEFs for the pre-ribosome associated GTPases remains to be determined.

Other proteins that are likely to participate directly in the structural reorganization of eukaryotic pre-ribosomes are the AAA-ATPases, Rix7p[26] and Rea1p/Mdn1p.[27,28] Other members of the AAA-ATPase family catalyze the restructuring or unfolding of diverse protein complexes (the name is derived from ATPases Associated with various cellular Activities) including snoRNPs.[29] For further examples see references 30 and 31. The problems of subunit refolding may be particularly acute for the large ribosomal subunit, since structural analyses reveal a very complex core RNA fold that lacks clear domains,[32,33] and it may therefore be significant that Rix7p and Rea1p are each required for 60S subunit synthesis. AAA-ATPases frequently function as hexamers[30] but Rea1p is itself a pseudo-hexamer of AAA-ATPase protomers and, at 560 kDa, is the largest ORF in the yeast genome. Other factors likely to aid rRNA folding and/or reorganization are the Lsm2–8p proteins.[34] These form a seven membered ring structure and promote changes in RNA structure and RNA-protein interactions in many contexts (reviewed in ref. 35). The homologous Hfq complex from *E. coli* also promotes RNA interactions[36,37] and regulates RNase activity on an mRNA substrate,[38] but no role in ribosome synthesis has been reported. Conversely, no specific role in eukaryotic ribosome synthesis has been reported for any of the many heat-shock protein chaperones, including the yeast homologues of DnaK (Hsp70 family) and GroEL (Hsp60 family).

The large-scale structural rearrangements of the pre-ribosomes that we envisage in eukaryotes may well require a physical framework. Candidates for proteins that might help form such a framework are

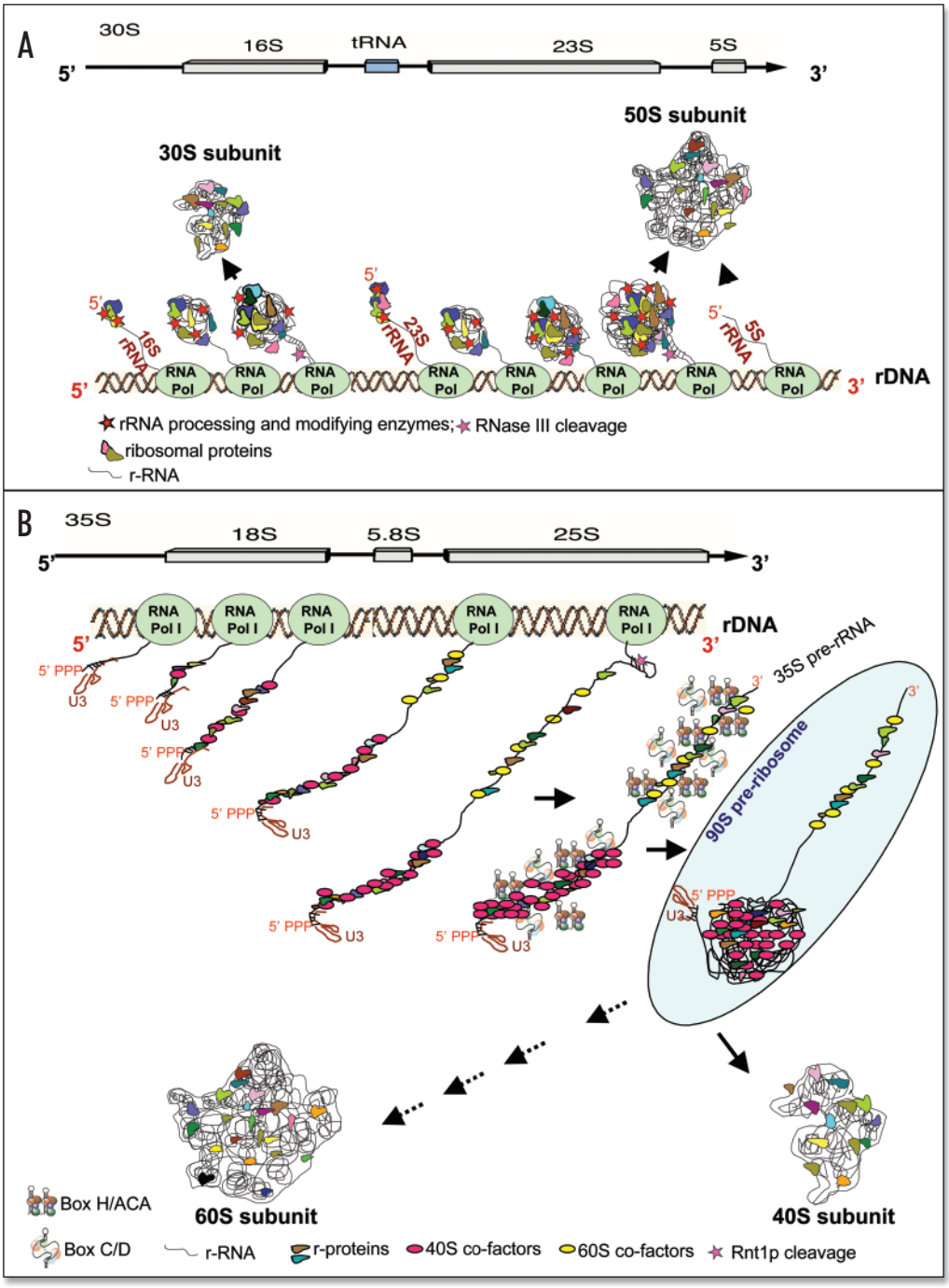

Figure 1. Models for ribosome synthesis in *E. coli* and *S. cerevisiae*. The pre-rRNAs have related structures in most organisms, with the small subunit 16S/18S rRNA and large subunit 23S/25-28S rRNA synthesized by processing from the same transcript. (A) In *E. coli* the rRNAs undergo largely cotranscriptional folding, maturation and assembly with the r-proteins. The precursors to the 16S and 23S rRNAs are cleaved from the nascent transcript by RNase III.[5] For clarity, subsequent maturation steps leading to the production of mature subunits are omitted. (B) In *S. cerevisiae*, the nascent transcript is also cleaved by RNase III (Rnt1p) releasing the 35S pre-rRNA, which then undergoes post-transcriptional snoRNP-directed modification. The early yeast pre-ribosomes that have been purified to date, termed the 90S pre-ribosomes or small-subunit processome,[41,66] contain several small subunit r-proteins and many factors required for 40S subunit synthesis, but largely lack 60S subunit r-proteins and processing factors. These purified early pre-ribosomes also lacked snoRNPs other than U3, but larger pre-ribosomes that contain the 35S pre-rRNA together with the modification guide snoRNAs are predicted to exist in vivo. We speculate that modification guide snoRNPs present in pre-ribosomal particles at the time of cell lysis, largely complete rRNA modification and dissociate from the pre-rRNA during the substantial period required for pre-ribosome purification. There is very little data on the timing of rRNA folding and small subunit r-protein binding in yeast. However, the requirement that the snoRNPs have access to the regions of the rRNAs that will form the core of the mature subunits suggests that the nascent transcripts must be maintained in a structure that is at least partially unfolded (see text for references).

Table 1 **Nonribosomal Proteins Potentially Involved in Remodeling Subunit Structure During Ribosome Synthesis**

E. coli		*S. cerevisiae*	
Putative helicases 30S		Putative helicases 40S	Fal1p (YDR021w) Dbp1p (YPL119c) Dbp4p/Hca1p (YJL033w) Dbp8p (YHR169W) Dhr1p (YMR128W) Dhr2p (YKL078W) Rok1p (YGL171W) Rrp3p (YHR065C)
Putative helicases 50S	DbpA CsdA SrmB	Putative helicases 60S	Dbp3p (YGL078C) Dbp7p (YKR024C) Dbp9p (YLR276C) Dbp10p (YDL031W) Drs1p (YLL008W) Has1p (YMR290C) Mak5p (YBR142w) Mtr4p/Dob1p (YJL050W) Spb4p (YFL002C) Rrb1p (YMR131c)
GTPases 30S	Era	GTPases 40S	Bms1p (YPL217C)
GTPases 50S	ObgE EngA	GTPases 60S	Nog1p (YPL093W) Nug1p (YER006W) Nog2p/Nug2p (YNR053C) Lsg1p/Kre35p (YGL099W) Ria1p/Efl1p (YNL163C)
Protein Chaperones 30S + 50S	DnaK		
Protein Chaperones 50S	GroEL		
Putative RNA chaperones 30S	RbfA RimM	Punative RNA chaperones AAA-ATPases 60S	Lsm2-8p complex Rea1p/Mdn1p; (YLR106C)
		HEAT repeat Proteins 40S	Rix7p (YLL034C) Utp10p (YJL109C) Utp20p (YBL004W) Noc4p (YPR144C)
		HEAT repeat Proteins 60S	Rrp12p (YPL012W)* Noc1p (YDR060W) Noc2p (YOR206W) Noc3p (YLR002C) Sda1p (YGR245C) Rrp12p (YPL012W)*

*Rrp12p participates in the maturation and export of both subunits.

a family of large, HEAT repeat proteins that are predicted to have a curved extended structure.[39,40] The Utp20p (280 kDa) and Utp10p (210 kDa) proteins are components of the early pre-ribosomes in which snoRNP-directed modification is believed to occur[41] and each is predicted to be long enough to wrap around the outside of most of a ribosomal subunit. In addition, many ribosome synthesis factors carry putative protein interaction domains, notably WD and TPR repeats and potential coiled-coil regions (Dlakic M, personal communication). Together these may form large, relatively rigid, protein complexes that provide the structural underpinning of the assembly system.

In *E. coli* all of the trans-acting factors are dispensable for ribosome assembly under optimal conditions, but their absence or mutation frequently leads to slowed ribosome synthesis with substantial pre-ribosome accumulation under restrictive conditions. In contrast, the large majority of yeast ribosome synthesis factors are strictly essential for ribosome synthesis and mutants do not accumulate substantial levels of pre-ribosomes. Most yeast processing mutants show a mild accumulation of one or more pre-rRNA species followed by their rapid and complete degradation. The exceptions are strains defective in the late, cytoplasmic processing of the 20S pre-rRNA to 18S rRNA.[42,43] These observations suggest that surveillance of defects in ribosome synthesis is much more stringent in yeast than *E. coli*. Defective yeast pre-rRNAs are degraded, at least in part, by the exosome complex of 3' to 5' exonucleases.[44] Defective rRNAs in *E. coli* are degraded by RNase R and PNPase,[45] which are homologous to exosome components, suggesting that the basic process has been conserved throughout evolution. How the degradation system identifies defects in ribosome or pre-ribosome structures that result from the absence or mutation of any single protein remains unclear.

Another major difference between *E. coli* and yeast is in the compartmentalization of the ribosome synthesis machinery. In *E. coli* all of the steps in ribosome synthesis occur in a single compartment. In eukaryotes this process is partitioned between physically discrete regions of the cell. Initial processing occurs in the dense fibrillar component of the nucleolus, with much of the subsequent subunit assembly in the granular region of the nucleolus. The pre-ribosomes are then released into the nucleoplasm, accompanied by the dissoci-

ation of many of the ribosome synthesis factors. Finally, the pre-ribosomes must be transported through the nuclear pore complexes to the cytoplasm where maturation into functional subunits takes place. Transfer of the pre-ribosomes between nucleolar compartments and release into the nucleoplasm may require specific factors.[46] Nucleo-cytoplasmic transport of 40S and 60S subunits in both yeast and vertebrates requires Xpo1p/Crm1p, a member of the large Importin-β or Karyopherin-β (Imp-β/Kap-β) family of transport factors (reviewed in refs.17 and 47). These share a structure comprised of HEAT repeats and have homologous binding sites for the small GTPase Ran (Gsp1/2p in yeast), which plays a key role in the directionality of nuclear import and export (reviewed in refs. 48 and 49). Xpo1p/Crm1p associates with the pre-60S subunit via an adaptor protein, Nmd3p, which may in turn bind to the 60S r-protein Rpl10p.[50-53] How Xpo1p/Crm1p interacts with the 40S subunit remains to be determined. Another family of HEAT-repeat proteins has been implicated in ribosome subunit export (see Table 1).[39,40] These proteins are directly associated with the pre-ribosomes, and we speculate that they help mediate transport of both subunits, perhaps following the initiation of export by Xpo1p/Crm1p. It is clear that the nuclear export of ribosomal subunits in eukaryotes involves numerous factors that will not be required in *E. coli*. Perhaps even more significantly, the physical separation of different steps in ribosome synthesis may have permitted the elaboration of systems that are too complex to work efficiently were all of the components simply to be mixed together. This may have been a major factor in the increased complexity of eukaryotic ribosome synthesis.

In eukaryotes, most newly synthesized r-proteins are imported into the nucleus prior to their association with the nascent pre-ribosomes, whereas r-proteins in *E. coli* probably bind to the nascent pre-rRNAs directly following their translation. Eukaryotic r-proteins are imported in complex with members of the Imp-β/Kap-β family (reviewed in refs. 48 and 49) and these interactions have additional functions in preventing premature or inappropriate RNA binding by the r-proteins.[54] Import of cargo proteins, including r-proteins, that are bound to Imp-β/Kap-β factors occurs in association with the GDP-bound form of Ran (reviewed in refs. 48 and 49). Release of the cargo from this complex requires the interaction of Ran-GDP with its GEF, RCC1 (Srm1p/Prp20p in yeast), which promotes exchange of Ran-GDP to Ran-GTP. It could be envisaged that this system does not simply deliver r-proteins into the nucleoplasm. Rather, r-proteins may be transferred to nucleolar carrier proteins, or directly delivered to the nascent pre-ribosomes. This offers a potential means of regulating the timing of r-protein assembly and preventing their premature association with the pre-ribosomes. Unfortunately the many recent proteomic analyses of yeast pre-ribosomes have not yet allowed the clear determination of the order of in vivo r-protein association.[19] No detailed r-protein assembly pathway is therefore available for eukaryotes, but older data show that there are at least early and late binding r-proteins.[67]

CONCLUSION

We envisage substantial differences in the organization of ribosome assembly between bacteria and eukaryotes. In *E. coli* folding and modification of the rRNA, assembly with the r-proteins and initial cleavage of the pre-rRNA are largely cotranscriptional (Fig. 1A). In contrast, the yeast rRNA precursors undergo post-transcriptional modification and processing, with the exception of the 3' cleavage that liberates the 35S pre-rRNA (Fig. 1B).[55] This large (7 Kb) RNA must initially be prevented from folding in order to allow access of the snoRNPs and other factors, to regions that are destined to form the compact cores of the ribosomal subunits. Following modification and snoRNP dissociation, the pre-ribosomes presumably undergo very extensive structural rearrangements. The numerous ATPases, helicases and GTPases presumably drive and monitor this reorganization. Many of the differences in the numbers of ribosome assembly factors in yeast and *E. coli* can potentially be attributed to the use of modification guide snoRNPs in eukaryotes. Modification guide sRNPs are also present in Archaea[56,57] and many other ribosome synthesis factors are closely conserved between eukaryotes and archaea. It seems likely that factors that act together in ribosome synthesis will show similar patterns of evolutionary conservation. Detailed analyses of the patterns of protein conservation between eukaryotes and archaea might therefore be useful in predicting functional interactions.

Connections between the ribosome synthesis machinery and cell cycle progression have recently emerged from analyses in yeast (see, for example, refs. 58–63) and long-standing observations link alterations in nucleolar morphology with malignant transformation in human cells (reviewed in ref. 64). Moreover, several RNA processing activities in addition to ribosome synthesis are localized to the eukaryotic nucleolus (reviewed in ref. 65). It appears that the large number of ribosome synthesis factors in eukaryotes has allowed the elaboration of many additional functions.

References

1. Lafontaine DL, Tollervey D. The function and synthesis of ribosomes. Nat Rev Mol Cell Biol 2001; 2:514-20.
2. Symmons MF, Williams MG, Luisi BF, Jones GH, Carpousis AJ. Running rings around RNA: A superfamily of phosphate-dependent RNases. Trends Biochem Sci 2002; 27:11-8.
3. Culver GM. Assembly of the 30S ribosomal subunit. Biopolymers 2003; 68:234-49.
4. Nierhaus KH. The assembly of prokaryotic ribosomes. Biochimie 1991; 73:739-55.
5. Srivastava AK, Schlessinger D. Mechanism and regulation of bacterial ribosomal RNA processing. Ann Rev Microbiol 1990; 44:105-29.
6. El Hage A, Sbai M, Alix JH. The chaperonin GroEL and other heat-shock proteins, besides DnaK, participate in ribosome biogenesis in *Escherichia coli*. Mol Gen Genet 2001; 264:796-808.
7. Charollais J, Pflieger D, Vinh J, Dreyfus M, Iost, I. The DEAD-box RNA helicase SrmB is involved in the assembly of 50S ribosomal subunits in *Escherichia coli*. Mol Microbiol 2003; 48:1253-65.
8. El Hage A, Alix JH. Authentic precursors to ribosomal subunits accumulate in *Escherichia coli* in the absence of functional DnaK chaperone. Mol Microbiol 2004; 51:189-201.
9. Pugh GE, Nicol SM, Fuller-Pace FV. Interaction of the *Escherichia coli* DEAD box protein DbpA with 23 S ribosomal RNA. J Mol Biol 1999; 292:771-8.
10. Diges CM, Uhlenbeck OC. *Escherichia coli* DbpA is an RNA helicase that requires hairpin 92 of 23S rRNA. EMBO J 2001; 20:5503-12.
11. Inoue K, Alsina J, Chen J, Inouye M. Suppression of defective ribosome assembly in a rbfA deletion mutant by overexpression of Era, an essential GTPase in *Escherichia coli*. Mol Microbiol 2003; 48:1005-16.
12. Xia B, Ke H, Shinde U, Inouye M. The role of RbfA in 16S rRNA processing and cell growth at low temperature in *Escherichia coli*. J Mol Biol 2003; 332:575-84.
13. Tan J, Jakob U, Bardwell JC. Overexpression of two different GTPases rescues a null mutation in a heat-induced rRNA methyltransferase. J Bacteriol 2002; 184:2692-8.
14. Lewicki BT, Margus T, Remme J, Nierhaus KH. Coupling of rRNA transcription and ribosomal assembly in vivo. Formation of active ribosomal subunits in *Escherichia coli* requires transcription of rRNA genes by host RNA polymerase which cannot be replaced by bacteriophage T7 RNA polymerase. J Mol Biol 1993; 231:581-93.
15. Venema J, Tollervey D. Ribosome synthesis In *Saccharomyces cerevisiae*. Ann Rev Gen 1999; 33:261-311.
16. Fatica A, Tollervey D. Making ribosomes. Curr Opin Cell Biol 2002; 14:313-8.
17. Tschochner H, Hurt E. Preribosomes on the road from the nucleolus to the cytoplasm. Trends Cell Biol 2003; 13:255-63.
18. Fromont-Racine M, Senger B, Saveanu C, Fasiolo F. Ribosome assembly in eukaryotes. Gene 2003; 313:17-42.
19. de la Cruz J, Kressler D, Linder P. Ribsomal subunit assembly. In: The Nucleolus. In: Olson MOJ, ed. KluwerAcademic / Plenum Publishers, 2003:262-90.
20. Szewczak LB, DeGregorio SJ, Strobel SA, Steitz JA. Exclusive interaction of the 15.5 kD protein with the terminal box C/D motif of a methylation guide snoRNP. Chem Biol 2002; 9:1095-107.
21. Kiss T. Small nucleolar RNAs: An abundant group of noncoding RNAs with diverse cellular functions. Cell 2002; 109:145-8.

22. Udem SA, Warner JR. Ribosomal RNA synthesis in Saccharomyces cerevisiae. J Mol Biol 1972; 65:227-42.
23. Rocak S, Linder P. DEAD-box proteins: The driving forces behind RNA metabolism. Nat Rev Mol Cell Biol 2004; 5:232-41.
24. Rodnina MV, Stark H, Savelsbergh A, Wieden HJ, Mohr D, Matassova NB, et al. GTPases mechanisms and functions of translation factors on the ribosome. Biol Chem 2000; 381:377-87.
25. Wilson DN, Blaha G, Connell SR, Ivanov PV, Jenke H, Stelzl U, et al. Protein synthesis at atomic resolution: Mechanistics of translation in the light of highly resolved structures for the ribosome. Curr Protein Pept Sci 2002; 3:1-53.
26. Gadal O, Strauss D, Braspenning J, Hoepfner D, Petfalski E, Philippsen P, et al. A nuclear AAA-type ATPase (Rix7p) is required for biogenesis and nuclear export of 60S ribosomal subunits. EMBO J 2001; 20:3695-704.
27. Nissan TA, Baßler J, Petfalski E, Tollervey D, Hurt E. 60S preribosome formation viewed from assembly in the nucleolus until export to the cytoplasm. EMBO J 2002; 21:5539-47.
28. Babler J, Grandi P, Gadal O, Leßmann T, Petfalski E, Tollervey D, et al. Identification of a 60S preribosomal particle that is closely linked to nuclear export. Mol Cell 2001; 8:517-29.
29. King TH, Decatur WA, Bertrand E, Maxwell ES, Fournier MJ. A well-connected and conserved nucleoplasmic helicase is required for production of box C/D and H/ACA snoRNAs and localization of snoRNP proteins. Mol Cell Biol 2001; 21:7731-46.
30. Lupas AN, Martin J. AAA proteins. Curr Opin Struct Biol 2002; 12:746-53.
31. Cheeseman IM, Desai A. Cell division: AAAtacking the mitotic spindle. Curr Biol 2004; 14:R70-2.
32. Ban N, Nissen P, Hansen J, Moore PB, Steitz TA. The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. Science 2000; 289:905-20.
33. Yusupov MM, Yusupova GZ, Baucom A, Lieberman K, Earnest TN, Cate JH, et al. Crystal structure of the ribosome at 5.5 A resolution. Science 2001; 292:883-96.
34. Kufel J, Allmang C, Petfalski E, Beggs J, Tollervey D. Lsm Proteins are required for normal processing and stability of ribosomal RNAs. J Biol Chem 2003; 278:2147-56.
35. He W, Parker R. Functions of Lsm proteins in mRNA degradation and splicing. Curr Opin Cell Biol 2000; 12:346-50.
36. Zhang A, Wassarman KM, Ortega J, Steven AC, Storz G. The Sm-like Hfq protein increases OxyS RNA interaction with target mRNAs. Mol Cell 2002; 9:11-22.
37. Moller T, Franch T, Hojrup P, Keene DR, Bachinger HP, Brennan RG, et al. Hfq. A bacterial Sm-like protein that mediates RNA-RNA interaction. Mol Cell 2002; 9:23-30.
38. Folichon M, Arluison V, Pellegrini O, Huntzinger E, Regnier P, Hajnsdorf E. The poly(A) binding protein Hfq protects RNA from RNase E and exoribonucleolytic degradation. Nucleic Acids Res 2003; 31:7302-10.
39. Dlakic M, Tollervey D. The Noc proteins involved in ribosome synthesis and export contain divergent HEAT repeats. RNA 2004; 10:351-4.
40. Oeffinger M, Dlakic M, Tollervey D. A preribosome-associated HEAT-repeat protein is required for export of both ribosomal subunits. Genes Dev 2004; 18:196-209.
41. Dragon F, Gallagher JE, Compagnone-Post PA, Mitchell BM, Porwancher KA, Wehner KA, et al. A large nucleolar U3 ribonucleoprotein required for 18S ribosomal RNA biogenesis. Nature 2002; 417:967-70.
42. Fatica A, Oeffinger M, Dlakic M, Tollervey D. Nob1p is required for cleavage of the 3' end of 18S rRNA. Mol Cell Biol 2003; 23:1798-807.
43. Vanrobays E, Gelugne JP, Gleizes PE, Caizergues-Ferrer M. Late cytoplasmic maturation of the small ribosomal subunit requires RIO proteins in Saccharomyces cerevisiae. Mol Cell Biol 2003; 23:2083-95.
44. Allmang C, Mitchell P, Petfalski E, Tollervey D. Degradation of ribosomal RNA precursors by the exosome. Nucleic Acids Res 2000; 28:1684-91.
45. Cheng Z-F, Deutscher MP. Quality control of ribosomal RNA mediated by polynucleotide phosphorylase and RNase R. Proc Natl Acad Sci USA 2003; 100:6388-93.
46. Milkereit P, Gadal O, Podtelejnikov A, Trumtel S, Gas N, Petfalski E, et al. Maturation and intranuclear transport of preribosomes requires Noc proteins. Cell 2001; 105:499-509.
47. Johnson AW, Lund E, Dahlberg J. Nuclear export of ribosomal subunits. Trends Biochem Sci 2002; 27:580-5.
48. Nakielny S, Dreyfuss G. Transport of proteins and RNAs in and out of the nucleus. Cell 1999; 99:677-90.
49. Weis K. Nucleocytoplasmic transport: Cargo trafficking across the border. Curr Opin Cell Biol 2002; 14:328-35.
50. Ho JH, Kallstrom G, Johnson AW. Nmd3p is a Crm1p-dependent adapter protein for nuclear export of the large ribosomal subunit. J Cell Biol 2000; 151:1057-66.
51. Gadal O, Strauss D, Kessl J, Trumpower B, Tollervey D, Hurt E. Nuclear export of 60s ribosomal subunits depends on Xpo1p and requires a nuclear export sequence-containing factor, Nmd3p, that associates with the large subunit protein Rpl10p. Mol Cell Biol 2001; 21:3405-15.
52. Thomas F, Kutay U. Biogenesis and nuclear export of ribosomal subunits in higher eukaryotes depend on the CRM1 export pathway. J Cell Sci 2003; 116:2409-19.
53. Trotta CR, Lund E, Kahan L, Johnson AW, Dahlberg JE. Coordinated nuclear export of 60S ribosomal subunits and NMD3 in vertebrates. EMBO J 2003; 22:2841-51.
54. Jakel S, Mingot J-M, Schwarzmaier P, Hartmann E, Gorlich D. Importins fulfil a dual function as nuclear import receptors and cytoplasmic chaperones for exposed basic domains. EMBO J 2002; 21:377-86.
55. Kufel J, Dichtl B, Tollervey D. Yeast Rnt1p is required for cleavage of the preribosomal RNA in the 3' ETS but not the 5' ETS. RNA 1999; 5:909-17.
56. Omer AD, Lowe TM, Russell AG, Ebhardt H, Eddy SR, Dennis PP. Homologs of small nucleolar RNAs in Archaea. Science 2000; 288:517-22.
57. Tang TH, Bachellerie JP, Rozhdestvensky T, Bortolin ML, Huber H, Drungowski M, et al. Identification of 86 candidates for small nonmessenger RNAs from the archaeon Archaeoglobus fulgidus. Proc Natl Acad Sci USA 2002; 99:7536-41.
58. Oeffinger M, Tollervey D. Yeast Nop15p is an RNA-binding protein required for prerRNA processing and cytokinesis. EMBO J 2003; 22:6573-83.
59. Jorgensen P, Nishikawa JL, Breitkreutz BJ, Tyers M. Systematic identification of pathways that couple cell growth and division in yeast. Science 2002; 297:395-400.
60. Du YC, Stillman B. Yph1p, an ORC-interacting protein. Potential links between cell proliferation control, DNA replication, and ribosome biogenesis. Cell 2002; 109:835-48.
61. Visintin R, Hwang ES, Amon A. Cfl1p prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. Nature 1999; 398:818-23.
62. Shou W, Seol JH, Shevchenko A, Baskerville C, Moazed D, Chen ZW, et al. Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. Cell 1999; 97:233-44.
63. Zhang Y, Yu Z, Fu X, Liang C. Noc3p, a bHLH protein, plays an integral role in the initiation of DNA replication in budding yeast. Cell 2002; 109:849-60.
64. Jorgensen P, Tyers M, Warner JR. Forging the factory: Ribosome synthesis and growth control in budding yeast. In Regulation of Cell Growth. M. Hall ed. Cold Spring Harbor Press 2004; In press.
65. Olson MO, Dundr M, Szebeni A. The nucleolus: An old factory with unexpected capabilities. Trends Cell Biol 2000; 10:189-96.
66. Grandi P, Rybin V, Bassler J, Petfalski E, Strauss D, Marzioch M, et al. 90S preribosomes include the 35S prerRNA, the U3 snoRNP, and 40S subunit processing factors but predominantly lack 60S synthesis factors. Mol Cell 2002; 10:105-15.
67. Kruiswijk T Planta RJ Krop JM. The course of the assembly of ribosomal subunits in yeast. Biochim Biophys Acta 1978; 517:378-89.